

Sorghum Improvement in the New Millennium



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Abstract

Sorghum is one of the most important cereal crops in the semi-arid tropics (SAT) of Asia, Africa and Latin America. Traditionally, it is grown for food, feed and fodder needs and of late it is emerging as an important bioenergy crop. Sorghum production is constrained by several biotic and abiotic stresses depending on the production environment. Although it is predominantly self-pollinated, the discovery of cytoplasmic male sterility (CMS) and genetic male-sterility (GMS) has enabled easy cross-pollination in sorghum. The ease of pollination control in sorghum has become a boon for sorghum researchers, thus enabling development of both pure-line varieties (as in self-pollinated crops) and hybrids and open-pollinated populations (as in cross-pollinated crops). Genetic enhancement of sorghum for economic traits *per se* and plant defense traits that stabilize the crop performance requires thorough knowledge about different end uses, nature and intensity of different production stresses, and sound theoretical and applied knowledge of genetic and crop breeding principles. Rapid progress in biotechnology research has provided powerful tools to sorghum researchers, which complement conventional crop improvement efforts to develop desired products. Production of pure and high quality seeds of genetically improved cultivars and their delivery to the target farmers in required quantities at the right time is the key for achieving high productivity in farmers' fields. The 32 chapters contributed by 37 specialists in this book is based on a joint training course on a range of topics including importance of sorghum, pollination control mechanisms, phenology of crop growth, germplasm diversity and utilization, genetic male sterility (GMS) and cytoplasmic male sterility (CMS) systems and their utilization, development of CMS-based male sterile lines (seed parents) and their fertility restorer lines (R-lines), development of GMS facilitated open-pollinated populations, heterosis theories and their harnessing, techniques to screen insect pests and diseases, seed production principles and practices, alternative uses of sorghum, linking producers and processors. This book serves as a valuable resource and will be of significant interest to those working on sorghum improvement.

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and CLL Gowda



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Science with a human face

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Foreword

Sorghum is one of the main staple foods for the world's poorest and most food-insecure people across the semi-arid tropics of the world, where it is mainly produced and consumed by rural poor. India contributes about 16% of the world's sorghum production. Despite remarkable achievements of sorghum programs in increasing productivity and production in the earlier period of the green revolution, the challenge of making sorghum profitable and attractive for small farmers still remains. The major challenge is to transform subsistence sorghum farming to commercial and profitable production. This requires reassessment of crop research needs in terms of current and future demand resolving specific production constraints, development of post-harvest processing and value-addition technologies, marketing techniques, and policies that may result in additional income and employment. All these have to be achieved without sacrificing the overall goal of attaining sustainable food and nutritional security, especially of the peasant farmers in dryland and remote areas, and the poor urban consumers of millets. Needless to state that emphasizing industrial uses of sorghum is essential to promote its production and marketing. Special emphasis should also be given to feed and fodder uses as the demand for meat and milk are increasing.

The potential yield of sorghum especially of hybrids exceeds 16 tons per hectare. However, actual yield in farmers' field in India is only about 1.0 ton/ha and even less in Africa. Moreover, sorghum is pushed continuously to less favorable soil and deprived of labor and purchased inputs in the wake of progress made by other crops like maize and Bt. Cotton.

Keeping all the above in view, the collaborative training on "*Sorghum hybrids and hybrid parents*" was most timely and relevant where National Research Centre for Sorghum and International Crops Research Institute for the Semi-Arid Tropics scientists devised this training module for both national and international trainees. I am sure the contents of this training book on sorghum will be a handy source to all the sorghum researchers and extension workers throughout the world. I congratulate the course coordinators and the editors for their valuable contribution.

N Seetharama

Preface

Sorghum (*Sorghum bicolor* L. Moench) is one of the most important cereal crops grown in semi-arid tropics of the world. It is traditionally grown as food-fodder crop at subsistence levels by resource-poor farmers with limited inputs. In recent years, it is emerging as a potential alternative feed, and bioenergy crop, thanks to rapid growth in the poultry industry and tremendous demand for fuel-grade ethanol following a mix of policies to blend petrol with ethanol made by several fossil-fuel importing countries with the twin objective of reducing air pollution and their import bills. Thus, sorghum is becoming truly a '4F' crop—food, fodder, feed and fuel. In addition, its resilience to high temperatures and drought makes it a climate ready crop.

Sorghum is grown in areas challenged by vagaries of environment such as biotic and abiotic stresses. Developing cultivars resilient to these challenges and diverse end uses is the key to improving sorghum productivity in farmers' fields to make sorghum cultivation a profitable and attractive venture. A thorough knowledge on the nature and intensity of these challenges and various alternative uses and keeping abreast of latest developments in these fields is essential for scientific improvement of sorghum.

Considering that seed is the most vital of all the inputs for any crop, so it is for sorghum, development of effective seed systems is of utmost importance for realizing impacts of genetic improvement efforts. Human resource development is an important component of sorghum research and development programs. It is equally important to constantly upgrade the skills and knowledge of the sorghum scientists to keep pace with rapid strides made both in conventional and biotechnological tools for crop improvement to achieve maximum impact on on-farm productivity of sorghum. Partnership research and development is the way forward for harnessing the potential of sorghum for multiple commercial uses.

Under this premise, I am extremely happy to write the foreword for this all important book that is being published in no better time than this. I am delighted to note that this book is the first of its kind and is prepared by internationally renowned sorghum scientists of all disciplines from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India and the National Research Centre for Sorghum (NRCS), Rajendranagar, Hyderabad, Andhra Pradesh, India based on the International Training Course jointly organized by ICRISAT and NRCS. This book is yet another proof of ICRISAT's commitment to partnership mode of science-based crop research for impacts.

This book provides exhaustive information on all aspects of sorghum improvement. I am sure this book will serve as an excellent reference to sorghum researchers, technicians, and all those involved in sorghum improvement.

William D Dar

Acknowledgments

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About this book

Sorghum is gaining importance in the light of new market opportunities for bioethanol, feed, industrial starch and nutritious food. The traditional food and fodder value of sorghum is intact and is likely to increase in the light of climate change. Adaptation to varying and harsh climatic conditions, high water and input use efficiency, high biomass from a short life cycle, availability of stable male sterility systems and easy cross-pollination are beneficial to sorghum production and its improvement. Heterosis for grain and stover yields is well established in sorghum and it is one of the first crops where commercial hybrids have been developed. The concerted efforts of NARS and the private sector over the years have made hybrids popular with farmers all over the world. In Africa, the seed industry is steadily developing, and this will help spread hybrids on the continent.

In spite of the high heterosis with hybrid technology, global sorghum productivity is hovering at 1.3 t ha⁻¹ compared to the genetic potential of 3 t ha⁻¹. Production of pure and high quality seeds of genetically improved cultivars and their delivery to the target farmers in required quantity at the right time is critical for achieving high productivity on farmers' fields. There is a compelling need to upgrade the skills of sorghum scientists and keep them abreast of the latest developments in sorghum research in order to increase the pace and efficiency of the sorghum improvement programs and their impacts on farmers' fields.

This book is developed based on the joint training course organized for sorghum scientists—public and private sectors—from India (16) and other countries [the Philippines (1) and Sudan (1)] at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India and National Research Centre for Sorghum (NRCS), Rajendranagar, Hyderabad, India during 6-17 February 2007. The course was co-ordinated by Belum VS Reddy, A Ashok Kumar and P Sanjana Reddy from ICRISAT and C Aruna, AV Umakant and Vilas A Tonapi from NRCS. The papers presented during the course are published in this book and describe the theoretical and practical aspects of sorghum improvement in the light of continuing and new challenges and novel technological options added to the armory. We hope that this book will be of use to scientists and technicians involved in sorghum genetic improvement and seed production and marketing.

Editors

Abbreviations and Acronyms

ABI	Agri Business Incubator
ABU	Ahmadu Bello University
AFLP	Amplified fragment length polymorphism
AHT	Advanced hybrid trial
AICSIP	All India Coordinated Sorghum Improvement Project
ALAD	Arid Lands Agricultural Development
ANGRAU	Acharya NG Ranga Agricultural University
APPF	Andhra Pradesh Poultry Federation
ARC	Agricultural Research Corporation
ARS	Agriculture Research Station
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
BLIPs	Best linear unbiased predictors
CBD	Complete block design
CFCF	Cell-free culture filtrates
CGIAR	Consultative Group on International Agricultural Research
CGR	Compound growth rate
CIAT	Centro Internacional de Agricultura Tropical
CIMMYT	Centro Internacional de Mejoramiento del Maíz y del Trigo
CIP	Composite interval mapping
CLSV	Curly leaf spot virus
CMS	Cytoplasmic male sterility
CRD	Completely randomized design
CRSP	Collaborative Research Support Program
CSC	Central Seed Committee
DAC	Department of Agriculture and Cooperation
DAE	Days after emergence
DArT	Diversity arrays technology
DBT	Department of Biotechnology
DFID	Department for International Development
DH	Doubled-haploid
DMD	Dry matter digestibility
DSSF	Design of seed store facility
DUS	Distinctiveness, uniformity and stability
EARCAL	East Africa Regional Cereals and Legumes
EARSAM	East Africa Regional Sorghum and Millets

EDV	Essentially derived variety
EPA	Environment Protection Act
ECA	Eastern and Central Africa
ESIP	Ethiopian Sorghum Improvement Program
FAO	Food and Agriculture Organization
FFA	Federation of Farmers Associations
FIIRO	Federal Institute for Industrial Research
GCA	General combining ability
GCD	Global Crop Diversity
GCV	Genotypic coefficient of variance
GEAC	Genetic Engineering Approval Committee
GIS	Geographical Information System
GMOs	Genetically Modified Organisms
GMR	Grain mold resistance
GMS	Genetic male-sterility
GOI	Government of India
GRIN	Germplasm Resources Information Network
HCN	Hydrocyanic acid
HPR	Host plant resistance
HTP	High-throughput
IADB	Inter American Development Bank
IAR	Institute of Agricultural Research
IBD	Incomplete block design
IBPGR	International Board on Plant Genetic Resources
IBSC	Institutional Bio-safety Committee
ICAR	Indian Council of Agricultural Research
ICBA	International Centre for Biosaline Agriculture
ICRISAT	International Crops Research Institute for the Semi-Arid Tropics
IAC	ICRISAT's Asia center
ICSA	ICRISAT sorghum A-line
ICSB	ICRISAT sorghum B-line
IDM	Integrated disease management
IDMRS	ICRISAT Data Management Retrieval System
INTSORMIL	International Sorghum and Millet Collaborative Research Support Program
IPGRI	International Plant Genetic Resources Institute
IRAT	Institut de Recherche Agronomiques Tropicales
ISTA	International Seed Testing Agency
ISR	Induced systemic resistance

IVDMD	In vitro dry matter digestibility
KSU	Kansas State University
LASIP	Latin American Sorghum Improvement Program
LAAS	Liaoning Academy of Agricultural Sciences
LOD	Logarithm of odds
LRAHPT	Landrace advanced hybrids and parents trial
LSD	Latin square design
LTS	Long-term storage
MAS	Marker-assisted selection
MSP	Minimum support price
MTA	Material Transfer Agreement
MTS	Medium-term storage
NAGS	National Active Germplasm Sites
NARS	National agricultural research systems
NBPGR	National Bureau of Plant Genetic Resources
NGB	National Genebank
NPGS	National Plant Germplasm System
NRCPB	National Research Centre on Plant Biotechnology
NRCS	National Research Centre for Sorghum
NSB	National Seed Board
NSC	National Seed Corporation
OAU	Organization of African Unity
PBR	Plant breeders rights
PCA	Principal component analysis
PCR	Polymerase chain reaction
PCV	Phenotypic Coefficient of variance
PDS	Public distribution system
PFT	Poultry feed trials
PGMR	Panicle grain mold rating
PIC	Prior informed consent
PGR	Plant genetic resources
PPVFRA	Protection of Plant Variety and Farmers Rights Act
QTL	Quantitative trait loci
R&D	Research and development
RAPD	Random Amplification of Polymorphic DNA
RCGM	Review Committee on Genetic Manipulation
REML	Restrict maximum likelihood

RFLP	Restriction fragment length polymorphism
RIL	Random inbred line
RH	Relative humidity
RCBD	Randomized complete block design
RCV	Reference collection of varieties
RRS	Reciprocal recurrent selection
SAAPRI	Serere Agricultural and Animal Production Research Institute
SADC	South African Development Committee
SAFGRAD	Semi-Arid Food Grain Research and Development
SAR	Systemic acquired resistance
SAT	Semi-arid tropics
SAUs	State Agricultural Universities
SIM	Simple interval mapping
SMIP	Sorghum and Millet Improvement Program
SNP	Single nucleotide polymorphism
SPV	Sorghum preliminary variety
SSC	State Seed Corporations
SSR	Simple sequence repeat
STMS	Sequence-tagged microsatellite site
STRC	Scientific Technical and Research Commission
STS	Short-term storage
TCL	Truthfully labeled seed
TRGV	True relative genetic variance
TRIP	Trade Related Aspects of Intellectual Property Rights Agreement
TSS	Total soluble sugars
TZ	Tetrazolium test
USA	United States of America
USAID	United States Agency of International Development
USDA	United States Department of Agriculture
VCU	Value for cultivation and usage
VTM	Variable tandem repeat
WCA	West and Central Africa
WTO	World Trade Organization

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Sorghum cultivation in India: Past and future

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Introduction

Sorghum, a dryland cereal crop, is mainly grown in the Deccan Plateau, Central and Western India apart from a few patches in Northern India. It is nutritionally superior to other fine cereals such as rice and wheat with high fiber content, minerals and slow digestibility. As sorghum is generally cultivated in nutrient-poor soils in frequently drought-prone areas, it offers food and fodder security through risk aversion on sustainable basis. Traditionally, sorghum is grown for food and fodder purposes. In view of decreasing demand for sorghum [rainy season (*kharif*) sorghum grain in particular] as a food crop, it is increasingly diverted for various alternative uses such as animal feed, poultry feed, potable alcohol from grain. Of late, sweet sorghum with juicy stalks and high sugar content is emerging as a potential alternative feedstock for ethanol production to meet the increased demand for ethanol following Government of India's policy to blend petrol with ethanol, with the twin objective of reducing air pollution and import bill for the country.

Status of sorghum in the world vs India

India is the largest sorghum grower in the world (area of 9.18 m ha during 2005–06) followed by Nigeria and Sudan. In terms of sorghum production, the situation is reverse. India ranked seventh among the nine countries where sorghum is grown on more than 1 m ha. During 2005–06, China topped in productivity with 3,960 kg ha⁻¹. Indian sorghum productivity during the same period constituted 20% of productivity of China or 60% of the global productivity (Table 1).

Table 1: Comparison of Indian sorghum production with the rest of the world.

Countries	Area (m ha)			Production (m t)			Productivity (kg ha ⁻¹)		
	1981	2005	% #	1981	2005	% #	1981	2005	% #
World	44.89	44.37	-1.16	65.52	58.99	-9.97	1458	1330	-8.779
Nigeria	2.68	7.07	163.51	3.28	8.03	144.52	1461	1135	-22.31
Sudan	3.05	7.03	130.19	2.27	4.01	76.42	725	565	-22.06
Mexico	1.49	1.9	27.43	4.99	6.59	32.04	3343	3471	3.82
USA	5.27	2.71	-48.61	19.16	10.67	-44.30	3618	4000	10.55
China	2.83	0.66	-76.66	7.03	2.6	-63.04	2493	3975	59.44
India	16.36	9.18	-42.97	11.38	7.47	-34.36	695	801	15.25
B. Faso	1.05	1.52	44.76	0.62	1.47	137.10	589	969	64.51

Change in TE 2005 over TE 1981; Source: FAO database.

The area under sorghum has declined in every continent except in Africa and Mexico, while global production and productivity have declined except in China, Burkina Faso and India. The increase in sorghum productivity over a period of about two decades in India (15%) is much lower than in China (59%). However, it is far better than the world's average (-9%).

Status of sorghum cultivation in India

Sorghum is grown in 9.19 million ha with a production of 7.47 million tons and an average productivity of 816 kg ha⁻¹ during 2005-06. *Kharif* sorghum occupied 4.11 million ha area while *rabi* sorghum was cultivated in relatively more acreage of 5.07 million ha (during 2005). Post-rainy season (*rabi*) sorghum area is slightly higher (56% of the total sorghum) than *kharif* sorghum area (44% of the total sorghum) while the *kharif* production is 10% higher than *rabi* production.

Trends in sorghum cultivation in India

Area changes: In India, sorghum was one of the major cereal staples during 1960s, occupied an area of 18 million ha during the same decade, which almost remained unchanged throughout the 60s. Almost 1 m ha area was lost between 1970 and 1973 and another 1 m ha between 1988 and 1989. Since 1990 the reduction in area continued rapidly resulting in current area of 8.78 million ha. Therefore, during the last 15 years, a total area of 7.22 million ha has been diverted to other crops. The compound growth rate in sorghum area from 1967-2005 is -2.35% per annum. This decline is more pronounced in *kharif* (-3.76%) compared to *rabi* (-1.17%) due to availability of more crop alternatives in the former than in the latter. However, in the recent past, the negative growth in both *kharif* and *rabi* area has reduced (Table 2). The decline in overall time period has serious repercussions on the cropping systems and the food security of these dryland regions of the country.

Table 2. All India compound growth rates of area, production and yield of principal crops.

Crop	Sea- son	1967-68 to 2005-06			1980-81 to 2005-06			1990 to 2005-06			2000-01 to 2005-06		
		A	P	Y	A	P	Y	A	P	Y	A	P	Y
Sorghum	K	-3.76	-1.63	2.20	-4.39	-3.18	1.26	-4.94	-4.91	0.04	-4.12	-5.56	-1.50
	R	-1.17	2.28	3.48	-0.92	0.22	1.15	-0.63	0.10	0.73	-0.85	-2.31	-1.47
	T	-2.38	0.10	2.55	-2.79	-1.95	0.87	-2.86	-3.01	-0.16	-2.40	-4.16	-1.81
Rice		-1.74	-0.49	1.27	0.40	2.31	1.90	0.11	1.07	0.96	-0.34	0.62	0.96
Wheat		0.68	0.57	-0.11	0.75	2.95	2.18	0.72	1.99	1.26	0.05	0.95	0.89

A = Area, P = Production, Y = Yield; K = Kharif; R = Rabi; T = Total

Source: Agricultural Statistics at a Glance (2005), MoA, GoI, New Delhi.

Factors for declining area

Several factors have contributed to decline in sorghum area (especially rainy season sorghum). These are: (i) lack of competitiveness/low remuneration for rainy season sorghum grains (ii) change in consumer preference for food grains in favor of fine cereals such as rice and wheat (iii) discriminative government policies (lack of both input subsidies and output price incentives which otherwise provided for rice and wheat, (iv) price competition from oil seed, pulse and commercial crops (such as cotton, soybean, groundnut, sunflower, etc) and (v) increase in land area under irrigation, which led to shift in commercial crops.

However, sorghum is a risk aversion crop and cannot be completely eliminated from cropping systems as it is a sustainable fodder source for meeting huge livestock demand under water scarce/drought conditions, which is a common feature of the semi-arid tropics (SAT). Further, it is a good choice as a rotation crop to maintain soil fertility and manage pests.

Productivity trends

The improvement in *kharif* sorghum's productivity for the period 1967–2005 and in other sub-periods is lower than that of rice and wheat for the corresponding period (Table 3). Genetic improvement was the major reason for this productivity



Fig 1. An improved *Kharif* grain hybrid from Indian national program.

improvement. Advances in productivity achieved from the first commercial hybrid, CSH 1 to the more recently evolved hybrid, CSH 16 (Fig. 1) and CSH 23 are significant in respect of both grain and fodder yields. *Kharif* sorghum productivity rose from 546 kg ha⁻¹ in 1970 to 957 kg ha⁻¹ in 2005, reflecting a 75% increase. Compound Growth Rate (CGR) of *kharif* sorghum productivity is to the tune of 3.55% during the period from 1967 to 1980, which surpassed those of all other dryland crops (and even rice and wheat) during the same period.



Fig. 2. Popular *rabi* cultivar in India.

The improvement in *rabi* sorghum productivity over the period of time is marginal and is not pronounced as much as in *kharif* sorghum. Unlike in *kharif* sorghum, factors responsible for the *rabi* sorghum productivity increase are not clearly identifiable. High yielding varieties are certainly not a principal factor as much of the *rabi* sorghum area is still grown with traditional landraces or improved varieties such as M 35-1 (Fig. 2). Adoption of improved crop management technologies with better soil moisture conservation techniques appear to contribute to the productivity improvement.

State-wise trends in sorghum area, production and productivity

State-wise area, production and yield of sorghum during 2005 are given in Table 3. Currently, Maharashtra is the largest sorghum producer with 1.51 million ha area under *kharif* and 3.10 million ha under *rabi* followed by Karnataka whose total sorghum area is 1.59 million ha (1.31 million ha is under *rabi* and the rest under *kharif*).

Gujarat, Andhra Pradesh and Madhya Pradesh states witnessed highest decline in sorghum area during the period from 1970 to 2005. However, improvement in productivity is phenomenal in these states during the same period. Sorghum area declined though at a lower magnitude (24 and 46% respectively) in major sorghum growing states such as Maharashtra and Karnataka also. Maharashtra is the only state where the production changes are positive and significant during the above mentioned period.

Table 3: Changes in sorghum scenario in major sorghum growing states in India.

States	Area (m ha)			Production (m t)			Yield (kg ha ⁻¹)		
	1970	2005	% #	1970	2005	% #	1970	2005	% #
Maharashtra	6.06	4.61	-24	2.79	3.79	36	456	764	68
Karnataka	2.94	1.59	-46	1.82	1.15	-37	628	729	16
Andhra Pradesh	2.66	0.48	-82	1.21	0.59	-51	455	1154	154
Madhya Pradesh	2.44	0.6	-75	1.54	0.72	-53	629	1128	79
Gujarat	1.32	0.13	-90	0.40	0.13	-68	304	1073	253
Tamilnadu	0.72	0.49	-32	0.53	0.49	-8	740	795	7
Rajasthan	1.10	0.62	-43	0.40	0.29	-27	350	439	25
Uttar Pradesh	0.76	0.27	-64	0.46	0.27	-41	603	961	59
All India	18.24	9.19	-50	9.21	7.47	-19	504	816	62

Change in TE 2005 over TE 1970; Source: Directorate of Economics and Statistics, Government of India.

Consumption trend

Importance of sorghum as the staple grain source is steadily declining. *Per capita* consumption of sorghum, in rural India steeply declined from 1.59 kg month⁻¹ in 1973 to 0.45 kg month⁻¹ in 2003. However, regional differences exist. This decline is also significant in Maharashtra where the grain is traditionally being used as the staple food.

Utilization of sorghum grain

Rabi sorghum grain is highly valued as food grain, and expensive for use as industrial raw material. The utilization of *kharif* sorghum grain as a raw material in various industries is increasing, given the limited prospects of rainy season (*kharif*) sorghum for human consumption. The main industries currently using sorghum in India are the poultry feed, animal feed and alcohol distilleries. At present poultry feed sector is using approximately 1.3 million t annually; animal feed sector uses about 0.45 million t followed by alcohol distillers (about 0.092 million t). The estimation for the future demand shows that poultry feed industry is going to be the major industry which will absorb huge quantity of sorghum (4.0 million t), followed by dairy animal feed industry (0.6 million t) by 2010 A.D. This estimate is based on the current trends, but if government policy permits the use of sorghum grain for potable alcohol production, about 4 million t may be required for the brewing industry alone.

Policy interventions to promote sorghum cultivation

Sorghum grain, especially *rabi* grain, should be included in the public distribution system (PDS) as a supplement to rice and wheat. This will help popularize sorghum grains in places where it is largely grown. Unlike *rabi* sorghum grains, *kharif* sorghum grains have lower shelf life. Therefore, *kharif* sorghum grains should be distributed within three months of its procurement, after which regular rice and wheat supply may be resumed. Minimum support price (MSP) of sorghum should be fixed based on cost of cultivation. The government should intervene whenever the market price goes below MSP to ensure marginal farmers a fair price for their produce. Popularization of sorghum as a health food is to be taken up on a large scale through the mass media. Cultivation of sweet sorghum should be promoted for use as alternative feed stock for the production of ethanol by announcing tax-holiday for five years.

Summary

The sorghum-based economy can be effectively revived and made viable despite threats from competing crops. There are opportunities galore for the commercialization of sorghum today. Despite the decline of the value of sorghum grain for human food, it is still the most preferred cereal in the areas where it is traditionally grown. If only policy support is garnered, the role of sorghum as food, fodder, and industrial raw material will be expanded. Sorghum production should be re-oriented to equip dryland farmers to face the free market forces in the face of globalization.

Reproductive biology and breeding behavior of sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] belongs to the family Gramineae, and the genus Sorghum. It is an important cereal staple and forage crop of the semi-arid tropics of the Indian sub-continent and several African regions. Notwithstanding several biotic and abiotic constraints, sorghum productivity is low (0.8 t ha^{-1}) in the world. Adoption of seed-based technologies in the form of improved cultivars developed through genetic enhancement is a low cost option compared to management options for increasing sorghum productivity in farmers' fields. Experience has shown that adoption level and diffusion rate of improved cultivars is higher than those of improved crop management practices and hence have greater impact on increasing productivity. An understanding of floral biology, pollination control mechanisms and seed development is essential for designing effective breeding strategies and suitable breeding methods for systematic genetic improvement of sorghum. Breeding methods and procedures to be employed for genetic enhancement of any crop species (sorghum is no exception to this) are largely determined by its mode of reproduction. In this article, floral biology and reproductive biology in sorghum is described.

Reproductive biology of sorghum

Panicle initiation

Sorghum is a short day plant, and blooming is hastened by short days and long nights. However, varieties differ in their photoperiod sensitivity (Quinby and Karper 1947). In traditional varieties, reproductive stage is initiated when day lengths return to 12 hours. Floral initiation takes place 30 to 40 days after germination. Usually, the floral initial is 15 to 30 cm above the ground when the plants are about 50 to 75 cm tall (House 1980). Floral initiation marks the end of the vegetative phase. The time required for transformation from the vegetative primordial to reproductive primordial is largely influenced by the genotype and the environment. The grand growth period in sorghum follows the formation of a floral bud and consists largely

of cell enlargement. Hybrids take less time to reach panicle initiation, more days to expand the panicle and a longer grain filling period than their parents (Maiti 1996).

Panicle emergence

During the period of rapid cell elongation, floral initial develops into an inflorescence. About 6 to 10 days before flowering, developing inflorescence inside the leaf sheath of flag leaf will appear as a boot shaped structure. This will occur in about 55 days from germination in a variety that flowers in 60 to 65 days. Sorghum usually flowers in 55 to more than 70 days in warm climates, but flowering may range from 30 days to more than 100 days.

Panicle structure

Inflorescence is a raceme, which consists of one or several spikelets (Fig. 3). It may be short, compact, loose or open, composed of a central axis that bears whorls of primary branches on every node. The racemes vary in length according to the number of nodes and the length of the internodes. Each primary branch bears secondary branches, which in turn bear spikelets. The central axis of the panicle, the rachis, is completely hidden by the density of the panicle branches in some, while it is completely exposed in others. The spikelet usually occurs in pairs, one being sessile and the second borne on a short pedicel, except the terminal sessile spikelet, which is accompanied by two pedicelled spikelets. On the pedicelled spikelet, the pedicels vary in length from 0.5 to 3.0 mm, and usually are very similar to the internodes. The first and second glumes of every spikelet enclose two florets; the lower one is sterile and is represented by a lemma, and the upper fertile floret has a lemma and palea. Two lodicules are placed on



Fig. 3. A typical sorghum panicle with anthesis in progress.

either side of the ovary at its base. Androecium consists of one whorl of three stamens. The anthers are attached at the base of the ovule by a very fine filament and are versatile and yellowish. Gynoecium is centrally placed and consists of two pistils with one ovule from which two feathery stigmas protrude. Many of these floral characters, such as anther color, stigma color, stigma length, length of pedicel, etc, are important distinctiveness, uniformity and stability (DUS) testing traits.

Sessile spikelets: The sessile spikelet contains a perfect flower. It varies in shape from lanceolate to almost round and ovate and is sometimes depressed in the middle. The color is green at flowering, which changes to different colors like straw, cream, yellow, red, brown, purple, or almost black at grain maturity. The intensity and extent of coloring on the glumes is variable. Glumes vary from quite hairy to almost hairless. The seed may be enclosed by the glume or may protrude from it, being just visible to almost completely exposed.

Pedicelled spikelets: These are much narrower than the sessile spikelets, usually lanceolate in shape. They can be smaller or longer than the sessile spikelets and sometimes the same size as the sessile spikelets. They possess only anthers but occasionally have a rudimentary ovary and empty glumes.

Anthesis and pollination

Anthesis starts with the exertion of the complete panicle. Flowers begin to open two days after complete emergence of the panicle. Floret opening or anthesis is achieved by swelling of the lodicules, and is followed by the exertion of anthers and stigmas between the lemma and palea. The sorghum head begins to flower at its tip and anthesis takes place successively downward over a period of 4 or 5 days. Anthesis takes place first in the sessile spikelets from top to bottom of the inflorescence. It takes about 6 days for completion of anthesis in the panicle with maximum flowering at 3 or 4 days after anthesis begins. Flowering proceeds downwards to the base in a horizontal plane on the panicle. When flowering of the sessile spikelets is halfway down the panicle, pedicellate spikelets start opening at the top of the panicle and proceed downwards. The flowering phase of pedicellate spikelets overtakes that of sessile spikelets before they reach the base of the inflorescence (Maiti 1996). Anthesis takes place during the morning hours, and frequently occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. It normally starts around midnight and proceeds upto 1000 depending on the cultivar, location and weather. Maximum flowering is observed between 0600 and 0900. Because all heads in a field do not flower at the same time, pollen is usually available for a period of 10 to 15 days. At the time of flowering, the glumes open and all the three anthers fall free, while the two stigmas protrude, each on a stiff style. The anthers dehisce when they are dry and pollen is blown into the air. Pollen in the anthers remains alive several hours after pollen shedding. Flowers remain open for 30

to 90 min. Dehiscence of the anthers for pollen diffusion takes place through the apical pore. The pollen drifts to the stigma, where it germinates; the pollen tube, with two nuclei, grows down the style, to fertilize the egg and form a $2n$ nucleus. Glumes close shortly after pollination, though the empty anthers and stigmas still protrude (except in the long glumed types). The florets of some of the very long glumed types do not open for fertilization—a phenomenon known as cleistogamy.

The discovery of cytoplasmic-nuclear male sterility (CMS) in sorghum has enabled the development and commercialization of hybrids. A good male-sterile plant will not develop anthers, but in some instances dark-colored shriveled anthers with no viable pollen will appear. Partially fertile panicles are also observed, although the viable pollen quantity in such panicles is less than that in normal fertile panicles.

Breeding behavior: Sorghum is predominantly a self pollinating crop and natural cross pollination varies from 0.6 to 6% depending on the genotype, panicle type and wind direction and velocity. Stigmas exposed before the anthers dehisce are subjected to cross pollination. Pollination for crossing purposes should start soon after normal pollen shedding is completed during morning hours. Hand pollination should begin around 0930 to 1000 and can be extended up to 1130 to 1230 on a foggy morning (House 1980).

Seed development

Seeds are borne on raceme branches on the panicles. After fertilization, the ovule begins to develop as a light green, almost cream-colored sphere; after about 10 days it begins to take size and becomes darker green. The development of grains follows a sequence of stages comprising milky, soft dough, hard dough to the final physiological maturity, when a black layer is formed at the hilar region due to the formation of callus tissue. It takes about 30 days for the seeds to reach maximum dry weight (physiological maturity). The seeds begin to turn from green to the different colors depending on the genotype at the time of maturity. The seeds contain about 30% moisture at physiological maturity; the seed moisture level reduces to about 10 to 15% at 20 to 25 days after attaining physiological maturity (House 1980). During this period, they lose up to 10% of dry weight. The seed can be harvested at any time from physiological maturity to seed dryness; however, seed with more than 12% moisture must be dried before storage. The seeds harvested and dried at physiological maturity have good quality and fetches higher market price (Audilakshmi et al. 2005).

Lower leaves begin to die and dry up during this period. By the time the grain begins to dry, four or five of the lower leaves dry up and drop from the plant. There is a distinct genetic difference in the rate of senescence of remaining leaves. All leaves may be dried at grain maturity; or the plant may remain green.



Fig. 4. Variability in grain shape.

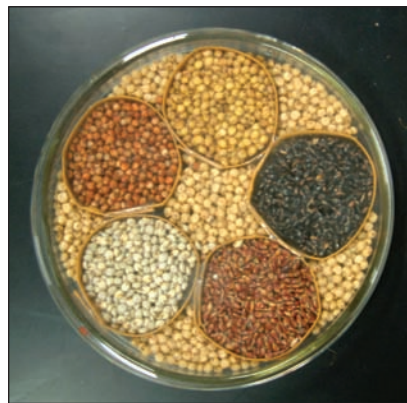


Fig. 5. Variability in grain color.

Greater genotypic variability is seen in seed morphology of different taxonomic groups. Their morphology differs widely in size, shape, orientation of seed inside the glumes (Fig. 4), types of glume, pigmentation and pattern of the hilar structure, and size of the embryonic region (Maiti 1996). The pericarp color (red, brown, white, yellow, cream) of seeds vary tremendously and may be a dull to pearly lustrous (Fig. 5). The testa may also be colored, usually dark red to dark brown. The endosperm is usually white, and occasionally may be yellow. The embryo mark (scutellum) varies in length from about one-half to two-thirds the length of the grain, and is elliptic to elliptic oblong, concave to flat, or (rarely) convex. The hilum frequently turns dark at about the time when the seed reaches physiological maturity. The seed endosperm varies from soft with little corneous portion to a solid corneous. Seed size varies from very small (less than 1 g 100 seeds⁻¹) to large (5 to 6 g 100 seeds⁻¹). Depending on the orientation of seeds within glumes, five basic races of *sorghum bicolor*, ie, *bicolor*, *guinea*, *caudatum*, *kafir* and *durra* are recognized (Dahlberg 2000) (Table 4).

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Table 4: The floral characters of different races of sorghum.

Trait	Bicolor	Guinea	Caudatum	Kafir	Durra
Panicles	Medium sized open panicles with long, slightly stiff rachis branches	Long, loose, glabrous, and pendulous	Medium to large, oblong, dense to slightly open, hairy with stout peduncle with rigid primary branches	Erect, elongated, mostly semi compact and cylindrical panicles	Stiff, dense, compact, ovate to oblong, covered with dense pubescence; branches short, semi-erect, hairy; rachis elongated or hidden; peduncles often recurved, but occasionally erect
Glumes	Long, clasping, thick and coriaceous with obscure nerves, lower glumes are depressed and hairy	Involute, opening widely, hairy; awns conspicuous	Coriaceous, shorter than the large grain, pubescent	Glossy at maturity, moderately coriaceous and much shorter than grains	Coriaceous on the lower half and slightly to strongly depressed with a central transverse wrinkle, tends to be lightly pigmented
Spikelets	Pedicellate-persistent	Pedicellate-both persistent and deciduous; Sessile-open when mature, thus exposing the grain	Pedicellate-deciduous; Sessile-ovate to elliptical	Sessile-hairy	Pedicellate- large, persistent; Sessile-Obvoate elliptic or rhomboidal
Grains	Elliptic to subglobose, enclosed by the glumes, persistently attached to the panicle, pigmented	Small to medium, biconvex and nearly ovate, some flattened, slightly pigmented	Flat on one side and round or bulging on the other (turtle backed), chalky white or pigmented	Broadly elliptic, sometimes compressed, flattened or biconvex	Medium sized to large, biconvex with broad tip, wedge-shaped base.

Emasculation and selfing techniques in sorghum

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Emasculation technique

Items needed: Scissors, secateur, needle, forceps, butter paper bags (7 cm×3 cm×15 cm), paper clips, stapler and marking pen (Fig. 6).



Fig. 6. Removal of anthers from a bisexual flower during emasculation.

Procedure

- From the desired parental line, choose a panicle that has just started anthesis
- Clip off the florets which have completed anthesis, with a secateur or scissors
- Remove primary or secondary and tertiary-branch rachises in the lower portion of the panicle, leaving about 200–300 florets in the central portion of the panicle just below the clipped florets

- Clip off all the pedicillate (sterile) florets from the central portion, leaving only the sessile (fertile) florets
- Thin out the sessile florets by clipping off some of the tertiary rachises to make it easier to hold the sessile florets during emasculation
- Grasp the sessile floret to be emasculated between the thumb and the forefinger
- Insert a blunt needle between the glumes below the middle portion of the floret, and move it slowly around the inner surface of the glumes so as to break the stamen filaments
- Lift the needle out and upwards, slowly pushing the detached anthers out of the floret
- After emasculating, cover with a butter paper bag and clip or staple it. These bags should have date of emasculation written on them.

Precautions

Care should be taken that the glume closest to the pedicillate spikelet is held facing away from the worker.

Trimming should be done so that the individual sessile florets remain uniformly spread along the panicle branch.

Selfing technique

Items needed: Kraft paper bags, scissors, paper clips, stapler and marking pen.

Procedure

- Remove odd or off-type or rogue plants from the plot before they reach the boot leaf stage
- When a few florets have opened at the tip of the panicle, snip off the flowered florets
- Cut the flag leaf at the base
- Record the date of selfing on the selfing bag
- Put the bag (with the date) over the panicle, taking care to see that the whole of the panicle is covered by the bag, and that the bag also covers about 5–8 cm of the peduncle
- Make sure the peduncle stays in the center of the mouth of the bag wrapped over by the folded corners of the paper bag on either side

- Either staple the folded corners of the paper bag or put a paper clip, taking care to see that the bag holds the peduncle tightly.

Ten to 15 days after bagging, the bags can be removed from the panicle. The same bags can be stapled around the peduncles to mark selfing.

Precautions

Bags can be blown away by the wind or can be damaged by rain. Care must be taken to replace them immediately, recording the information originally written on the bags. Periodic inspection of the selfed plots is essential to detect damaged bags.

Characterizing phenology and growth stages of sorghum hybrids

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the principal cereal grown for food, feed, fodder and fuel around the world in over 45.8 m ha, with production and productivity of 59.6 m t and 1.30 t ha⁻¹, respectively (FAO 2004). In India, it is cultivated over 9.50 m ha with a production of 7.73 m t and productivity of 0.77 t ha⁻¹. The major production constraints that limit sorghum productivity are: abiotic (nutrient deficiency and drought stresses, water logging, temperature extremities, soil salinity and acidity, etc) and biotic (shoot fly, stem borer, head bugs, grain mold, foliar diseases, charcoal rot, etc). An understanding of phenology and growth stages is handy for systematic crop genetic improvement and for developing crop management technologies for maximizing productivity. An attempt has been made to describe phenology and growth stages in sorghum hybrids in this article.

Vanderlip and Reeves (1972) described the growth stages in temperate sorghums on a 0–9 scale. The growth stages of temperate sorghums are not adequate to characterize the Indian tropical sorghums, where the growing conditions and seasons are different from those of temperate countries. In this article, phenology and growth stages of tropical sorghum hybrids have been described (Rao et al. 2004).

Identification of growth stages and their duration

Growth stages of Indian tropical sorghums have been characterized on a '0' (emergence) to '9' (physiological maturity) scale (Table 5). The duration of these growth stages may vary with planting date, genotype and location (latitude).

Stage 0 (Emergence): Emergence is considered to have occurred when the seedlings are seen above the soil surface. This can be identified when the coleoptile is visible at the soil surface, which takes about 4 days. Furthermore, sorghum emergence will vary depending on depth of planting, seed vigor and soil moisture, temperature and physico-chemical characteristics (Fig. 7).

Management guide: Planting should be timed to synchronize adequate moisture in the top 0–15 cm soil profile to enable good emergence. Planting within 10 days

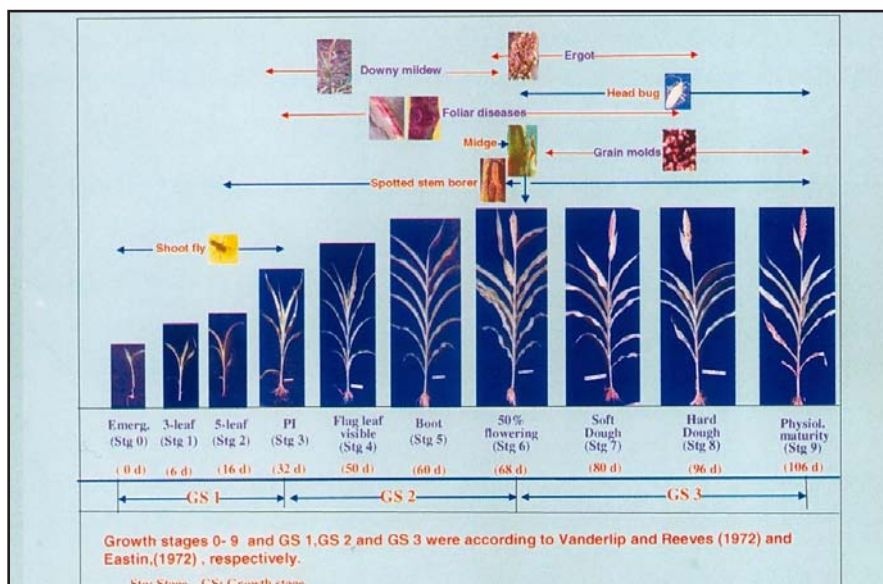


Fig. 7. Pictorial view of occurrence of insect pests and diseases at various growth stages.

after the onset of monsoon avoids shoot fly infestation. The use of pre-emergence herbicide, atrazine (@ 1.0 kg ha⁻¹) immediately after planting will prevent weed growth. Light sprinkler irrigation, if available, weakens the crust and allows rapid seedling emergence.

Table 5. Identification of growth stage characteristics in sorghum.

Growth stage number	Days from emergence	Duration (days)	Identification characteristics
0	0	0	Emergence: Coleoptile is visible at soil surface (first leaf is seen with a round tip).
1	6	6	3-leaf stage: Collar of 3 rd leaf visible.
2	16	10	5-leaf stage: Collar of 5 th leaf visible.
3	32	16	Growing point differentiation (panicle initiation): Approximately 9-leaf stage by previous criteria.
4	50	18	Flag leaf visible: Tip of flag leaf (final leaf) visible in the whorl.
5	60	10	Boot: Head extends into flag leaf sheath.
6	68	8	50% flowering: Half of the plant has completed pollination from the tip downward.
7	80	12	Soft dough: Squeezing kernel between fingers results in little or no milk.
8	96	16	Hard dough: Seed cannot be compressed between fingers.
9	106	10	Physiological maturity: Black layer (spot) appears on the hilum, at the base of the seed.

N.B. Planting to emergence takes 4 days.

Stage 1 (3-leaf stage): This stage is identified when the seedlings have three fully expanded leaves, and the collar of 3 leaves is clearly visible, which occur at 6 days after emergence (DAE) (Table 5) and the seedlings grow to a height of 20 cm (Fig. 8).



Management guide: Since seedlings are quite small with less leaf area, growth rate of weeds is higher, capitalizing on initial soil moisture and basal fertilizers applied for crop growth. Therefore, weed control during this stage is crucial for increasing crop yields. Seedlings are infested by shoot fly at this stage.

Fig. 8. 3-leaf stage.

Stage 2 (5-leaf stage): This stage is identified by the appearance of visible collar in all the 5 leaves, continuous visibility of first leaf with round tip, at 16 DAE (Table 5). The seedlings enter into 'grand period of growth' at this stage. The seedlings grow to a height of 50 cm (Fig. 9).



Fig. 9. 5-leaf stage.

Management guide: Shoot fly, weed competition, nutrient and water stress reduce the crop stand. Prolonged cold, wet and cloudy weather causes purple coloring on the leaf sheath and blades, besides iron chlorosis. Crop should be thinned to one seedling per hill to reduce seedling

competition for sunshine, soil water and soil nutrients. Soil application of carbofuran 3G (20 kg ha⁻¹) or phorate 10G (15 kg ha⁻¹) for shoot fly; and need-based application of carbofuran 3G or phorate 10G inside the plant whorls (@ 8 and 12 kg ha⁻¹) at 30 and 45 DAE protects the plant from stem borer damage.

Stage 3 (panicle initiation stage): This stage is identified at 32 DAE, when the meristem transforms from vegetative (leaf producing) to reproductive phase (panicle producing) (Table 5). Seedlings grow to a height of 95–100 cm. Panicle initiation can be observed by splitting the stalk with a sharp knife and examining under compound microscope (Fig. 10). During this stage, seedlings develop 9–10 leaves, depending upon maturity group, and the basal 2–3 leaves may become senescent. Culm growth increases rapidly following this stage.

Management guide: Seedling growth and nutrient uptake are rapid during this stage. Top-dressing of nitrogen fertilizer is recommended to hasten the panicle

growth. Inter-cultivation and weeding promotes root growth and conserves the soil moisture. The crop may be susceptible to spotted stem borer resulting in deadhearts due to larval damage to the growing tip and stem tunneling. Stem borer can be managed with the application of carbofuran granules inside the plant whorls as indicated earlier.



Fig. 10. Panicle initiation stage.

Stage 4 [Flag leaf (final leaf) visible]: The stage is identified at 50 DAE, and 18 days from stage 3. The stage can be recognized by observing the appearance of tip of flag leaf in the whorl (Table 5). Plants exhibit rapid leaf and culm elongation during this stage. All the leaves except the top 3–4 are expanded, and the basal 3–5 leaves may be dropped due to senescence. Plants grow to a height of 115–120 cm.

Management guide: Severe water, nutrient and insect pests damage reduces the potential seed number plant⁻¹. Inter-cultivation should be avoided to prevent pruning of expanding root system, loss of soil water and nutrient uptake. Continuous stem tunneling by the spotted stem borer may delay the emergence of flag leaf.

Stage 5 (boot stage): The stage can be identified at 60 DAE and 10 days from stage 4 as a swollen flag leaf sheath enclosing the panicle, which gives the appearance of boot shape (Table 5). Flag leaf is the last leaf to emerge from the growing tip and at this stage all the leaves will have maximum leaf area. This is the last stage of vegetative growth with developed panicles in the boot leaf (Fig. 11). Plants grow to a height of 125–130 cm. Plant experiences high water demand and hence plant response to irrigation is greatest at this stage.



Fig. 11. Boot leaf stage.

Management guide: Culm elongation continues. Severe drought stress during this stage may shorten the peduncle length and prevent complete exertion of the panicle. This leads to reduced number of seeds in the panicle. Due to stem tunneling by stem borer, panicle exertion may be either affected or delayed.

Protective irrigation under moisture stress over a period of >10 days help realize maximum yields.

Stage 6 (50% flowering): It can be identified when 50% of the plants in the field are in anthesis, and takes about 68–70 DAE and 8 days from stage 5 (Table 5). Plants grow to a height of 150–160 cm. Flowering typically starts 5–7 days after panicle exertion and progress from the tip to bottom of the panicle. The crop is said to be at 50% flowering when anthesis occurs on 50% of the plants in the field (Fig. 12). Flowering duration (from starting to end) usually takes 4–9 days.



Management guide: At this stage, the crop is sensitive to moisture stress. Moisture stress results in poor seed filling. Choosing appropriate maturity duration cultivars and planting time depending on the rainfall pattern is important to maximize yield. During this stage, midges will get attracted due to flowering panicles, and lay eggs inside the florets resulting in poor/no seed setting. Head bug is another insect pest that affects grain development. These pests can be managed by spraying of endosulfan 35EC or carbaryl 50 SP (@ 1 l in 500 l water ha⁻¹).

Fig. 12. Panicle with flowering in progress.

Stage 7 (soft dough stage): Following flowering stage, seed development progresses from milky stage through soft dough stage, which can be identified when kernel is squeezed between fingers for the presence of little or no milk. This stage can be identified at 80 DAE and 12 days after flowering (Fig. 13). This stage signals the end of culm elongation. About 8 to 10 functional leaves are observed and may vary with the cultivar. Plants grow to a height of about 170 cm.



Management guide: Crop yields depend on the rate of biomass accumulation and duration for grain filling. High humid conditions and prolonged rainfall following flowering result in grain mold, and cause loss in grain weight and quality. Head bug population buildup may increase rapidly, if unchecked, it may result in grain shriveling and predispose the seed to grain mold infections. Spraying malathion 10D (@ 20 kg ha⁻¹) helps manage this pest.

Fig. 13. Soft dough stage.

Stage 8 (hard dough stage): At this stage, the seed cannot be compressed between fingers, and it takes about 96 to 106 DAE to attain this stage (Fig. 14). Plants become susceptible to lodging and charcoal rot if the crop suffers from severe moisture stress. Lodging also occurs by defoliation due to insect pests and leaf diseases during flowering through hard dough stage. Also, heavy rain or hail driven by wind may cause lodging.

Management guide: Appropriate soil conservation measures can help mitigate drought stress and hence charcoal rot infection. Adequate application of farm yard manure helps retain soil moisture for longer periods, besides improving soil physico-chemical characteristics and promoting beneficial soil microorganisms during this stage. Because of extensive stem tunneling by the stem borer, the root system may also be predisposed to charcoal rot infections. Therefore, management of stem borer enhances crop yield. Cloudy weather with prolonged wet spells of rain during this stage causes grain discoloration and loss in grain weight due to grain molds. The use of appropriate maturity duration cultivars and those with genetic tolerance to grain mold are best ways to minimize damage due to grain mold.



Fig. 14. Hard dough stage.

Stage 9 (physiological maturity): This stage can be identified when a dark spot (black layer) appears at the basal portion of seed. The appearance of this spot signals the end of photosynthate supply to the seed. Physiological maturity occurs in about 106 DAE and 10 days after hard dough stage (Fig. 15). Seed moisture content at this stage varies between 25% and 35% and seeds lose maximum moisture. The crop could be harvested at 20% seed moisture content. However, the seeds must be dried to 14% moisture content for safe storage. On an average, 1,000 seeds weigh 25 g, but may range from 13 to 40 g.

Management guide: To reap the maximum yield and quality of marketable produce, the crop should be harvested immediately after physiological maturity to avoid grain mold incidence and crop lodging. Grain mold incidence to some extent can be controlled by spraying captan (0.3%) plus dithane M-45 (0.3%) thrice at 10-day intervals during grain filling stage. Harvested grains are predisposed to storage pests when kept for long term



Fig. 15. Physiological maturity.

storage. The seed may be treated with malathion 10% dust (@ 2 g kg⁻¹ seed to protect from storage pests.

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Designing a sorghum genetic improvement program

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Introduction

For ages farmers have exercised selection through saving and carrying forward the grain from healthy plants as seed for sowing the next crop. Plant breeders further augmented/enhanced the crop productivity, thus mediating directed evolution. The art of plant breeding lies in the breeder's skills in observing plants with unique economic, environmental, nutritional and aesthetic characteristics. In this article, we briefly describe various factors that need to be considered in launching a crop improvement program with emphasis on sorghum.

Economic importance

Sorghum is cultivated in over 45 million hectares in 92 countries traditionally and primarily for grain both as food (Africa and India) and as animal feed (developed countries, China, Australia, etc) and stalks as animal fodder. Of late, sweet sorghum juice is emerging as potential feedstock source for bioethanol production for use in blending petrol with dual objectives of reducing air pollution and dependency on fossil fuel. Thus, the main types of sorghums that a breeder should visualize are grain sorghum, dual-purpose sorghum, fodder sorghum and sweet sorghum.

Basic characteristics

Yield potential: Generally, sorghum can be cultivated in fields of varying fertility levels. Sorghum responds well to fertilizers. Selection of cultivars suitable for target environment and the duration that match with the rainfall pattern coupled with the use of recommended production package helps realize maximum productivity potential of the improved cultivars.

Water requirement: Sorghum requires less moisture for growth compared to maize (Table 6). It has a fibrous root system. Low rainfall conditions encourage root development; roots grow deeper even up to 1.5 m under receding soil moisture and able to extract moisture from a greater volume of soil. Traditionally, it is cultivated as a rainfed crop in rainy season in areas receiving 600 to 1000 mm rainfall in

many countries in May/June plantings. However, in postrainy season in India, it is cultivated in *vertisols* after cessation of rains when crop matures by utilizing receding soil moisture in September/October plantings.

Table 6. Water use efficiency of sorghum in comparison to maize.

Crop	Water use efficiency (kg water/kg dry matter)
Sorghum (Lima, 1998)	310
Maize (Chapman and Carter 1976)	370

Temperature requirement: It can be cultivated in temperatures ranging from 43°C (maximum) to 15°C (minimum); but growth and germination occur in some varieties even in temperatures as low as 12°C (minimum).

Germplasm base

Archaeological evidence suggests that sorghum domestication dates back to about 3000 BC (Doggett 1965a). Ethiopia/Sudan—Eastern Horn of Africa is considered to be the primary center of origin while India is considered to be the secondary center of origin. These centers of origin provide the greatest variability for breeders to select further. Harlan and de Wet (1972) classified sorghum by recognizing five basic races (*bicolor*, *guinea*, *caudatum*, *kafir* and *durra*) and their 10 hybrid races (eg, *caudatum-durra*, etc) based on the orientation of glumes over the developing grain. These races are known to differ significantly not only for grain quality traits but also for yield potential. It is established that inter-racial hybrids have greater heterosis than those of intra-racial hybrids. The knowledge on characteristics of these races becomes handy for the breeder for systematic genetic improvement of sorghum for traits of interest.

Breeding behavior

A breeder should know the breeding behavior of sorghum before launching a breeding program as breeding methods largely depend on the pollination control mechanisms. The inflorescence in sorghum is called panicle with racemes on tertiary rachis each with one or several spikelets. One spikelet is always sessile and the other pedicillate except the terminal sessile spikelet, which is accompanied by two pedicillate spikelets. The sessile spikelets have both male (androecium) and female (gynaecium) parts, and the pedicillate are usually male or female in sex. Outcrossing occurs to an extent of 5–20% depending on the weather conditions and genotypes. However, it is usually handled as self-pollinated species in breeding.

Outcrossing is mediated by the wind. Anthers mature first before stigmas and comes out of glume (called protandry), but there is variation among landraces. Anthesis (flower opening) begins from florets at the top to those at the base of the panicle usually in the morning hours after 0800 (House 1985). Outcrossing in sorghum can be facilitated by the use of genetic male-sterility. There are nearly eight different recessive genes in homozygous condition that contribute to male sterility. Among these, ms_3 and ms_7 genes are more stable and are being deployed/maintained in various populations. Cytoplasmic-nuclear male sterility (CMS) system also facilitates outcrossing in sorghum. CMS systems have facilitated development of commercial hybrids in sorghum. As many as six CMS systems are being maintained at ICRISAT. These are A_1 , A_2 , A_3 , $A_4(g)$, $A_4(vzm)$ and $A_4(M)$.

Adaptation

Sorghum has considerable potential to adapt itself to varied environmental conditions and hence, can be bred for broad adaptability. Materials bred at ICRISAT-Patancheru have done exceedingly well not only in India but in other countries as well. For example, ICSV 112, a variety bred at ICRISAT, Patancheru has been released in India, Zimbabwe, Zambia and the Central/Latin American countries. Similarly, CSH 9, a hybrid bred in the Indian Sorghum Program and released for cultivation in India, performed well in eastern and southern African countries.

Once the material is selected for broad adaptability, further improvement in yield gains can be obtained through breeding for regional adaptation, and then for specific adaptation within the region and finally for threshold traits within the specifically adapted materials. This strategy helped ICRISAT to maximize the utilization of germplasm in breeding program and enhance the yield potential significantly (Fig. 16).

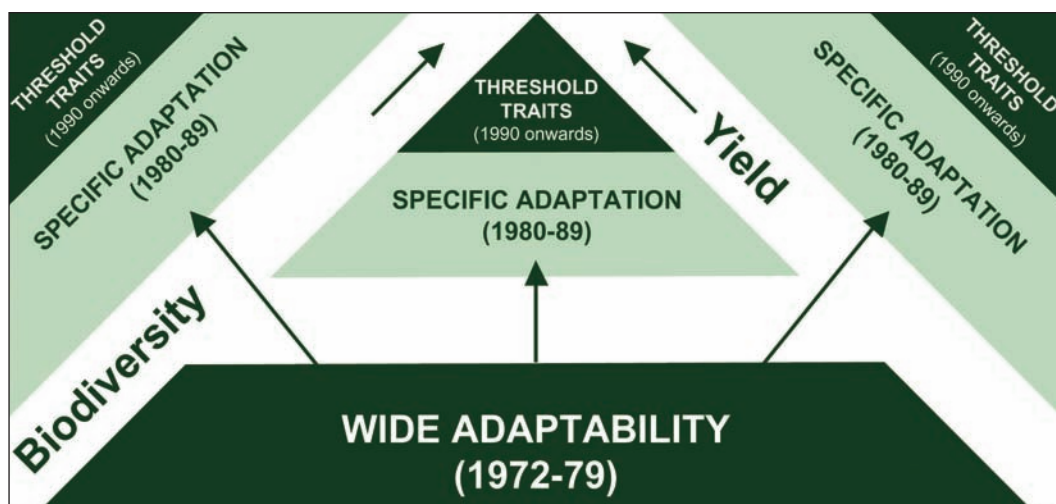


Fig. 16. Adaptation, germplasm utilization and yield levels.

Abiotic and biotic stresses

Information on the factors that limit the productivity needs to be gathered and they should be prioritized based on their importance in the target region and the availability of resources. Tolerance to drought, salinity and acidity among abiotic stresses and shoot fly, stem borer and headbug among the pests and grain mold, anthracnose, rust, leaf blight and downy mildew among diseases are important yield limiting factors and their importance vary depending on the season and location. Therefore, there is a need to develop efficient screening techniques and identify tolerance/resistance to various yield-limiting factors.

Target materials

In sorghum, target materials could be varieties or hybrids. Hybrids are popular with farmers in countries like USA, India, China and Australia. Hybrids have heterosis for grain and biomass yield, better resistance and adaptation to varied environmental conditions. The cytoplasmic-nuclear male sterility system has made it possible to produce hybrids for commercial cultivation. Further, private sector seed companies have taken up the seed production, distribution and sales in these countries helping farmers have easy access to the seed of improved cultivars. Parental lines performance has positive relationship with hybrids performance (Rao and House 1972; Mukherjee 1995); therefore parents need to be improved to improve hybrids. Heterosis in hybrids is proportional to the divergence between the parents involved (Crow and Kimura 1970). Therefore, the genepools for developing A/B-lines (female parents) development and R-lines (male parent) developments need to be handled separately to maintain divergence between them. In many of the countries in Africa (except Nigeria and Ghana), varieties are the target materials. Hybrids did not pick up because of poor seed systems in place.

Grain and fodder quality traits

Grain quality traits such as bold grain with thin pericarp and luster with semi-corneous endosperm are important for making “*roti*”, while hard grain (small grain size) contribute to lessening grain mold damage. Grain nutritional traits selection programs, such as, for high protein or lysine are not successful in sorghum. Heritabilities of fodder digestibility, high protein and less fiber content are reasonably high and it is possible to breed for high grain yield and high fodder quality.

Genetics and tools

Information on the genetics of various` traits of interest is important in designing a breeding program. For example, if a trait is under the control of a few genes, one

may use backcrosses or pedigree breeding. On the other hand, if traits are under the control of a large number of traits, population improvement with appropriate recurrent selection schemes with ms_3/ms_7 gene may be followed. The latter method is time and resource consuming. The researchers are under pressure to deliver improved cultivars within a short period, say 4 to 6 years. So, commonly, breeders follow the pedigree method of breeding.

The new science tools—marker assisted selection or genetic engineering can also be deployed in sorghum wherever resources and expertise are available. For example, markers for resistance to shoot fly and *Striga* and stay-green traits are identified in sorghum, and applying them in practical breeding is underway both in the Indian Sorghum Program as well as at ICRISAT. Genetic engineering to transfer Bt genes to control stem borer is also underway in these programs.

Resources and team

The scope of a breeding program depends on the resources available and the extent and the expertise of team members. When the resources are limited, for example, one can conveniently use simple mass selection in (ms_3) population for all traits that can be observed before flowering. Examples are mass selection for resistance to shoot fly, high tiller number, midrib color (brown) intensity, etc. On the other hand, if the resources permit, one may go for a full-fledged population improvement program or pedigree method of breeding. Also, new science tools may be followed provided resources and expertise are available.

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Breeding methods in sorghum

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Introduction

Plant breeding is a technology based on art and science of engineering crop cultivars based on sound genetic principles. The breeding methods depend on the pollination control mechanisms and cultivar options. Considering that sorghum is predominantly a self-pollinated crop, breeding methods that are being followed in sorghum are those that are designed for self-pollinated crops. In this article, breeding methods that have been adopted for genetic improvement of sorghum have been reviewed and presented.

Pure line selection

Pure line selection is practiced in two situations (1) when there is a need to develop a variety from a land race population, and (2) while developing a variety from a segregating population. The local landraces from Maharashtra were collected and single plant selections were selfed for a couple of generations and the performance for grain and stover yields was compared. The line showing better performance than the check variety is released. In case of segregating populations, the individual plants are heterozygous in the beginning and attain homozygosity in successive generations. Individual plant selections have to be carried out for at least 5-6 generations to achieve the desired level of homozygosity of a pure line. Higher number of plants (3,000–10,000) of segregating population is evaluated and selection is practiced to obtain desired plants.

Mass selection

Mass selection differs from pure line selection, wherein a number of desirable plants (instead of only one), are selected and compositing is done on the harvested seed to produce the next generation (Allard 1960). This method has a few drawbacks, such as, it is not known whether the plants being grouped are homogenous and some of them if heterogenous would segregate further in following generations, and repeated selection would be required (Sharma 1988). Mass selection is practiced to purify a variety. A large number of single plants are selected from impure variety population, each line progeny tested and similar type progenies

bulked to form the pure seed lot. The success of the method depends upon high heritability, that is, the presence of additive gene action and minimal influence of genotype \times environment interaction on the expression of the selected trait. The advantage of mass selection is that a variety developed by this procedure will be phenotypically uniform for agronomic purposes and identification, but will carry a considerable genetic variability for quantitative traits, which is useful for slow and gradual improvement over time.

Hybridization-based methods

The term hybridization refers to crossing of two genetically different individuals as it combines the traits of two varieties and provides an opportunity to select plants with desirable features of both parents through recombination in the segregating progenies. As the natural variability was exploited completely and varieties developed by mass or pure line selections, there was a need to create new variability by making artificial hybrids to make any further dent in developing improved varieties. Plant breeders use two methods to deal with segregating populations, one is pedigree method and the other is bulk method. The pedigree method is widely used and in this method the records of the ancestry or pedigree of each progeny is maintained. With the pedigree system, the F_2 generation represents the first opportunity for selection. Selection for superiority is based on the vigor and other agricultural features of progenies (families). In F_2 , selection is limited to individuals. In F_3 and subsequent generations, until a reasonable level of genetic homozygosity is reached, selection is practiced both within and between families. Therefore, selection is practiced among families until the number of progenies has been reduced to the point where comprehensive evaluation trials can be undertaken. The bulk breeding consists of parental hybridization, planting a number of seeds (plants) each generation, harvesting the plants in bulk and planting a sample of seeds the following year. Bulk population breeding is an economic method of obtaining homozygous lines in self-fertilized crops. Several hundred or thousand F_2 plants are used for further advancement. Normally, natural selection is allowed to proceed for at least three generations, F_2 , F_3 and F_4 . Single plant selections are planned in F_5 and selected plants are grown as family and in F_7 evaluated for yield. Harlan and Pope (1922) suggested the backcross method to conserve the yielding ability of a well adapted variety, while incorporating resistance from unadapted parent. The backcross method is particularly suited for transferring specific genes to a good variety, which is deficient in one or a few traits. In this method, recurrent backcrosses are made to the more desirable parent while selection is practiced for the characters being transferred from the donor parent.

Choice of parents: The choice of parents for hybridization programs is critical for its success and requires careful and critical evaluation of potential parents for

various attributes such as yielding ability, disease resistance, adaptation, quality of the produce and morphological features relevant to crop management practices. Since new strains are intended to have superior yield potential than the existing varieties, one of the parents is invariably the adapted variety of the area. The other parent is primarily chosen for complimenting the specific weakness of the variety, which needs to be replaced. The general combining ability of a parent is likely to be reflected adequately in the parental performance of the trait. Besides selection of the parents on the yield performance and general and specific combining abilities in the partial diallel crosses or line × tester crosses, it is desirable to analyze the potential parents for principal components such as panicle length, number of primary/secondary branches, grain per primary branch, and grain size.

Sometimes the breeder makes crosses between elite × elite lines to accumulate high yielding genes from both elite parents. This should be taken up only when both the elite parents' pedigree and yield traits contributing for yield are different. In sorghum, high yielding variety, CSV 15 was developed by crossing two elite parents, SPV 475 and SPV 462. Selecting parents with diverse genetic background is essential to make any improvement.

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Techniques to screen sorghums for resistance to insect pests

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Introduction

Sorghum is an important cereal crop in Asia, Africa, Americas and Australia. Grain yields on farmers' fields in Asia and Africa are generally low (500–800 kg ha⁻¹) mainly due to insect pest damage. Nearly 150 insect species have been reported as pests on sorghum (Sharma 1993), of which sorghum shoot fly (*Atherigona soccata*), stem borers (*Chilo partellus*, *Busseola fusca*, *Eldana saccharina* and *Diatraea* spp.), armyworms (*Mythimna separata*, *Spodoptera frugiperda* and *S. exempta*), shoot bug (*Peregrinus maidis*), aphids (*Schizaphis graminum* and *Melanaphis sacchari*), spider mites (*Oligonychus* spp.), grasshoppers and locusts (*Hieroglyphus*, *Oedaleus*, *Aliopus*, *Schistocerca*, and *Locusta*), sorghum midge (*Stenodiplosis sorghicola*), mirid head bugs (*Calocoris angustatus* and *Eurystylus oldi*), and head caterpillars (*Helicoverpa*, *Eublemma*, *Cryptoblabes*, *Pyroderces*, and *Nola*) are the major pests worldwide. Other insects reported to be damaging sorghum are of regional/local importance. Annual losses due to insect pests differ in magnitude on a regional basis. They have been estimated to be \$1,089 million in the semi-arid tropics (SAT), \$250 million in United States, and \$80 million in Australia (ICRISAT 1992). In India, nearly 32% of sorghum crop is lost due to insect pests (Borad and Mittal 1983).

Host-plant resistance is one of the most effective means of pest management in sorghum. It is compatible with other methods of pest control, there is no cost involvement for the farmers, and is environment-friendly. There are over 36,700 sorghum germplasm accessions in the genebank at ICRISAT, which serves as a global repository of the sorghum germplasm. We have undertaken an extensive exercise to screen the sorghum germplasm collection for resistance to the key sorghum pests such as sorghum shoot fly, spotted stem borer, sorghum midge and head bugs. A brief description of the biology, nature of damage by the target insect pests, and the techniques followed to evaluate for resistance to these insect species are described below.

Sorghum shoot fly, *Atherigona soccata*

Biology and nature of damage

Sorghum shoot fly, *A. soccata* is a key pest of sorghum in Asia, Africa and the Mediterranean Europe. Shoot fly females lay cigar shaped eggs singly on the lower surface of the leaves at 1 to 7-leaf stage, ie, 5 to 25 days after seedling emergence. Eggs hatch in 1 to 2 days, and the larva moves along the shoot to the growing point. The larva cuts the growing point, resulting in wilting and drying of the central leaf, known as a deadheart (Fig. 17). The deadheart can be pulled out easily, and produces a bad smell. Normally, the damage occurs one week to four weeks after seedling emergence. The damaged plants produce side tillers, which may also be attacked. Larval development is completed in 8 to 10 days and pupation takes place mostly in the soil. The pupal period lasts for 8 days. The entire life cycle is completed in 17 to 21 days. The shoot fly population begins to increase



Fig. 17. Formation of deadhearts in sorghum due to shootfly damage.

in July, peaks in August-September, and declines thereafter. Infestations are high when sorghum plantings are staggered due to erratic rainfall. Shoot fly infestations are normally high in the postrainy season crop planted in September to October. Temperatures above 35°C and below 18°C, and continuous rainfall reduces shoot fly survival. During the off-season, the insect survives on alternate hosts such as *Echinochloa colonum.*, *E. procer*a, *Cymbopogon* sp., *Paspalum scrobiculatum*, *Pennisetum glaucum*, and on volunteer/fodder sorghum.

Resistance-screening techniques

Interlard-fishmeal technique (multi-choice field-screening). Adequate shoot fly density for resistance screening can be achieved by manipulating the sowing date, using infester rows, and spreading fishmeal (which attracts the shoot flies) in the field (Plate 1). Shoot fly abundance can be monitored through fishmeal-baited traps to determine the periods of peak abundance of the shoot fly. This information can be used for planting the test material so that the susceptible stage of the crop coincides with the optimum shoot fly pressure. Late-sown crops are subjected to high shoot fly infestation. At ICRISAT-Patancheru, sowing test material in

mid-July in the rainy season, and during October in the postrainy season is effective in screening for resistance to shoot fly. The interlard-fishmeal technique, which is useful for increasing shoot fly abundance under field conditions, involves planting four rows of a susceptible cultivar (such as CSH 1 or CSH 5), sown 20 days before the sowing of test material. Fishmeal is spread uniformly 1 week after seedling emergence or kept in plastic bags in the interlards to attract shoot flies from the surrounding areas. One generation of the shoot fly is completed on interlards, and the emerging flies infest the test material. The same procedure can also be adopted for the test material as well (Taneja and Leuschner 1985a, Sharma et al. 1992).

No-choice-cage-screening technique. To confirm resistance to the observed under field conditions, and to study the resistance mechanisms, the cage-screening technique developed by Soto (1972) has been modified to simulate field conditions. The shoot flies are collected from fishmeal-baited traps in the field (Sharma et al. 1992). Shoot flies are collected in the morning, and are separated from other dipteran flies. The cage-screening technique can be used for multiple- or no-choice tests. For a multiple-choice test, the test genotypes are sown in the field in 3.4×2 m beds, with a row spacing of 15 cm. Ten days after seedling emergence, the plants are covered with a 3.4×2×1 m screened cage, and the shoot flies are introduced into the cage. Eggs and deadhearts are recorded after 1 week. For a no-choice test, only one genotype is sown in 1×1 m beds. Six beds can be covered with a 2×3×0.5 m cage having six compartments. Twenty shoot flies are released into each compartment, and observations are recorded as described above. Rapid screening can also be carried out using a top-cage technique. This system consists of two plastic trays (40×30×14 cm), one for sowing the test material and the other (a top-cage fitted with fine wire-mesh) is clamped over the lower tray, thus forming a cage. Ten days after seedling emergence, the top-cage is assembled and 20 flies are released into each cage through an opening. Observations are recorded as described below.

Damage evaluation for resistance screening. Record the number of eggs and the plants with eggs, plants with deadhearts, and the total number of plants at 14 and 21 days after seedling emergence. Record the number of tillers, and tillers with panicles at maturity as a measure of genotype's recovery resistance. Grain yield under protected and unprotected conditions can also be used as a measure of resistance to sorghum shoot fly.

Spotted stem borer, *Chilo partellus*

Spotted stem borer, *Chilo partellus* is common in Asia and East and Southern Africa. The first indication of stem borer infestation is the appearance of small-elongated windows in whorl leaves where the young larvae have eaten the upper surface of

the leaves. Later, the plant presents a ragged appearance as the severity of damage increases. The third-instar larvae migrate to the base of the plant, bore into the shoot, and damage the growing point resulting in the production of a deadheart. Normally, two leaves dry up as a result of stem borer damage. Larvae continue to feed inside the stem throughout the crop growth. Extensive tunneling of the stem and peduncle leads to drying up of the panicle, production of a partially chaffy panicle or peduncle breakage (Fig. 18a & b). Stem borer

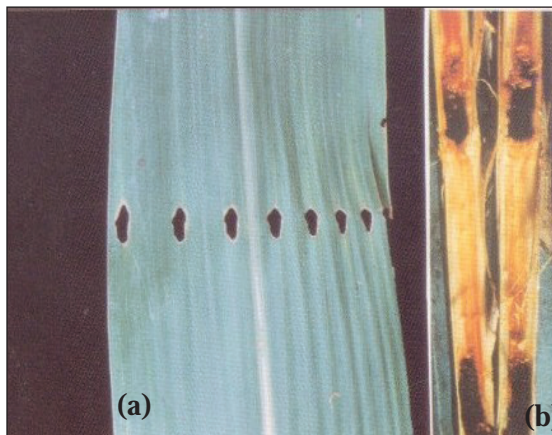


Fig. 18. Leaf damage (a) and stem tunneling (b) by spotted stem borer in sorghum.

infestation starts about 20 days after seedling emergence, and deadhearts appear on 30 to 40 day old-crop. A female lays up to 500 eggs in batches of 10 to 80 near the midrib on the under surface of the leaves. Eggs hatch in 4 to 5 days. The larval development is completed in 19 to 27 days. Pupation takes place inside the stem and the adults emerge in 7 to 10 days. During the off-season, the larvae diapause in plant stalks and stubbles. With the onset of rainy season, the larvae pupate and the adults emerge in 7 days. In northern India, moth catch in light traps begins to increase during the last week of July and peaks during August to September, while in southern India, the peak in moth catches has been recorded during January to February.

Resistance-screening techniques

Techniques to screen for resistance to spotted stem borer have been described by several workers (Jotwani 1978, Taneja and Leuschner 1985b, Sharma et al. 1992). The following techniques may be followed to screen for resistance to stem borer under natural and artificial infestation.

Screening under natural infestation

Hot-spots. Hot-spot locations, where the pest populations are known to occur naturally and regularly at levels that often result in severe damage, are ideal to test large numbers of germplasm accessions. Hot-spot locations for *C. partellus* are Hisar in Haryana and Warangal in Andhra Pradesh, India; Agfoi and Baidoa in Somalia; Panmure and Mezarbani in Zimbabwe; Kiboko in Kenya; and Golden Valley in Zambia.

Sowing date. To screen for resistance under natural infestation, especially at the hot-spot locations, adjust the sowing date of the crop such that the crop is at a susceptible stage when the stem borer abundance is at its peak. Determine the periods of maximum borer abundance through pheromone traps, light traps, or by monitoring borer infestation in the crop planted at regular intervals. In northern India, *C. partellus* is most abundant in August to September, and the crop sown between the 1st and 3rd week of July suffers maximum stem borer damage. At ICRISAT-Patancheru, a maximum number of moths in the light traps have been recorded during September, followed by smaller peaks during November and February.

Mass rearing and artificial infestation

Mass rearing. Artificial infestation with laboratory-reared insects has been successfully used for several pest species, including *C. partellus*. Several diets have been developed for mass rearing of *C. partellus*. An artificial diet to rear *C. partellus* has been standardized at ICRISAT (Taneja and Leuschner 1985b). Most of the ingredients of this diet [Fraction A: water 2000 mL, *kabuli* chickpea flour 438.4 g, brewer's yeast 32.0 g, sorbic acid 4.0 g, vitamin E (Viteolin capsules) 4.6 g, methyl parahydroxy benzoate 6.4 g, ascorbic acid 10.4 g, and sorghum leaf powder 160.0 g. Fraction B: Agar-agar 40.8 g, water 1600 mL, and formaldehyde (40%) 3.2 mL] This amount is enough to prepare 15 jars with 300 g diet each. For preparing sorghum leaf powder, collect leaves from a susceptible cultivar (such as CSH 1) from 35 to 40-day-old plants. Wash the leaves, dry in sunshine or in an oven at 65°C, grind them into a fine powder, and autoclave for 15 min at 120°C at 5 kg cm⁻² pressure. Store the leaf powder in a sealed container in a cool dry place. Blend the ingredients of fraction A (except the sorghum leaf powder) for 1 min. Soak the sorghum leaf powder in warm water (70°C) and blend with fraction A for 2 min. Boil agar-agar (fraction B) in 1.6 L of water, cool it to 40°C, combine with formaldehyde and fraction A, and blend for 3 min. Pour 300 g diet in a one-liter plastic jar. Allow the diet in the jar to cool to room temperature. Place about 100 eggs at the black-head stage in each jar, and keep the jars in a dark room for 2 days. This discourages the photopositive behavior of 1st-instar larvae, and they settle on the diet for feeding. After 2 days, transfer the jars to the rearing room, maintained at 28±1°C, 60 to 70% relative humidity (RH), and 12 h photoperiod. On artificial diet, the larval period lasts for 22 to 28 days and the pupal period for 5 to 6 days. Moth emergence begins 30 days after larval inoculation, and continues up to the 40th day. Females emerge 2 to 3 days later than the males. The sex ratio is close to 1:1. Average moth emergence from this diet is 70 to 75%, with a maximum of up to 90%. Most of the moths emerge in 30 to 40 days after larval inoculation.

The moths are collected with the help of aspirators attached to a vacuum cleaner or with the help of hand-held aspirators. Collect the male and female moths separately (males are smaller in size with dark forewings and pointed abdomen), and transfer them to the egg-laying cages. The oviposition cage is a wire-framed (36 mm holes) cylindrical cage (25 cm high and 25 cm in diameter). A fine georgette cloth with 6×6 mm holes at regular intervals is fitted around the outer side of the cage. A sheet white glycine paper (25×80 cm) is wrapped around the cage to serve as an oviposition site. Two plastic saucers covered with a mosquito net are placed at the bottom and the top of the cage. Release 50 pairs of moths in each oviposition cage. A female lays 10 to 12 egg masses (500 to 600 eggs) over a period of 4 days. Most eggs are laid on the 2nd and 3rd day after emergence. Replace the glycine paper daily. Feed the moths with water using a cotton swab. Egg hatching is drastically reduced when relative humidity falls below 50%. To obtain high humidity, hang the glycine papers containing egg masses on a rod in a plastic bucket containing water. Cover the plastic bucket with a lid. Store the eggs at 26±1°C. Under these conditions, the embryo matures to the black-head stage within 4 days. For long-term storage, keep black-head stage eggs at 10°C. This delays egg hatching up to 15 days. This is quite helpful in timely infestation of the test material.

Field infestation. For field infestation, the Bazooka applicator, developed at the International Maize and Wheat Improvement Center (CIMMYT) (CIMMYT 1977), has been modified to suit the requirements for infesting sorghum. Take 500 black-head stage egg masses along with 85 g of poppy seeds (*Papaver* sp.) or corn cob grits, and keep them overnight in a plastic jar with a tightly fitted lid. In the morning, mix the 1st-instar larvae with the carrier and transfer them into the plastic bottle of the Bazooka. Infest 15 to 20-day-old plants in the field individually by placing the nozzle of the Bazooka close to the leaf whorl. With a single stroke, 5 to 7 larvae are released into each plant whorl, which are sufficient to cause appreciable leaf feeding and >90% deadhearts in the susceptible genotypes. Deadheart formation decreases progressively as the infestation is delayed. For stem and peduncle tunneling, plants may be infested between 25 to 35 days after seedling emergence. Infest the crop in the morning between 0800 and 1100 to avoid larval mortality due to high temperature. Rotate the Bazooka applicator after every 10 plants to ensure uniformity in larval distribution. The number of larvae per plant can be regulated by varying the number of egg masses mixed with the carrier in each Bazooka. A second infestation may be required if it rains immediately after first infestation. Shoot fly infestation interferes with screening for resistance to stem borer. Spray fenvalerate or endosulfan to suppress shoot fly infestation 1 week before artificial infestation with stem borer. Also, it is helpful to sow the test material early in the season when shoot fly infestation is negligible.

Damage evaluation for resistance screening

Stem borer attack in sorghum causes leaf damage, deadheart formation, stem and peduncle tunneling, and production of chaffy panicles. Leaf injury is the first larval feeding symptom, and is related to yield loss only under severe infestation. Stem tunneling adversely affects the quantity and quality of fodder, but is not correlated with reduction in grain yield under many situations. Peduncle damage could be critical if there are winds of high enough velocity to break the peduncle. Deadheart formation is the most important criterion for differentiating degrees of resistance, and is directly related to loss in grain yield. The following observations may be recorded to evaluate for resistance to stem borer.

Leaf feeding. Record the rate of leaf feeding 2 weeks after artificial infestation, and 4 to 5 weeks after crop emergence under natural infestation. Record the total number of plants, the number of plants showing the leaf-feeding symptoms, and the leaf-feeding score on a 1 to 9 scale (1=<10% leaf area damaged, 2=11–20, 3=21–30, 4=31–40, 5=41–50, 6=51–60, 7=61–70, 8=71–80, and 9= >81% leaf area damaged).

Deadhearts. Record plants with deadhearts 3 weeks after artificial infestation, and 4 to 6 weeks after crop emergence under natural infestation. Record the total number of plants, plants showing borer deadhearts, and the visual score (1 to 9 scale) as described for leaf feeding score (1 = <10% plants with deadhearts, and 9 = >80% plants with deadhearts).

Chaffy panicles. At crop harvest, record observations on the number of partial and completely chaffy panicles, the number of broken panicles, and the visual score (1 to 9 scale) for chaffy/broken panicles, and grain mass per 100 grains.

Recovery resistance. Record the number of plants with tillers and the number of tillers with productive panicles. Evaluate for recovery resistance on a 1 to 5 scale (1=>80% plants with 2 to 3 uniform and productive tillers, and 5=<20% plants with one or nil non-uniform productive tillers).

Stem tunneling. At maturity, record plant height and the peduncle length of five plants at random in each plot. Measure the stem and peduncle tunneling separately and express it as a percentage of stem/peduncle length.

Shoot bug, *Peregrinus maidis*

Shoot bug, *Peregrinus maidis* is a common pest of sorghum in India, parts of Africa, West Indies and the Philippines. It sucks sap from the leaf whorls. The damaged plants become stunted. In case of severe infestation, the top leaves start

drying up first, extending gradually to the lower leaves, and the plant may die. The leaves curl and present a tanned appearance. They also secrete honeydew on which the sooty molds grow. Infestation at the later stages of plant growth may twist the top leaves, and inhibit panicle emergence. Its infestations are more severe under drought conditions. It is a serious pest of sorghum in the post-rainy season in India. The female is yellowish brown, and the male dark brown. Wings may be longer or shorter than the abdomen. Long winged forms have transparent wings. Females are larger than the males. The nymphs and adults live in groups in plant whorls, and on the inner side of leaf sheaths. The females make a slit in the upper surface of the mid-rib and insert the eggs in groups of 1 to 4, and cover them with a white waxy substance. A female lays up to 100 eggs in 7 days. The eggs are white, elongate, cylindrical and taper at the ends. Egg incubation period is 1 to 7 days. There are 7 nymphal instars, and the development is completed in 16 days.

Evaluation for resistance

Screening for resistance to shoot bug can be carried out under natural infestation in field or in the greenhouse. For this purpose, the material should be planted during late rainy season in July or early post-rainy season in October. Appropriate resistant and susceptible checks should be included in the trials. The material should be protected against shoot fly and stem borer through whorl application of carbofuran granules during the seedling stage. Shoot bug infestation/damage should be evaluated on the main plants. Shoot bug infestation can also be created under greenhouse conditions, by using leaf cages or by confining the shoot bug females to the whorl leaves. Data may be recorded on the numbers of shoot bugs produced in 15 days. For this purpose, the leaf cages can be fixed on 3rd or 4th leaf from the bottom, or infestation carried out in the whorl leaves, and the plants placed away from each other.

Damage evaluation

Shoot bug density. Just before emergence of the flag leaf, ie, 45 days after seedling emergence, evaluate the test genotypes for shoot bug resistance by recording insect density. For this purpose, count the number of shoot bugs in the whorls of five plants tagged at random in each plot.

Plant damage ratings. Shoot bug damage to the plants can be evaluated at the panicle emergence stage on a 1 to 9 scale as follows:

1. A few shoot bugs present in the leaf whorls and no apparent damage to the leaves.
2. One to two central leaves showing damage symptoms, and 10–20% of the infested leaf area damaged.

3. Two to three leaves showing damage symptoms, and 20–30% of the leaves damaged.
4. Three to four leaves showing damage symptoms, and 30–40% of the leaves damaged.
5. Four to five leaves showing damage symptoms, and 40–50% of the leaves damaged.
6. Five to six leaves showing damage symptoms, and 50–60% of the leaf area damaged, and no panicle emergence in some plants.
7. Six to seven leaves showing damage symptoms, 60–70% of the leaf area damaged, and 50% plants with panicle emergence.
8. Seven to eight leaves showing damage symptoms, 70–80% of the leaf area damaged, and a few plants with panicle emergence.
9. Most of the leaves showing shoot bug infestation > 80% and plants with a twisted appearance and no panicle emergence.

Grain yield. Record grain yield of the genotypes being tested. The test material can be maintained under infested and noninfested conditions. Harvest all panicles from the middle row(s) at maturity, and record panicle and grain mass. Express the loss in grain yield in the infested plots or panicles as a percentage of the grain yield in noninfested plots or panicles.

Sugarcane aphid, *Melanaphis sacchari*

The sugarcane aphid, *Melanaphis sacchari* occurs in Asia, Africa and America. It prefers to feed on the under surface of older leaves (Fig. 19). The damage proceeds from lower to the upper leaves. The adults and nymphs are yellow. They suck sap from the lower surface of leaves, and this leads to stunted plant growth. The damage is more severe in crop under drought stress, resulting in drying of leaves and plant mortality. The aphids secrete honeydew, which falls on the ground, on which sooty molds grow. Their numbers increase rapidly at the end of the rainy season during dry spells. This aphid also reproduces by parthenogenesis. Each female gives birth to 60 to 100 nymphs in 13 to 20 days. The life cycle is completed in 6 to 7 days during the dry season. Its populations are high during the postrainy season in India.



Fig. 19. Aphid infestation in sorghum.

Screening for resistance

Screening for resistance to sugarcane aphid can be carried out under natural infestation in the field. For this purpose, the material should be planted during late rainy season in July or early postrainy season in October. Appropriate resistant and susceptible checks should be included in the trials. The material should be protected against shoot fly and stem borer through whorl application of carbofuran granules, and aphid infestation/damage should be evaluated on the main plants only. Aphid infestation can also be created under greenhouse conditions. Aphid multiplication and growth rates can be studied using leaf cages by confining aphid females with the leaves, and counting the numbers of aphids produced in 15 days. For this purpose, the leaf cages can be fixed on 3rd or 4th leaf from the bottom.

Damage evaluation

Aphid density. At the flag leaf stage or 50 days after seedling emergence, evaluate the test genotypes for aphid resistance by recording aphid density. For this purpose, count the number of aphids in a unit area (3×3 cm) on the mid-portion of three leaves per plant (3rd, 5th and 7th leaf), and record observations on five plants tagged at random in each plot.

Leaf damage rating. Leaf damage due to aphid feeding can be assessed at the milk-dough stage of grain development using a 1 to 9 scale.

1. A few aphids present on the lower 1–2 leaves, with no apparent damage to the leaves.
2. Lower 1 to 2 leaves showing aphid infestation, and 10–20% of the infested leaves/ area showing damage symptoms.
3. Lower 1 to 3 leaves showing aphid infestation, and 20–30% of the infested leaves/area showing damage symptoms, with moderate leaves of honeydew/ black molds on the leaves/soil.
4. Lower 1 to 4 leaves showing aphid infestation, and 30–40% of the infested leaves/area showing damage symptoms, with moderate leaves of honeydew/ black molds on the leaves/soil.
5. Lower 1 to 5 leaves showing aphid infestation, and 40–50% of the infested leaves/area showing damage symptoms, with moderate levels of honeydew/ black molds on the leaves/soil.
6. Aphid infestation up to 6th leaf, and 50–60% of the infested leaves/area showing damage symptoms, and heavy honeydew/black molds on the leaves, and on soil below.
7. Aphid infestation up to 7th leaf, and 60–70% of the infested leaves/area

showing damage symptoms, and heavy honeydew/black molds on the leaves, and on soil below.

8. Aphid infestation up to 8th leaf, and 70–80% of the infested leaves/area showing damage symptoms, and heavy honeydew/black molds on the leaves, and on soil below.
9. Heavy aphid infestation up to the flag leaf, and >80% of the leaves showing aphid damage (drying up symptoms), heavy honeydew/black molds on the leaves, and on soil below.

Grain yield. Record grain yield of the genotypes being tested. The test material can be maintained under infested and noninfested conditions. Harvest all panicles from the middle row(s) at maturity, and record panicle and grain mass. Express the loss in grain yield in the infested plots or panicles as a percentage of the grain yield in noninfested plots or panicles.

Sorghum midge, *Stenodiplosis sorghicola*

Sorghum midge, *Stenodiplosis sorghicola* larvae feed on the developing ovary resulting in production of chaffy spikelets. Females lay eggs in panicles at flowering during the morning hours. The damaged panicles present a blasted appearance. Midge damaged spikelets have a pupal case attached to the glumes or have a small exit hole of the midge parasite on the upper glume. Adults emerge between 0600 to 1100. Mating takes place within one hour after emergence. Generally, the males emerge one hour earlier than the females, and hover around the spikelets where the females are about to emerge. Males die after mating while the females proceed in search of sorghum panicles at flowering for oviposition. Females lay 30 to 100 eggs singly in the spikelets at anthesis during the morning hours, and die by the afternoon. Eggs hatch in 1 to 4 days. The larvae suck the sap from the developing ovaries and complete development in 7 to 12 days. Larvae pupate inside the glumes, and the pupal period lasts for 3 to 8 days. Adults live for 4 to 48 h. The population builds up 2 to 3 months after the onset of monsoon rains, and maximum density occurs during September to October. A small proportion of the larvae enter diapause in the spikelets in each generation, which may last as long as 3 to 4 years. The larval diapause is terminated by warm and humid weather (25 to 30°C, and > 60% relative humidity).

The major difficulties in identifying source material with stable resistance against sorghum midge includes: a) variation in the flowering of sorghum cultivars in relation to midge incidence, b) day-to-day variation in midge populations, c) competition with other insects such as head bugs, d) parasitization and predation by natural enemies; and e) sensitivity of midge flies to temperature and relative humidity. A large proportion of lines selected as less susceptible under natural conditions

comprise early- and late-flowering escapes. Because of these problems, genotypes rated as resistant under natural infestation often turn out to be susceptible in the following seasons or at other locations. Techniques to screen for midge resistance have been described by Jotwani (1978), Page (1979) and Sharma et al. (1988a,b, 1992).

Resistance-screening techniques

Field screening techniques (multi-choice conditions)

Hot-spots. Hot-spot locations are useful to screen for resistance to sorghum midge. Hot-spot locations for sorghum midge are Dharwad, Bhavanisagar and Pantnagar in India, Sotuba in Mali, Farako Bâ in Burkina Faso, Alupe in Kenya, and Kano in Nigeria. Midge infestations are also high at several locations in Australia, the USA and Latin America.

Sowing date. To screen test the material for resistance to sorghum midge under natural conditions, it is necessary to determine the appropriate time for sowing at different locations. Determine the periods of maximum midge density through fortnightly sowings of a susceptible cultivar. Adjust sowing dates so that the flowering of the test material coincides with greatest insect density. At ICRISAT-Patancheru, maximum midge damage has been observed in the crop planted during the 3rd week of July. The peak in midge density occurs during October, and a second but smaller peak has been observed during March in the postrainy season, for which planting is carried out during mid-December.

Infester row technique. Midge abundance can be increased through infester rows and spreading sorghum panicles containing diapausing midge larvae in the infester rows (Sharma et al. 1988a). Sow infester rows of susceptible cultivars such as CSH 1 and CSH 5 (1:1 mixture) 20 days before the test material. Alternatively, early-flowering (40 to 45 days) lines (IS 802, IS 13249 and IS 24439) can be sown along with the test material. Plant four infester rows of the susceptible cultivar after every 16 rows of the test material. Collect midge-infested chaffy panicles containing diapausing midge larvae at the end of the cropping season, store in gunny bags or in bins under dry conditions until the next season. Moisten the panicles for 10 to 15 days to stimulate the termination of larval diapause. Spread midge-infested sorghum panicles containing diapausing midge larvae at the flag leaf stage of the infester rows. Adults emerging from the diapausing larvae serve as a starter infestation in the infester rows to supplement the natural population. Midge population multiplies for 1 to 2 generations on the infester rows before infesting the test material. This technique increases the midge damage by 3 to 5 times. Infester rows alone are also effective in increasing midge infestation.

Sprinkler irrigation. High relative humidity is important for adult emergence, oviposition and subsequent damage. Use overhead sprinkler irrigation to increase relative humidity in midge-screening trials during the postrainy season or periods of low relative humidity. Operate sprinkler irrigation daily between 1500 to 1600 from panicle emergence to the grain-filling stage of the crop. Sprinkler irrigation between 1500 to 1600 does not affect oviposition by the midge females because peak midge activity and oviposition occur between 0730 and 1100. Midge damage increases significantly with the use of sprinkler irrigation.

Selective use of insecticides to control other insects. Head bug, *Calocoris angustatus* and the midge parasitoid, *Tetrastichus diplosidis* are the two major insects limiting midge abundance in resistance screening trials. Head bugs damage the sorghum panicles from emergence to hard-dough stage and compete for food with the sorghum midge. They also prey on the ovipositing midge females at flowering, while *T. diplosidis* is an efficient parasite of sorghum midge at some locations. Spray less persistent and contact insecticides such as carbaryl and malathion to control head bugs at the complete-anthesis to milk stage (Sharma and Leuschner 1987). The sorghum midge larvae feeding inside the glumes are not affected by the contact insecticides sprayed after flowering.

Split sowings and grouping the material according to maturity and height. Group the test material according to maturity (early, medium and late) and height (dwarf, medium and tall) for proper comparisons, and avoid the shading effect from tall genotypes. Sow the test material twice at a 15-day interval to minimize the chances of escape from midge damage. Split sowing of the test material increases the efficiency of selection for midge resistance. Plant population also affects the insect density per unit area, and in some cases influences the incidence and survival rate of insects. The level of midge damage has been observed to be higher at lower planting densities.

No-choice headcage technique

Caging midge flies with sorghum panicles permits screening for midge resistance under uniform insect pressure. A headcage technique has been developed at ICRISAT-Patancheru. It consists of a cylindrical wire frame made of 1.5 mm diameter galvanized iron wire. The loop attached to the top ring rests around the tip of the panicle, and the extensions of the vertical bars at the lower ring are tied around the peduncle with a piece of G.I. wire or electric wiring clips. Select sorghum panicles at 25 to 50% anthesis stage, and remove spikelets with dried-up anthers at the top and immature ones at the bottom of the panicle so that only the spikelets at anthesis are exposed to the midge flies for oviposition. Place the wire-framed cage around the sorghum panicle and cover it with a blue cloth bag (20 cm wide

and 40 cm long). The cloth bag at the top has an extension (5 cm in diameter, 10 cm long) to release the midges inside the cage. Collect 20 adult female midges in a plastic bottle (a 200 ml aspirator) between 0800 and 1100 from flowering sorghum panicles (only female midges visit the flowering sorghum panicles and these are collected for use in infestation). Release 40 midges into each cage, and repeat the operation the next day. Infest 5 to 10 panicles in each genotype, depending upon the stage of material and the resources available. Midge damage decreases as the time of collection and release advances from 0830 to 1230. Examine the cages 5 to 7 days after infestation and remove any other insects such as head bugs, panicle-feeding caterpillars and predatory spiders from inside the cage. Remove the cages 15 days after infestation and evaluate the midge damage. The headcage technique is quite simple, easy to operate, and can be used on a fairly large scale to confirm the field resistance of selected genotypes. Changing weather conditions influence midge activity and can affect midge damage under the headcage. In general, it is a thorough test for use in resistance screening, and is particularly applicable in identifying stable and durable resistance.

Damage evaluation for resistance screening

Feeding by the midge larva inside the glumes leads to sterile or chaffy spikelets. However, the symptoms (chaffiness) of natural sterility and extensive grain damage by sucking insects are superficially similar to the damage caused by sorghum midge. The midge-infested panicles have either small white pupal cases attached to the tip of damaged spikelets or have small parasite exit holes in the glumes. Genotypes flowering on different dates should be tagged with different-colored labels or tapes or marked with paint along with panicles of resistant and susceptible checks for proper comparison. Selection for resistance should be based in relation to reaction of resistant and susceptible checks flowering on the same day.

Chaffy spikelets. This is the most appropriate criterion by which to evaluate sorghum lines for midge resistance. Tag five panicles in each genotype at half-anthesis stage. Record midge damage in 250 spikelets at 15 days after flowering or at maturity. Collect five primary branches each from the top, middle and bottom portions of each panicle, bulk the samples from all the five tagged panicles in a genotype, remove secondary branches from the primary branches, and mix the sample thoroughly. Pick up the secondary branches at random and count the number of chaffy spikelets in a sample of 250 spikelets. In samples collected at the milk stage, squeeze the chaffy spikelets between the thumb and first finger or with forceps, and record the number of spikelets producing a red ooze (this indicates midge damage). Chaffy spikelets with early-instar larvae at times may not produce a red ooze. Express the data as a percentage of chaffy or midge-damaged spikelets.

Visual damage rating. At crop maturity, evaluate midge damage on a 1 to 9 scale where 1=<10%, 2=11–20%, 3=21–30%, 4=31–40%, 5=41–50%, 6=51–60%, 7=61–70%, 8=71–80% and 9=>81% midge-damaged spikelets.

Grain yield. Record grain yield from the genotypes being tested. The test material can be maintained under infested and noninfested conditions by using cloth bags or sprayed with insecticides at flowering to control the sorghum midge. Harvest all panicles from the middle row(s) at the time of maturity and record the panicle and grain weight. Express the loss in grain yield in the infested plots or panicles as a percentage of the grain yield in non-infested plots or panicles.

Head bug, *Calocoris angustatus*

Head bug, *Calocoris angustatus* is a serious pest of grain sorghum in India. The nymphs and adults suck the sap from the developing grain. The damage starts as soon as the panicle emerges from the boot leaf. High levels of head bug damage lead to tanning and shriveling of the grain. Head bug damage leads to both qualitative and quantitative losses in grain yield. Head bug damage spoils the grain quality, and renders the food unfit for human consumption. Such grain also shows poor seed germination. Head bug damage also increases the severity of grain molds. Head bug females lay eggs inside the spikelets from panicle emergence to post-anthesis. A female lays 150 to 200 eggs, and the eggs hatch in 5 to 7 days. Nymphal development is completed in 15 to 17 days. Nymphs feed on milky and soft-dough grains, and this results in tanning and shriveling of the grain. Its infestations are high during August-September in the rainy season. During the off-season, the bugs feed on fodder sorghum. There is no evidence of diapause. Techniques to screen for resistance to head bugs have been discussed by Sharma and Lopez (1992) and Sharma et al. (1992).

Resistance-screening techniques

Field screening

Screening for head bug resistance can be carried out under field conditions during periods of maximum bug density. Screening for head bug resistance under field conditions is influenced by: a) variation in flowering, b) fluctuations in bug density, and c) the effect of weather conditions on the bug population build-up and damage. Early- and late-flowering cultivars normally escape head bug damage, while those flowering in mid-season are exposed to very high bug infestation. The following techniques can be used to increase the screening efficiency for head bug resistance under field conditions.

Hot-spots. In India, ICRISAT-Patancheru, Bhavanisagar, Kovilpatti, Coimbatore, Palem and Dharwad are the hot-spot locations to screen for resistance to head bugs. At ICRISAT- Patancheru, head bug density is very high during September to October.

Sowing date. Adjust sowing dates such that flowering of the test material coincides with maximum head bug density. Determine the periods of maximum head bug abundance through fortnightly sowings. Maximum bug numbers at ICRISAT-Patancheru have been recorded during September and a second but smaller peak has been recorded during March. Crops sown during the second week of July suffer the maximum head bug damage. At Bhavanisagar, the peak in head bug density occurs during May to June, and the optimum time to sow the test material for resistance screening is during the second fortnight of February.

Infester-row technique. Sow infester rows of mixed-maturity cultivars 20 days earlier than the test material. Alternatively, sow early-flowering (40 to 45 days) sorghums (IS 802, IS 13249 and IS 24439) as infester rows along with the test material. Sow four rows of a susceptible cultivar after every 16 rows of the test material. Collect head bugs from other fields and spread them in the infester rows at the panicle emergence to augment bug abundance. Sow the test material in two sets, at an interval of 10 to 15 days to reduce the chances of escape in the early- and late-flowering lines. For better results, group the test material according to maturity and height. The sowing date of each maturity group can also be suitably adjusted so that flowering occurs during peak activity period of the head bugs.

No-choice headcage technique

To overcome the problem of variation in flowering among the test cultivars, and fluctuations in insect abundance, the headcage technique developed for midge resistance screening has been found to be useful to screen for resistance to head bugs as well. This technique also permits to monitor the increase in head bug population in the infested panicles under no-choice conditions in relation to different infestation levels and stages of panicle development. Select 5 to 10 panicles at the top-anthesis stage in each plot/genotype, and tie the headcage around the sorghum panicle and cover it with a white muslin cloth bag. Collect bugs in muslin cloth bags from sorghum panicles at the milk stage, and separate the adult males and females (males are smaller and darker in color than the females). Collect 10 head bug pairs in a 200-ml plastic bottle aspirator and release them inside the cage. Examine the infested panicles after 1 week and remove panicle-feeding caterpillars or predatory spiders if any. Remove the muslin cloth bag along with the bugs 20 days after infestation, kill the bugs with ethyl acetate or benzene (2 ml bag⁻¹), or keep the bags in deep-freeze for 30 min. Count the total number of bugs

in each cage. Evaluate the panicles for head bug damage at maturity as described under damage evaluation.

Damage evaluation for resistance screening

Sorghum head bugs suck the sap from developing ovary and result in shriveling and tanning of the grains. Some grains may also remain undeveloped. Damage symptoms are normally evident on some or all of the grains. Head bug damage is generally higher inside the panicle. In some cases, a portion of the panicle may be more damaged than the rest, and some grains may be normal, while others show damage symptoms. Head bug damage can be evaluated by the following criteria.

Head bug counts. Tag five panicles at random in each genotype at the half-anthesis stage. Sample the panicles for head bugs at 20 days after flowering or infestation in a polyethylene or muslin cloth bag containing a cotton swab soaked in 2 ml of ethyl acetate or benzene. Count the total number of adults and nymphs.

Grain damage rating. Evaluate head bug damage at maturity on a 1 to 9 scale (1 = all grains fully developed with a few feeding punctures, 2 = grain fully developed and with feeding punctures, 3 = grains showing slight tanning or browning, 4 = most grains with feeding punctures and a few showing slight shriveling, 5 = grains showing slight shriveling and browning, 6 = grains showing more than 50% shriveling and tanned, 7 = most of the grain highly shriveled and having a dark brown coloration, 8 = grain highly shriveled and slightly visible outside the glumes, and 9 = most of the grains highly shriveled and almost invisible outside the glumes).

Grain yield. Harvest all panicles from the middle row(s) of each plot or genotype at maturity and record panicle and grain weight in each plot or panicle. Plots or panicles of lines being tested can also be maintained under infested and uninfested conditions by using cloth bags to exclude the head bugs. Measuring grain yield and grain quality parameters under insecticide-protected and infested conditions can also be used as a measure of genotypic resistance to head bugs. Express the loss in grain yield of infested plots or panicles as a percentage of the grain yield in non-infested plots or panicles.

Grain weight and floaters. Take a sample of 1,000 grains at random from each replication or panicle. Equilibrate the moisture content (24 h at 37°C), and record the grain weight. Prepare a sodium nitrate solution of a specific density of 1.31 in a beaker. Place the 1,000-grain sample in the beaker containing sodium nitrate solution, and count the number of grains floating on the surface, and express them as a percentage of the total number of grains.

Germination test. Take 100 grains at random from each replication or panicle and place them between the folds of a water-soaked filter paper in a Petri dish. Keep

the Petri dishes in an incubator at $27\pm 1^{\circ}\text{C}$ or at room temperature in the laboratory. Record the percentage of grains with radical and plumule emergence after 72 h. Data on grain hardness, 1,000-grain weight, percentage floaters, and percentage germination should be recorded only when the researcher intends to collect more data for in-depth studies on head bug resistance.

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Screening techniques for resistance to sorghum diseases

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the host of many diseases that are caused by fungi, bacteria, viruses, nematodes and parasitic plants. These diseases are also classified according to the crop stages at which they occur, such as seedling diseases, foliar diseases, root and stalk diseases, leaf sheath diseases, panicle and grain diseases, and storage diseases. According to the symptoms, the diseases are classified into seedling blight, root and stalk rots, leaf blight, leaf spot, rust, smut, ergot, wilt, downy mildew, grain mold, leaf stripe, leaf streak, leaf mosaic, etc. Of over 50 diseases reported, only a few are economically important globally. There are several diseases that are regionally and locally important in specific ecosystems.

At ICRISAT, globally important diseases, which include grain mold, anthracnose, leaf blight, downy mildew, charcoal rot, rust, ergot and *Striga* are being addressed. The current research priority at ICRISAT, however, is only grain mold in Asia (Fig. 20). The main strategy of disease management at ICRISAT is mainly through host plant resistance (HPR), which is economical, environment-friendly and technically feasible at farmers' level, although expensive at the research level. Disease management through HPR involves sound knowledge of biology and epidemiology of the disease (Bandyopadhyay et al. 2000), availability of pure culture of the pathogen, effective inoculation technique, an appropriate disease rating scale, availability of large variable germplasm, adequate laboratory, greenhouse and field facilities, and a competent pathology-breeding team.

Over the years, ICRISAT has developed/refined screening techniques for grain mold (Bandyopadhyay and Mughogo 1988, Thakur et al. 2006), anthracnose (Pande et al. 1994), leaf blight, downy mildew (Pande et al. 1997), charcoal rot and *Striga*. For some of these diseases, both greenhouse and field screening techniques are available. The basic underlying principle in developing a disease screening technique is to provide adequate pathogen inoculum at the right growth stage of the crop under congenial conditions for infection and disease development to clearly distinguish between resistant and susceptible plants. Once the resistant



Fig. 20. Grain mold resistant red (left), white (middle) and susceptible (right) sorghum panicles.

plants/lines are identified, the resistance is confirmed by screening their next generation plants. Resistance stability is determined by growing the lines in different environments through multilocation testing. Stable resistance sources are used in breeding program to generate disease resistant hybrid parents and varieties. It is often desirable to study inheritance of resistance and number of genes involved before using such sources in breeding program. A number of resistant lines for each of the major diseases are identified and several of those have been effectively utilized in developing hybrid parental lines and varieties.

In this article, the techniques to screen for some of the important diseases are briefly discussed. These techniques could be modified to some extent to suit the particular climate and location conditions.

Screening technique for sorghum grain mold

Field screening

- Classify the test entries into different maturity groups, and plant the test entries and known susceptible lines in such a way that the flowering coincides with the probable rainy days.
- Tag uniformly-growing 10 plants/entries/replicates at flowering time for recording the disease incidence.

- Provide over-head sprinkler irrigation for about 30 min each at noon and evening (between 1700 and 1800) on rain free days from flowering until physiological maturity to provide high humidity (>90% RH) for infection (Fig. 21).
- Monitor physiological maturity stage (black layer formation at the hilum region of grains) of each line, and also monitor grain mold development in the susceptible check entries in each maturity group.
- Record the panicle grain mold rating (PGMR) on each panicle of the tagged plants at the right physiological maturity stage using the following 1 to 5 grain mold severity rating scale.
 - 1 = no infection – highly resistant
 - 2 = 1–10% grain molded on a panicle – resistant
 - 3 = 11–25% grain molded on a panicle – moderately resistant
 - 4 = 26–50% grain molded on a panicle – susceptible
 - 5 = >50% grain molded on a panicle – highly susceptible
- Also record the percent grain infection by individual fungi on a panicle based on the typical symptoms produced by each fungus.
- Harvest the panicles soon after recording PGMR, dry and thresh them.



Fig. 21. Sprinkler irrigation to facilitate grain mold infection.

- Compute the data for PGMR for each set of materials and subject to statistical analyses to find significant differences between entries and classify lines into resistant and susceptible classes.
- Also record data for each line on days to 50% flowering, plant height, panicle type, glumes color, glumes coverage of grain, and grain color at physiological maturity stage.
- Identify the resistant lines with acceptable agronomic/morphological traits for utilization in breeding program.

Screening technique for anthracnose and leaf blight

Greenhouse screening

- Grow the fungal isolates in 0.1% oatmeal broth in a rotary shaker (25°C, 125 rpm, with cool fluorescent light) for 10 days.
- Make aqueous conidial suspension and filter through a double-layered muslin cloth to separate conidia from mycelial mat. Adjust spore concentration of 1×10^5 with the help of haemocytometer. Add two drops of Tween-20 to 100 ml of each inoculum, just before inoculation.
- Spray-inoculate 21-day old pot-grown plants with the help of a hand held atomizer. Inoculated plants are air-dried, and transferred to humidity chamber (25°C, RH >95%) for 24 h.
- Shift the inoculated plants to the greenhouse (25°C, RH >90%) on benches and provide the mist to maintain high humidity.
- Record data for latent period (time in days for appearance of first chlorotic/necrotic lesion) from the 3rd day after inoculation.
- Record data for disease reaction and disease severity 14 days after inoculation using the following scale

Grade	Disease severity	Reaction type
1	No visible symptoms/chlorotic flecks	Highly resistant (HR)
2	Up to 10% leaf area covered with lesions	Resistant (R)
3	11–25% leaf area covered with lesions	Moderately resistant (MR)
4	26–50% leaf area covered with lesions	Susceptible (S)
5	>50% leaf area covered with lesions	Highly susceptible (HS)

Field screening

- Plant known susceptible entries in every 9th row in a field as infector rows.
- Plant test entries in between the infector rows.
- Grow isolates on autoclaved sorghum grains in flasks for 10 days (grains soaked overnight in water, decanted, filled in flasks, autoclaved for 1h, cooled, and inoculated with specific isolates) with 12-h photoperiod.
 - Take out grain culture in trays and air-dry for 30 minutes.
 - Place 2–3 infested grains per plant in whorls of 21-day old plants.
 - Inoculations should be preferably done in the evening hours, followed by light sprinkling with water or good irrigation to provide adequate moisture for infection.
 - Tag 10 plants/entry/rep for recording the disease reactions.
 - Record disease reaction (based on R, MR and S) and disease severity (based on 1–5 scale) at soft-dough stage.

Screening technique for sorghum downy mildew

Greenhouse

- Maintain the downy mildew inoculum on the susceptible genotypes in a greenhouse (Fig. 22).
- Prepare the potting medium (a mix of black soil, sand and FYM @ 2:1:1) and autoclave at 121.6°C for 1h a cycle, 2 cycles with an interval of 24h.
- Fill the pots of 6" diameter and plant the pots with the test entries.
- Plant known susceptible entries as controls.
- Harvest systemically infected leaves and wash them using cotton-swabs, dry them for some time to remove the surface water.
- Keep the infected leaves in moist chambers in such a way that the lower side of the leaf is facing up to facilitate sporulation.
- Incubate the leaves in moist chambers at 20°C for 6h with >90% RH under dark conditions.
- Harvest the conidia in ice-cold water using a camel hairbrush to prevent the germination of the conidia.

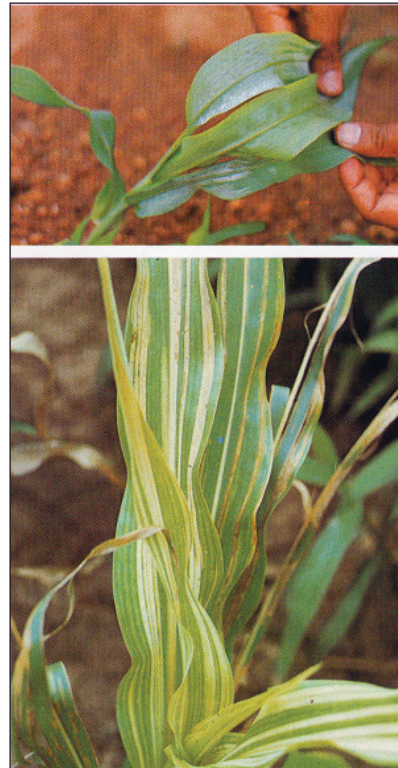


Fig. 22. Downy mildew infection in sorghum.

- Filter the conidial suspension through a double-layered muslin cloth, and adjust the conidial concentration to 1×10^5 conidia ml⁻¹.
- Spray-inoculate the seedlings at the 1st leaf stage and incubate the inoculated seedlings at 20°C for 16–20 h by providing high humidity.
- Transfer the pots to a greenhouse maintained at 25°C for 2 weeks.
- Record total and diseased seedlings to calculate the percent disease incidence.

Field screening

A downy mildew nursery consists of the following three components:

- **Infector rows:** Plant the known susceptible cultivar/line as infector rows on every 9th row.
- Inoculate the infector rows at the 1st leaf stage with conidial inoculum maintained in the greenhouse.
- Maintain high humidity (>90%) for the infection-development for 3 weeks by perfo/furrow irrigation.
- Thin the plants in the infector rows by leaving the infected plants for good growth of the plants in the infector rows.
- **Test materials:** Plant the test entries 3 weeks after the infector rows when they show >70% infection.
- **Indicator rows:** Plant the known susceptible cultivars/lines along with the test entries as indicator rows to compare the disease pressure.
- Provide perfo-irrigation for maintaining the high humidity required for infection development for 2–3 weeks.
- Record the total and diseased plants at pretiltering stage (30 DAE) and at soft-dough stage.
- Calculate the percent disease incidence based on total and diseased plants at soft dough stage.

Development of sick-plot:

- Mix the DM-infected leaf tissue (containing the oospores) in the soil and plant the known susceptible entries to find the disease pressure.
- Plant the test entries along with indicator rows (as controls) in the sick-soil when it shows >50% infection in the susceptible lines.
- Maintain high humidity by furrow/sprinkler irrigation for infection development in the test rows.

- Record total and diseased plants at pre-tillering and soft dough stage in both test entries and controls.
- Calculate the percent disease incidence based on total and diseased plants at soft dough stage.

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Field screening technique for charcoal rot resistance in sorghum

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Introduction

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is an economically important disease of sorghum having worldwide distribution. In India, it assumes significance because of large area (around 5.0 million ha) grown with sorghum during the post-rainy (winter) season where this disease interferes with grain and stover (dry fodder) quality. Stover of winter sorghum is used for cattle and thus it is as valuable as grain in India. The disease reduces grain yield (up to 64% in hybrid) and reduces stover quality. Loss in grain yield is mainly due to lodging of the crop and loss in stover quality (and yield) is due to rotting and decaying of the stalk (Mughogho and Pande 1984).

In sorghum, *M. phaseolina* causes three-phase symptoms viz., seedling blight, root rot and stalk rot. If infection occurs before the emergence of secondary roots, the plants die. Less severely infected seedlings, however, survive and establish secondary roots and under favorable conditions of sufficient soil moisture grow to mature plants. Under the conditions of moisture and temperature stresses that often coincide with the onset of flowering, the entire host-defense-system is weakened and activity of *M. phaseolina* increases many fold leading to rapid and extensive rotting of root and stalk. The pathogen produces at least six phytotoxins of which phaseolinone is the major product. Phytotoxins injure cell protoplast and prime the infected plants towards more severe disease in later stages (Kuti et al. 1997). Phaseolinone can cause anemia in mice (LD_{50} 0.98 g/kg body wt) and has the potential to cause health hazard in animals (Bhattacharya et al. 1994). High degree of variations in pathogenic properties in *M. phaseolina* and very strong relation of the disease with yield and environmental stresses particularly moisture and temperature makes the task of evaluating host resistance more challenging (Das et al. 2008).

Screening methods

Three screening methods have been developed and these are described below.

Sick-plot method

- Grow test lines in *Macrophomina* sick-plot in replicated trials (usual plot size is 2 rows×5 m length).
- Maintain uniform plant spacing to allow uniform moisture stress after flowering.
- Record data on time to 50% flowering, plant stand and plant height at flowering.
- Record the number of charcoal rot infected and lodging plants, lesion length, number of node crossed by lesion, and grain yield at harvest.
- Count the plants showing charcoal rot symptoms and calculate percent incidence.
- Score individual plants for charcoal rot severity on a 1–5 scale, where
 - 1 = one internode invaded, but rot does not pass through any nodal area;
 - 2 = two internodes;
 - 3 = three internodes;
 - 4 = more than three internodes; and
 - 5 = most internodes extensively invaded, shredding of stalk and death of plant).
- Compare charcoal rot incidence and severity of test lines with that of the known resistant and susceptible checks to identify resistant lines

Precautions

- The *M. phaseolina* sick plot must have high inoculum density (100-200 microsclerotia g⁻¹ soil) to avoid escape.
- The crop should experience sufficient moisture stress after flowering (stress can also be increased by removing the flag leaf after flowering).
- The comparison of test entries must be done within similar maturity (early genotypes have greater chance of escaping disease because of relatively less soil moisture stress) and yield groups.

Merits

- Useful for initial screening of large number of test entries.
- The method simulates the natural infection process that starts in the root under natural field conditions, therefore, more reliable.

- It counts natural defense in the root. Infection starts at the root level where phytotoxins, enzymes and other injurious metabolites of the pathogen acts on the host tissues for a long time and make it more prone to severe disease.

Demerits

- It requires well maintained sick-plot and cannot be screened in any sorghum growing field.

Toothpick method

This method was originally developed for maize and later adopted to sorghum.

- Insert toothpick infested with virulent isolate of *M. phaseolina* into the second internode of the stalk at 10-15 days after 50% flowering.
- Inoculate 5-10 plants (uniform in growth, height, flowering and spacing) per row in each replication.
- Withhold irrigation at 50% flowering to ensure adequate moisture stress.
- After 30 days of inoculation, split open each stalk and observe for the presence of charcoal rot symptom.
- Count the plants showing symptom and score individual plants using the 1-5 scale (as given in sick plot method).
- Compare the charcoal rot rating of test lines with that of the known resistant and susceptible checks to identify resistant lines.

Precautions

- The isolate (*M. phaseolina*) used must be in a virulent state as in the sick-plot method.

Merits

- Screening can be done in any sorghum growing field in any location during postrainy season.
- No additional cost for maintenance of sick plot is required.
- Chances of disease escape are relatively low.
- Useful for studying pathogenic variations of large number of isolates using different genotypes.

Demerits

The method does not simulate the natural infection process that starts in the root.

- It totally defies natural defense system of the root and ignores natural disease progress route.

- Charcoal rot severity observed under toothpick inoculation is usually less than that under natural infection.
- It is time and labor intensive as individual plants need to be inoculated.

In vitro screening of seedling

In this method sensitivity of seedling-tissues to phaseolinone, a predominant exotoxin produced by *M. phaseolina* in cell free culture filtrate is tested.

- Make cell-free culture filtrates (CFCF) of *M. phaseolina* by growing the fungus in Czpek-Dox broth for 15 days at 32°C and high intensity light conditions followed by filtering of the broth using 0.2m syringe filters.
- Standardize the CFCF by using host differentials viz., R16 as susceptible check and B35 as resistant check. The concentration at which all the R16 seedlings are killed in 10 days is considered as the working concentration.
- Grow seedlings of test lines along with controls in sterile conditions on MS0 medium for 7 days. Add the standardized CFCF to the medium with 15 seedlings for each treatment and grow in light at 28°C.
- Symptoms in the susceptible seedlings develop in 10 days (seedlings may be pin pricked for hastening the infection).
- Rank the test lines for resistance by comparing the performance with susceptible and resistant checks and the concentration of CFCF used.

Precautions

- Production of phaseolinone is influenced by external conditions of the culture; concentrations need to be standardized before the batch is used for the screening.

Merits

- Preliminary screening of large number of breeding and germplasm lines can be performed in a very short period of time.
- Economic, rapid and reproducible
- Useful for resistance rating in segregating populations.

Demerits

- Seedling resistance may not necessarily correlate with adult plant resistance due to inherently large variation observed in sorghum and hence it is suggested that the values are correlated with observations on field grown plants in endemic spots
- Lab-intensive and requires technical expertise to produce CFCF and testing.

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Heterosis – theories and harnessing in sorghum

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Introduction

The first two decades of the 20th century were marked by a flurry of genetic, cytogenetic and plant breeding research. In the year 1914, Shull proposed the term “heterosis” to describe the increase in vigor, development, growth and yield of the F₁ hybrid. This opened up the work on various theories explaining heterosis. To breeders, heterosis will generally mean a luxuriant, unfixable superiority of the hybrid as compared to parents in any of the economic traits.

The modern concept of heterosis may be classified as follows:

1. Direction
 - a. Positive heterosis
 - b. Negative heterosis
2. Expression
 - a. Luxuriant heterosis
 - b. Adaptive heterosis
 - c. Selective heterosis
 - d. Reproductive heterosis
3. Transmissibility through sexual phase
 - a. Labile heterosis
 - i. Heterozygous heterosis
 - ii. Heterokaryotic heterosis
 - b. Fixed heterosis
 - i. Balanced heterozygous heterosis
 - ii. Homoelogenous or homozygous heterosis

Evaluation of heterosis

Mid-parent heterosis (MP): $\{F_1 - [(P_1 + P_2)/2]\} / \{[(P_1 + P_2)/2]\} * 100$

where, F_1 is the mean value of a variable measured on F_1 , P_1 is the mean value of a variable measured on parent 1 and P_2 is the mean value of a variable measured on parent 2. This estimate usually reflects the effects of dominance and over dominance (epistasis also).

Better parent heterosis: $(F_1 - \text{better parent}) / \text{better parent} * 100$

This estimate would include only the over dominance effects.

Superiority over check (Standard heterosis): $(F_1 - \text{best variety}) / \text{best variety} * 100$

This estimate helps to explore the possibilities of exploiting it advantageously by producing hybrids.

Theories of heterosis

Geneticists, physiologists and biochemists explained the phenomenon of heterosis as follows.

Dominance hypothesis: The intercrossing of the lines would enable masking of the deleterious effects of the recessive alleles in the hybrids and the dominant alleles would express themselves. If each locus carries at least one dominant allele, the hybrid will have heterosis.

Overdominance hypothesis: Heterozygosity per se is necessary for the full expression of heterosis. The implication is that both the alleles perform different functions and the sum of their products is superior to the single product produced by either allele in homozygous state.

Physiological stimulus and initial capital: East (1908) and Shull (1908) suggested that heterozygosity provided some physiological stimulus that results in enlarged seed size (high initial capital), vigor and higher yield in the hybrids. The Heterozygosity was considered as the cause and heterosis was the end effect. However, it was noted that hybrids were not always found to have larger embryos and therefore it was concluded that it could not be the sole cause of heterosis.

Complementation at the cellular and sub cellular level: Growth and yield are the result of a series of reactions. Lack of potential of even one reaction in the long chain can influence the final produce. Individually, both the parents would have poor rate of synthesis, but a hybrid between these two parents would function better than either of the parents.

Balanced metabolism: Biochemical mechanisms under the control of genes determine the phenotype. Several enzymes were analysed but except a few

were not found to be heterotic. This led Hageman et al. (1967) to postulate that a “balanced metabolism” was the basis of heterosis.

Hormonal and other factors: Hormones and vitamins constitute an important group of chemicals that influence growth and development. However, there are no instances where heterotic amounts of growth regulators on unit basis have been observed.

Heterosis in natural populations

The main source of variation in population is mutation. It is maintained by (a) Balance between mutation and selection and (b) Heterotic balance.

Normally in populations, mutation and selection balance such that the frequency of the bb genotypes would remain at a level that could be reasonably attributed to the mutation rate of the allele B to b. However, in some populations, the frequency of bb genotypes could not be explained in terms of mutation rate. Such populations are said to be polymorphic. This is the situation when heterotic balance plays a part.

The well-known example is the sickle cell anemia. The sickle cell anemia condition is due to an allele in homozygous condition. If this is so, after some generations, the allele should disappear from the population and all the individuals should be normal, ie, AA genotype. However, in Africa, about 40% of the people in some tribes carry this allele ie, Aa genotype. It was found later that these individuals are resistant to malaria.

Besides individual locus heterotic balance, there are also studies on heterotic balance collectively at a large number of loci. Then a question arises – how many loci of a character could be heterotic in a population of species? The existence of overdominance is admitted if the number of heterozygous loci is very small and the viability of heterozygotes decreases with the increase in the number of heterozygous loci after the viability has reached optimum level.

Predicting the best hybrid combinations

Non-parental single crosses as applicable to maize: The performance of a double cross, say (A×B) (C×D) is given by the mean of the four non-parental single crosses. That is, yield of (A×B) (C×D) = $\frac{1}{4}$ (yield of A×C+A×D + B×C+B×D)

Grafius component method: It depends on the information collected about the principal yield components on parents. The steps are as follows:

The sequence of the development of yield components should be determined based on the physiological information. For example, Head – spikelets – seeds – weight.

The effects due to correlations between these components should be removed.

Let $X_1 \dots X_p$ be yield components

Let $Y_1 \dots Y_p$ be yield components after correcting for correlations

The $Y_1 \dots Y_p$ could be obtained as follows

$$Y_1 = X_1$$

$$Y_2 = X_2 - a_{21} Y_1$$

$$Y_3 = X_3 - a_{32} Y_2 - a_{31} Y_1 \text{ and so on}$$

$$A_{21} = \text{covariance } X_2 Y_1 / \text{variance } X_1$$

$$A_{31} = \text{covariance } X_3 Y_1 / \text{variance } X_1$$

$$A_{32} = (\text{covariance } X_3 Y_2 - a_{21} \times \text{covariance } X_3 Y_1) / \text{variance } X_1$$

This will give the corrected data of the parents.

By genetic analysis “true relative genetic variance” (TRGV) should be obtained.

Example:							
Trait	Original data of parents			Corrected data			TRGV
	Mean (M)	Variance (V)	(V/M)*100	M	V	(V/M)*100	
I set							
Heads/Plant	22.85	1.04	-	-	-	-	100
Spikelets/head	20.70	1.19	5.7	26.6	0.49	1.8	31.8
Seeds/spikelet	2.24	0.18	8.0	16.9	0.03	0.2	2.3
Weight/grain	37.15	108.99	293.4	476.9	0.003	0.01	0.01
II set							
Heads/Plant	3.83	0.53	-	-	-	-	100
Spikelets/head	16.30	1.93	11.85	14.7	1.9	12.5	105.3
Seeds/spikelet	1.29	0.03	2.32	1.5	0.02	1.3	56.9
Weight/grain	42.05	116.86	277.91	165.9	0.001	0.01	0.01
TRGV = values of 7 th column / values of 4 th column.							

Interpretations: In the I set, the variances of the 1st and 2nd characters of the untransformed data are more or less equal. However, TRGV indicates that 100–31.8 = 68.2% of the variation of 2nd character is due to the reflection of the effect of 1st character, namely heads. Therefore, selecting the parents based on the mean of heads/plant would give better prediction about the performance of the hybrid.

In the 2nd set, the effect of the first character on the 2nd character is nil. Thus, TRGV observed (105.3) is entirely due to spikelets/head. Hence, the prediction of the hybrid combinations, based on the mean of the 2nd trait, namely spikelets/head of parents would give a realistic picture.

Methods of fixing heterosis

Utilization of heterosis is temporary in the sense that each time we have to make fresh F₁. Some of the mechanisms known to fix heterosis are as follows:

Cloning: Vegetative propagation

Apomixis: Apomixis could be transferred by backcrossing to the parents that give best hybrids

Translocations: If such a mechanism is involved, the crossover chromatids would end up with duplications or deficiencies whose gametes would be lethal. As a result, the parental type of gametes would be restored. Thus, once the hybrid is made involving such a mechanism, it would be maintained year after year.

Inversion: The chromosomes resulting from crossing over would be either deficient in certain genes or contain certain genes in double. Their gametes would be lethal.

Synthetics: Possible in open pollinated species. One of the main objections is that there would be breaking down of the performance of these in later generations.

Polyploidy: Polyploids are often fertile and their offspring continue to show the heterosis arising from the combination of genes from the two parents. Almost half of the cultivated plants are amphiploid in origin.

Exploitation of heterosis in sorghum

In India, sorghum [*Sorghum bicolor* (L.) Moench] is an important grain and fodder crop grown in both rainy and postrainy seasons. While rainy season sorghum grain is used mostly for animal/poultry feed, postrainy season sorghum grain is used primarily for human consumption. Among the forage crops, sorghum offers great potential to supplement fodder resources because of its wide adaptation, rapid growth, and high green and dry fodder yields, and good quality (Pahuja et al. 2002). The Government of India's policy to blend 5% ethanol in petrol and likely increase of this proportion up to 10% will increase the demand for ethanol to blend with petrol. Sweet sorghum is an excellent alternative to sugarcane molasses for ethanol production, to cope with possible increased demand for ethanol. Large genetic variability is available for improving sorghum hybrid parents for grain, green

fodder and sweet stalk yields. Damages due to shoot fly in both rainy and postrainy seasons and grain mold in rainy season are the important production constraints and the genes conferring resistance to these constraints need to be deployed in high-yielding backgrounds.

The phenomenon of heterosis was observed in sorghum as early as 1927 (Conner and Karper 1927). Commercial exploitation of heterosis has been possible in sorghum owing to 1) the availability of a stable and heritable cytoplasmic-nuclear male sterility (CMS) mechanism (Stephens and Holland 1954), enabling large-scale, economic hybrid seed production and 2) sufficiently high magnitude of heterosis across a range of production environments for economic characters justifying the replacement of currently adapted 'homozygous' or 'pure-line' varieties. The hybrids, besides being superior for grain yield and other traits of interest, are stable across environments in all the types of sorghums; grain sorghum (rainy and postrainy seasons), forage sorghum and sweet sorghum.

Heterosis in rainy season grain sorghum

Substantial sorghum area (about 60%) in rainy season is covered by over 50 hybrids. Despite wide adaptability of hybrids, their farm level productivity (1 t ha^{-1}) is far lower than their potential (5 t ha^{-1}), as rainy season sorghums are frequently damaged by shoot fly and grain mold.

It is possible to develop hybrids with shoot fly resistance for rainy season adaptation. However, large G×E interaction for the development of trichomes, an important component trait conferring shoot fly resistance in rainy season, implies that breeding lines should be screened for shoot fly resistance in the season for which the hybrids are targeted. Investigations at ICRISAT have clearly demonstrated that high-yielding shoot fly resistant hybrids could be developed by crossing shoot fly resistant seed parents with landrace restorers or with improved resistant restorer lines and the probability of realizing heterotic hybrids for shoot fly resistance is higher when either both parents are resistant or at least the seed parent is resistant to shoot fly.

While breeding hybrid parents for developing hybrids with grain mold resistance (GMR) for rainy season, both seed parents as well as restorers need to be improved in separate programs as it has been clearly shown that resistant mechanisms present in hybrid parents complement to produce hybrids with higher levels of GMR, even when parents themselves may not be resistant (Reddy et al. 1992). Breeding for grain mold resistance however, was accompanied with reduction in grain size. Since farmers desire bold grain cultivars with enhanced GMR for rainy season adaptation, seed and restorer parents should be developed by crossing

bold grain, high yielding restorer/maintainer lines with new sources of GMR (as a long term strategy) as well as with high yielding GMR seed parents and restorers and selecting for GMR using refined screening technique in high yielding bold grain background (as a short term strategy).

Heterosis in postrainy season grain sorghum

The hybrids developed and released by Indian national program and ICRISAT did not attract the farmers as these hybrids lacked matching grain quality, and shoot fly resistance comparable to the most popular landrace variety, M 35-1. The landrace pollinator-based hybrids developed from a few female parents available prior to 1980s lacked marked heterosis, had threshing difficulties, and were too tall, a habit not amenable for increasing plant density and hence, this approach was not pursued further. However, extensive research at ICRISAT clearly demonstrated that landrace pollinator-based hybrids approach is the best way to develop hybrids with farmer-preferred traits for postrainy season adaptation.

Shoot fly resistance in landrace pollinator-based hybrids: Shoot fly resistance is required in both the parents or at least in seed parents in order to realize higher frequency of shoot fly resistant hybrids in postrainy season.

Temperature sensitivity in landrace pollinator-based hybrids: Seed setting ability (under bagging) in hybrids at low temperatures is critical to the postrainy season hybrids and requires greater attention to ascertain the differences among the landraces for their ability to restore fertility in hybrids, especially under low temperatures, normally observed in postrainy season.

Fertility restoration in landraces: Individual plant progenies of M 35-1 that restored A_1 , also restored A_2 CMS lines. However, the frequency of restoration and restoration ability of progenies of M 35-1 were dependent on the race from which the CMS lines were derived. Most of the landraces, including M 35-1, showed segregation for fertility restoration/sterility maintenance ability indicating the need to select for restoration ability within the landraces. This also explains the partial restoration observed when bulk pollen of M 35-1 was used by many workers. Both A_1 and A_2 CMS lines could be used for producing hybrids for postrainy season.

Role of nuclear genome (*durra* and *caudatum* types) on fertility restoration: Fertility restoration levels were lower on *durra*-derived A_1 CMS lines than *caudatum*-derived A_1 CMS lines. This finding has a bearing on developing CMS lines involving *caudatum*-based germplasm lines adapted to postrainy season and testing for fertility restoration in hybrids.

Performance of selected landrace pollinator-based hybrids: The selected landrace pollinator-based hybrids were significantly superior to M 35-1 and/or CSH 12R and were on par with CSH 13R for grain and fodder yields. They were significantly earlier in maturity, and had larger grain than CSH 13R. The hybrids involving *caudatum*-based female parents and *durra*-based landraces showed high heterosis for grain yield. The *caudatum*-derived female lines were adapted to rainy season. Rao et al. (1986) argued that by eliminating temperature sensitivity (in relation to development) in both male and female parents, greater success could be achieved in breeding hybrids for postrainy season. However, male sterile lines bred from landraces adapted to postrainy season, as indicated earlier, showed temperature-induced restorer inefficiency. Also, the hybrids developed from landraces crossed to female lines derived from M 35-1 did not show high heterosis.

Heterosis in landrace pollinator-based hybrids: Landrace-based hybrids were evaluated in an Advanced Hybrid Trial (AHT) during the 1993 postrainy season at ICRISAT, Patancheru and in Landrace advanced hybrids and parents trial (LRAHPT) during the 1995 postrainy season at Nandyal (Andhra Pradesh, India) and ICRISAT, Patancheru. Superiority over check for grain yield in AHT was recorded in 79% of the hybrids (range 3.8 to 49.2%) and in all the hybrids in LRAHPT (range 23.2 to 81.3%). For fodder yield, 45% of the hybrids (range 0.4 to 40%) in AHT and 97% of the hybrids (range 0.3 to 118%) in LRAHPT were heterotic.

Heterosis in forage sorghums

The forage sorghum developed and released/ marketed are mostly based on multicut sorghum-Sudan grass hybrids involving the available grain sorghum male sterile lines as seed parents and Sudan grass lines as restorers. The extent of heterosis for both green fodder and dry matter yields are substantial. There are many programs the world over targeting grain sorghum female lines improvement for forage traits. However, concerted efforts to improve pollinators for high tillering, biomass and foliar disease resistance are limited.

Heterosis in sweet sorghums

Sweet sorghums have a great potential for ethanol production by virtue of their high stem sugar concentrations, with a Brix value up to 24% (Almodares and Sepahi 1997). The predominant role of non-additive gene action for the characters plant height, stem girth, total soluble solids, millable stalk yield and extractable juice yield revealed the importance of heterosis breeding for improving the characters (Sankarapandian et al. 1994). Efforts are underway at ICRISAT, Patancheru to develop sweet sorghum hybrids with high Brix, high juice content and biomass.

Some breeding concepts in sorghum

Stability of male sterility: Stable male-sterile lines could be selected by evaluating the lines in environments where the day temperatures exceed 40°C during flowering.

Stability of restorers: Efficient and stable restorers could be selected by evaluating their testcrosses or hybrids under conditions where night temperatures are below 13°C during the flowering phase.

Diversification of hybrid parents: As a short-term strategy, CMS-based seed parents and restorers need to be diversified by creating separate gene pools through crossing between *guinea*-based B-lines and *durra*-based B-lines and between *caudatum*-based R-lines and *guinea/durra*-based R-lines for various selected traits.

Relationship between *per se* performance of parents and their combing ability: Parental *per se* performance and *gca* in sorghum is strongly correlated with hybrid performance.

Complementation of traits in hybrid combinations: Increased yield in grain sorghum hybrids gradually will depend less on heterosis *per se* and more on complementation of defensive traits from parents that confer yield stability. Therefore, improving the parental *gca* and/or *per se* performance for yield and yield constraints such as biotic and abiotic stresses, together with desired grain qualities are the keys to breeding hybrids parents.

Racial diversification of hybrid parents: F₁s made on *caudatum*-based seed parents with *durra*-based pollinators resulted in high heterosis under postrainy season condition.

Selection strategy for plant defense traits: Simultaneous selection for resistance and grain yield in early generation segregants starting from F₄ and converting those with maintainer reaction into male sterile lines would enable to develop male sterile lines with resistance to pests and diseases in the shortest possible period of four to five years (Reddy et al. 2003). Also, selection for resistance on family basis, and selecting individual single plants within the selected resistant families based on the grain yield have been found to be most effective.

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Development of male-sterile lines in sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth important cereal crop in the world after wheat, rice, maize and barley. Of late, it has emerged as 'fuel' crop in addition to its food, feed and fodder utilities. Sorghum is predominantly a self-pollinated crop and development of new 'varieties' is a natural option for crop improvement. However, there is 5 to 15% outcrossing in sorghum depending upon the wind direction, nature of genotype, and humidity (House 1985), which makes it amenable for use in population improvement and hybrid development to exploit the heterosis. Discovery of genetic male sterility (GMS) and cytoplasmic-nuclear male sterility (CMS) facilitated the application of recurrent selection procedures and hybrid cultivar development methods, respectively, in sorghum improvement. In this article, male-sterile line development using CMS in sorghum is described.

Cytoplasmic-nuclear male sterility

CMS has been used extensively to exploit heterosis in hybrid development on a large scale for commercial cultivation since 1960s. In the pre-hybrid era of early 1960s, the average sorghum productivity was 0.49 t ha⁻¹ in India, 0.66 t ha⁻¹ in China, 0.76 t ha⁻¹ in sub-Saharan Africa, 1.48 t ha⁻¹ in Australia, and 2.8 t ha⁻¹ in the USA, respectively. In the USA, Northern and Central America, where commercial hybrids were exploited, there was a 40% increase in productivity from early 1960s to early 1990s. A similar trend was noticed globally. The productivity increases were 47% in China and by 50% in India from early 1960s to early 1990s. However, the productivity remained static in Africa from 1960s to early 1990s (FAO 1960–1996), which can be directly attributed to non-exploitation of heterosis through hybrid development in Africa.

Origin of CMS

CMS is a physiological abnormality, resulting from a disharmonious interaction between the cytoplasmic factors (now widely identified as mitochondrial genetic factors) and nuclear genetic factors leading to the production of degenerated or non-viable pollen grains or non-dehiscent anthers with or without functional

pollen grains. Understandably, this disharmonious interaction is likely to be more pronounced in populations incorporating divergent sources of cytoplasm and nuclear genes (Reddy et al. 2003). Sorghum is no exception to this. For example, the A₁ CMS source in sorghum was identified in the F₂ population of cross Double Dwarf Yellow sooner Milo × Texas Blackhull *kafir* by Stephens and Holland (1954), in which the milo inbred belongs to *durra* race and is from Sudan and the Ethiopian border (Duncan et al. 1991), and the *kafir* inbred from Eastern Africa (House 1985). Twenty-five percent of male-sterile plants were observed in the F₂ generation of this cross if milo was the female parent. The male-sterile segregants from this cross produced male-sterile hybrids if crossed with the *kafir* parent and fully fertile hybrids if crossed with the milo parent. Thus, it was recognized that *kafir* could be used as a maintainer source of CMS. Since the progeny received the cytoplasm from the female, it was hypothesized that the milo parent had a male sterility-inducing cytoplasm and dominant nuclear genes for pollen fertility, whereas the combine *kafir* parent contained a normal (fertile) cytoplasm but recessive nuclear genes for male sterility. All progenies of the milo × *kafir* cross contained milo (sterility-inducing) cytoplasm, but those that also inherited the homozygous recessive genes from the *kafir* parent were male sterile. The male-sterile plants in the milo × combine *kafir* cross were used as females in repeated backcrossing with *kafir* as the male parent. At the end of seven backcrosses, the entire genome of *kafir* was transferred into the milo cytoplasm. This resulted in two morphologically similar versions of the combine *kafir* (CK 60) parent: a male-sterile combine *kafir* (CK 60A) and a male-fertile combine *kafir* (CK 60B). The male-sterile lines are designated as A-lines and their maintainer lines as B-lines.

Development of new hybrid parents (A-, B- and R-lines)

The lines that produce fertile F₁s when crossed with A-lines are called restorer lines or R-lines. The development of hybrid parents involves two steps: (1) identification of potential B- and R-lines; and (2) development of A-lines and R-lines.

Identification of B- and R-lines: Improved breeding lines, named/released varieties and landraces from the pollinator collection are the sources that can be used as pollen parents or pollinators. The hybrids obtained by crossing these pollinators with a male-sterile line, the testcrosses, are evaluated for the sterility maintenance or fertility restoration in them (Murty et al. 1994). This evaluation is usually sown in small plots (one or two rows of 2 m length). Examination of anther morphology may be a basis for classifying the hybrids as male-sterile or male-fertile; but it is not a sure way. A more reliable method is the bagging test, ie, covering 4-6 panicles with a paper bag before anthesis, and observing the seed-set after 2-3 weeks. (Similar to enclosing the panicles in selfing bags). The testcrosses are of the following four types:

1. Testcrosses exhibiting absolutely no seed-set on all the bagged panicles, ie, male sterility was maintained in these hybrids. The corresponding pollinator is classified as a maintainer or non-restorer or B-line. This could serve as a source of a new A-line.
2. Testcrosses with complete seed-set on all bagged panicles. The corresponding pollen parents are classified as potential restorer or R-lines. They can serve as male parents to produce hybrids.
3. Testcrosses with a partial seed-set on all the bagged panicles. The corresponding male parents are rejected from the program as they serve neither as restorers nor as maintainers.
4. Testcrosses with a full seed-set on some bagged panicles and no seed-set in others. The corresponding pollen parent of such a hybrid is said to be segregating for fertility-restoration or sterility-maintainer genes. Usually, such parents are not pursued further in a hybridization program, as they involve additional work of fixing the genes for fertility restoration/sterility.

Development of new A- and R-lines: Three criteria are used in the selection of parents for this purpose: genetic diversity, the *per se* performance of the lines, and the average performance of a line in crosses with other lines [called general combining ability (GCA)]. Experience in sorghum has shown that parents of diverse origin produce highly heterotic hybrids. It has also been found that *per se* performance of parents is positively correlated with the performance of the hybrids (Murty et al. 1994). Further, the general combining ability is more important than specific combining ability (the deviation from performance predicted on the basis of general combining ability) in sorghum. Further, shorter (usually 1.25-1.75 m) and high-yielding lines with sterility-maintenance ability are chosen for conversion into male-sterile lines. Taller lines (usually 1.75–2.50 m) with restorer reaction are chosen as R-lines.

The maintainers identified through the bagging test possess recessive genes for fertility restoration/ sterility maintenance but have a normal cytoplasm. The selected B-lines can be crossed with any recognized male-sterile line. The resulting F_1 s and the corresponding maintainers are sown alternately in small plots, and the hybrids are backcrossed repeatedly with the respective maintainer lines for six or seven generations using the corresponding maintainer lines as recurrent parents until male-sterile lines with appearance identical to the recurrent B-line parent are obtained. It is important that plant-to-plant crossing should be attempted in the backcrossing phase. This involves crossing individual male-sterile plants with individual plants of the recurrent parent that are morphologically similar to each other. This plant-to-plant method is useful to select out the partial sterility maintainers from the program. Also, it enables faster realization of A-lines with morphological traits similar to the maintainer line.

The A-lines thus obtained may be sown alternately with the respective B-lines, and the pollen (bulk) from the respective B-lines collected in separate bags may be put over the male-sterile panicles with emerged stigmas. The bags should be shaken thoroughly. Before pollination, these male-sterile panicles should be bagged as in selfing to prevent outcrossing with pollen from unwanted parents. Similarly, the B-lines should be selfed. The seed bulked within the A-lines will form the A-line seed. The B-line seed bulked within the line will form the B-line seed. Thus, A- and B-lines are maintained. It should be remembered that rouging should be carried out before selfing/pollination of A-/B-lines.

Once uniform A- and B- lines are produced, the stability of the male sterility in the A-lines may be evaluated by evaluating them in areas where the temperature at flowering reaches 42°C or more. Unstable A-lines become fertile at this temperature.

Seed production of A-, B- and R-lines

Small-scale production: R-line seed (identified through the bagging test of testcrosses) may be produced by sowing the seed in a plot of the desired size and selfing the plants after rouging out the off-types before and at flowering. Bulk harvesting of true-to-type panicles may be done. A plot of two rows of 4 m length, if maintained properly, may give about 2.0–2.5 kg seed.

Production of A- and B-lines involves several operations:

1. Sow A- and B-lines in the plot side by side. Usually, for every four rows of A-line, two rows of B-line are sown.
2. Carry out rouging regularly in the A-lines and B-lines before and during anthesis. Apart from off-types, pollen ‘shedders’ can be a problem in the A-lines [a pollen shedder is a fertile plant in the A-line that results from a breakdown of male sterility; in practice, however, B-line (fertile) plants which appear in the A-line plot due to mechanical mixing are also referred to as shedders]. These should be removed by inspecting the field everyday during anthesis.
3. Prune the florets of A-line with protruded anthers/stigmas at the tip of the panicles, and pull kraft paper bags over the panicles with the date of bagging recorded on them. Carry out a similar operation on the B-line.
4. After 4–6 days, collect pollen from the B-line panicles into the same bags used for selfing, and put these bags carefully over the respective A-line panicles, by slightly bending the A-line, and shake the panicles along with the pollen bags by holding the mouth of the bag tightly wrapped around the peduncle. Each pollen bag may be used to pollinate 2–3 panicles of the same A-line.

5. Cover the pollinated panicles with the same pollen bag or with a new one. The bag should carry information on the date of the first bagging and pollination, and an A×B mark indicating that it was pollinated by a B-line.
6. Pollination of A-lines with B-lines may be repeated again after the 6th or 7th day in order to pollinate all the florets in the entire panicle.
7. B-line panicles should be selfed by bagging after using their pollen to pollinate the A-line panicles.
8. Take out the bags 15–20 days after pollination/selfing, and staple them over the peduncle below the base of the panicles, as in selfing.
9. Rogue out plants at the time of harvest, and bulk harvest the panicles in A-lines and B-lines separately and label them clearly.

Precautions: Periodic replacement of damaged bags is essential.

Large-scale production: Large-scale production of A-, B- and R-lines is usually taken up in isolation plots (Chopra 1982).

1. **Production of R-line:** R-line is produced in an isolation field separated from other sorghum fields by at least 300 m. Periodic rouging of the off-types is essential. Bulk harvesting is done by taking true-to-type panicles.
2. **A- and B-lines:** Production of A- and B-lines is done by growing the A-line in four rows alternating with the corresponding B-line in two rows. Across all the rows in the entire field, it is recommended that a strip of 1 m length should be sown with the B-line. This is useful in providing pollen to the A-line panicles at the end of the rows. Rouging of the off-type plants and pollen shedders should be done during anthesis everyday. Open pollination by wind will ensure seed-set on the A-lines. Self-pollination takes place in the B-lines. Harvesting of A-line and B-line seed should be done separately. To avoid mechanical mixing, it is recommended that they should be harvested at different times, preferably one after the other.

Improving B-lines and A-lines

We have so far dealt with the procedure of developing A-lines from the B-lines identified from the pollinator collection through testcrossing. It is important to know the procedures involved in improving A- and B-lines in hybrid programs. It involves the following steps.

1. Identify the B-line(s) for improvement and the resistance source lines for stress factors or high yielding lines (depending on the objective) which may be fertility restorers/sterility maintainers.
2. Cross the B-line with the selected source line(s) and advance them to the F₂ generation.

3. Grow F_2 under the desired screening for resistance, and select for monogenic or oligogenic traits apart from resistance.
4. Grow selected F_3 progenies in head-to-rows under screening for stress factors of interest. Select plants with the desired combination of traits within the family selected for resistance and uniformity.
5. Testcross the selected segregants onto an A-line sown separately near the F_3 nursery under screening. Also self the selected segregants (pollinator) used in testcrossing.
6. Grow the testcross and the pollinator (F_4 s) in a block near the pollinator screening block. With experience, one can usually determine the male-sterile testcrosses by anther morphology at anthesis. Otherwise one should use the bagging test to identify male-sterile plants. Repeat step 4, ie, select families for resistance and select individual plants for crossing on the basis of agronomic desirability.
7. Backcross the male-sterile F_1 (A-line) panicles with pollen from the selected plants (as above) individual panicles as per the procedure outlines for pollination. The F_4 families selected for resistance should be used as pollinators. Harvest the backcrossed A-line panicles and selfed pollinators' panicles individually and pair them as per the pollination done.
8. Repeat steps 6 and 7 for six to seven generations. Care should be taken at every stage in the following areas: check male sterility on the basis of anther morphology and seed-set on a few panicles under bagging; also, always make plant-to-plant backcrossing, ie, the individual male-sterile panicles (2–3) selected for backcrossing should be similar in morphology to those individual plants of the pollinators selected for pollination.
9. At the stage when male-sterile lines resemble the respective maintainer lines and are uniform, they are called A- and B-lines. The B-lines may further be selected on the basis of their *per se* performance and resistance to the factors of interest.
10. Further selection of A-lines may depend on GCA tests for traits of interest. The selected A- and B-lines may thus be numbered with the year, followed by serial number and letters A or B to indicate male sterility or maintenance. For example, ICSA 95001 and ICSB 95001 indicate that these two represent one A and B pair, bred in the year 1995, and the line number is 1.
11. Maintenance of the selected A-lines is done as per the procedure outlined earlier.

The trait-based breeding approach followed at ICRISAT, Patancheru (1985–95) facilitated the use of lines of diverse origin and provided a range of male-sterile lines in varying genetic backgrounds. Resistance levels in each resistance group

vary from highly resistant to less susceptible. Grain yield level in these groups is compensated by resistance, and therefore they are on par with the best checks 296A/B, Tx 627 A/B or IC5A/B 101 for grain yield and agronomic desirability. Efforts are underway for pyramiding resistance, assessing grain yield and grain characters of these lines to use them in hybrid development in a big way. Similarly, the ongoing programs (1999 onwards) on the race-specific and alternate (non-milo) CMS specific diversification of A-/B-lines is providing dividends in terms of increased diversity. By now 39 A-/B-lines with A₁ background belonging to different races and 46 A-/B-lines with A₂ background in different races have been developed by ICRISAT, Patancheru to thoroughly exploit the diversity for hybrid development. Efforts are underway at ICRISAT, Patancheru for utilization of A₃ and A₄ cytoplasm for further diversification of hybrid parents. Emphasis is also given on development of hybrid parents for post-rainy season adaptation.

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Development of sorghum restorers and varieties

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Introduction

The discovery of stable and heritable cytoplasmic male sterility (CMS) system enabled exploitation of heterosis in sorghum. Since then, hybrids are often the only cultivar types that are developed and cultivated in developed countries. In countries such as India and China both pure-line varieties and hybrids are cultivar options. Hybrid breeding requires the development of CMS system-based seed parents/male-sterile (A-) line and restorer (R-line) parents which restore male-fertility in the hybrids. Stability of male-sterility, especially under high air temperature in A-lines and profuse pollen producing ability and tall plant stature in R-lines (compared to seed parents), among others, are important requirements for hybrid seed production. Those R-lines with high yielding ability and superior grain quality traits could be used as varieties for commercial cultivation. The A-lines are developed by repeated backcrossing of lines with male-sterility maintainer reaction onto a known A-line. The lines that restore male-fertility in the hybrids upon test-crossing with a known A-line are classified as restorers. The A-lines and R-lines/varieties are usually developed in separate programs. In this article, the research on the development of sorghum restorers/varieties in Indian sorghum improvement programs is reviewed briefly.

Historical preview

Restorers

The early efforts made under “Accelerated Hybrid Sorghum Project” to identify heterotic combinations among the A-lines and the converted dwarf lines, resulted in the development of the hybrids, CSH 1, CSH 2 and CSH 3. The R-lines of these hybrids had tropical parentage and were selected from yellow endosperm lines such as IS 84 (CSH 1) and IS 3691 (CSH 2 and CSH 3). CSH 2 and CSH 3 could not become popular because of the seed production problem due to shorter stature of their common male parent, IS 3691 compared to their respective A-lines.



CSH 15R

Post rainy season
hybrid

Fig. 23. A promising postrainy season hybrid developed by Indian national program.

Male parent improvement through selection in “temperate-tropical” crosses resulted in the development of CS 3541, which has been derived from a cross between IS 3541 (African zerazera)×IS 3675. The line CS 3541 also contributed to the development of male parents of CSH 5, CSH 6 and CSH 9. The R-line, 148/168 of the postrainy season hybrid, CSH 7R was derived from the cross IS 3687×Aispuri, an Indian variety. The R-line, PD 3-1-11 of CSH 8R was derived from the cross IS 84×BP 53 (Rao and Rana 1982).

Later three hybrids, CSH 10, CSH 11 and CSH 12R based on male sterile line, 296A and SB 1085, MR 750 and M 148-138 as R-lines were released. However, these hybrids could not be promoted due to seed production limitation and smaller seed size and quality. Another postrainy season adapted hybrid, CSH 13 is superior to the local variety for grain yield but inferior to M 35-1 for grain quality. Though there was no significant grain yield improvement, useful diversification for early maturity and higher fodder yield has been earlier achieved with the release of CSH 14 and CSH 13. The R-line of CSH 13 contributing to higher heterosis levels for fodder yield was developed from SC 108, an American elite line and SPV 126 (a tall mutant of CS 3541). The R-line (RS 585) of the hybrid CSH 15 was derived from crosses between postrainy season adapted local variety and CS 3541, an elite rainy season adapted variety (Fig. 23).

In view of seed production problems and stagnating yield levels, the need for diversification of hybrid parents was felt. As a result, a high yielding hybrid, CSH 16 was developed from new MS line 27A and the R-line C 43. This hybrid showed improvement in grain mold tolerance as the grain mold resistant genes from Ethiopian germplasm line IS 23549 were introduced to its male parent (Audilakshmi et al. 2003). The R-lines [RS 673 (SPV 544 × S GIRL-MR-1)] and Indore 12 of the hybrids CSH 17 and CSH 18 were derived from crosses between SSV 53 and SPV 475. CSH 19R was a postrainy season-based hybrid developed using R 354 as male parent. CSH 23 was the latest hybrid released in 2005 which matures in 103 days and is tolerant to terminal drought. The male parent (RS 627) of this hybrid is derived from crosses between rainy×postrainy season adapted breeding lines.

Varieties

In India, the varietal improvement program was initiated in the 1930s. The locals were tall, late-maturing, and generally photosensitive and characterized by localized adaptation and low harvest index. Local×local hybridization followed by selection resulted in varieties having marginal increase in grain yield (Rao 1971). Since production environment, constraints, end-product utilization and hence cultivar requirements in rainy and postrainy seasons are quite different, separate programs were initiated to develop varieties for rainy and postrainy season adaptations.

Rainy season adaptation: Varietal improvement was achieved by introducing temperate and tropical germplasm. The first variety, CSV 1 is a direct introduction of line IS 3924 from the USA. By crossing temperate and tropical germplasm, the varieties, CSV 2 and CSV 3 were developed. The variety CSV 4, which was used as restorer (CS 3541) of 3 most popular hybrids, CSH 5, 6 and 9, became highly popular. CSV 5 was another variety derived from cross between Indian local and US line IS 3687. It showed resistance for *Striga*. The variety, CSV 10, which became popular for higher fodder value was developed from a cross between Texas elite variety SC 108 and the Indian elite variety CS 3541. SPV 462 was another variety that became very popular for its higher grain and fodder yields with good grain quality. This variety was derived from multiple cross involving IS 2947 and IS 3687 from the USA and IS 1151 and BP 53, the local lines from Maharashtra and Gujarat states of India. CSV 13, yet another high yielding variety with medium height was developed from multiple cross having exotic and local parentage. Another variety, CSV 15 was developed from the segregating population derived from the cross between SPV 462 and CSV 13. It is a dual purpose variety having grain yield comparable to that of hybrid CSH 5 and fodder yield comparable to CSH 10.

Postrainy season adaptation: Several varieties such as CSV 7R, CSV 8R and CSV 14R were developed using selections from segregating populations derived from the crosses among Indian locals, M 35-1 and IS 2644 with American germplasm lines. Marginal improvement was achieved for grain yield over the most popular landrace variety M 35-1. The variety, CSV 216R released in 2000 is a landrace selection from postrainy germplasm from Maharashtra. CSV 18 is the latest postrainy season variety released in 2005 derived from a cross CR 4×IS 18370. The list of varieties and hybrids released through the All-India Coordinated Sorghum Improvement Project are given in Annexures 1–4. Most of these varieties could not become popular as they did not match shoot fly resistance level and grain quality of M 35-1.

Development of new restorers/varieties

Identification of potential male parents/varieties

In sorghum, experience has shown that the parental origin has a significant bearing on the extent of heterosis and productivity of the hybrid and it is important to choose parents of diverse origins when making experimental hybrids. Most sorghum hybrid breeding programs divide their parental line development programs into two distinct groups (A/B and R-lines) to maximize heterosis between parents of the two groups (Rooney and Smith 2000). Potential restorers are identified by crossing promising lines from a pollen parent collection which could consist of world collection of germplasm, cultivars, genetic stocks in advanced generations, etc, to a male-sterile line and assessing their corresponding hybrids in small plots of an observation nursery. It is recommended that test crossing of new selections be carried out in early segregating generations like F_3 or F_4 (Andrews et al. 1996; Murty et al. 1994). This is necessary because restorer genes can be lost during recombination and segregation during the process of inbreeding and selection. A few plants of each test cross are subjected to a bagging test before flowering and observed for seed set under the bag after a few weeks. The hybrids exhibiting complete seed set under the bag are called fertile hybrids and the corresponding pollen parents are classified as potential R-lines, which could be useful in producing hybrids and for direct use as varieties, if they possess desirable agronomic traits. Lines that produce high yielding hybrids with appropriate agronomic characters are advanced for additional testing. These R-lines are hybridized among themselves and with several potential A-lines to identify hybrids with specific combining ability.

Restorer line evaluation

Evaluation of performance of R-lines with respect to seed production is important. It should start shedding pollen prior to the emergence of stigmas in the A-line, and must continue to shed the pollen throughout the flowering of A-lines. The R-lines should be taller than A-lines. It should also completely restore male fertility, even under low temperatures (Andrews et al. 1996, Rooney and Smith 2000). The genetic variation for anther formation on pedicel flowers that mature later than the anthers on the sessile flowers should be exploited. R-lines with this useful feature have, in effect, two successive phases of pollen shedding from the same panicle and thereby ensure continuous pollen supply. The R-lines should also have higher grain yield ability as experience has shown direct relationship between parental and hybrid performances. The general combining ability of R-lines has been considered to have greater value than specific combining ability of the hybrids.

Breeding methods for development of R-lines/varieties

Although both short-term (pedigree method and backcross) and long-term breeding methods (population improvement methods) are available, short-term breeding methods are most commonly used considering pressure for producing finished products in the shortest possible time.

Short term program: Pedigree selection (Fig. 24) among the segregating populations derived from carefully selected parents has been the most popular method for developing R-lines. Breeding new R-lines has increasingly become dependent on elite R \times elite R crosses. The continued use of such crosses progressively narrows genetic base of resulting R-lines. Therefore, use of diverse germplasm for generating useful variation is essential for developing R-lines and hence hybrids with broad genetic base. Broadening genetic base of hybrids is the key for sustainable production.

Backcross method is normally used to incorporate specific traits like seed size, seed shape, through repeated backcrossing of donor parent with recipient parent, which is deficient in specific traits, but otherwise possess elite agronomic background. At NRCS, improvement of AKR 354, (R-line of post-rainy season adapted hybrid

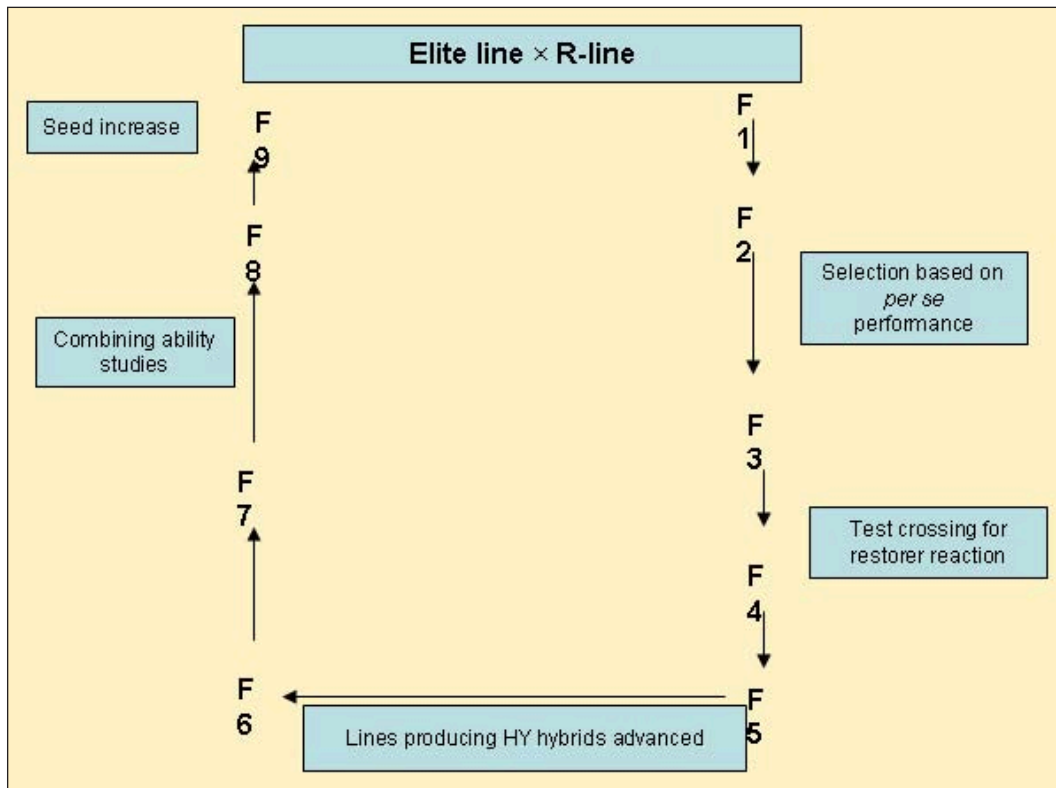


Fig. 24. Development of new restorer lines by pedigree method.

CSH 19R) for grain quality is in progress since the quality of the hybrid CSH 19R is not acceptable to the consumers. AKR 354 has small grain (on average 2.47 g/100 grains), grain shape is elliptical at both dorsal and profile view and grain is non-lustrous. AKR 354 was backcrossed with rainy season and postrainy season adapted elite lines having bold, circular and lustrous grain (Table 7). Out of 200 promising derivatives, 49 derivatives showed 100 grain weight of 3.5 to 4 g and 35 had lustrous grains and 30 had round grains (Audilakshmi, personal communication).

Long term program: When the objective is to introgress new desirable genes distributed in many source lines, population improvement methods are used. Population improvement methods, besides offering greater opportunities for recombination to break linkages between desired and undesired traits, provides scope for increased utilization of biotic and abiotic stress resistant, but agronomically non-elite source germplasm lines. Population improvement provides a long-term breeding strategy to derive diverse and broad genetic-based superior varieties/hybrid parents. Therefore, a comprehensive crop improvement strategy has to combine both short- and long-term programs for continuous improvement of economic traits (Reddy et al. 2006). The population improvement procedure involves selection of component parents with high GCA, incorporation of genetic male sterility, intercrossing and random mating among parents following recurrent selection schemes. At ICRISAT-Patancheru, 19 trait-specific populations were developed and are being maintained. While population improvement programs are not the most common in sorghum breeding, they are an important source of genetic variation and improved traits (Rooney and Smith 2000).

New approaches to develop R-lines/varieties

Development of heterotic groups: While development of heterotic groups for breeding maize hybrid parents is an established approach, such an approach in sorghum is still in its infancy. Most of the available B- and R-lines are genetically related. As a result, level of realized yield heterosis has been limited. Therefore, development of heterotic groups is expected to realize maximum heterosis.

Exploitation of different sorghum races for improving hybrid vigor: In sorghum, though there exists 5 different races (*bicolor*, *caudatum*, *durra*, *guinea* and *kafir*), only *caudatum* followed by *durra* races have been exploited to a great extent for the development of parental lines and for exploitation of hybrid vigor in commercial hybrids. It has been found that other races like *guinea*, *kafir* do possess genes for yield and other traits of interest and needs to be exploited to enhance heterosis levels in commercial hybrids.

Table 7. Performance of selective AKR 354 derivatives for grain traits*.

Pedigree	Generation	Test weight (g/100 seed)	Seed shape*		
			Dorsal view	Profile view	Grain luster
{(AKR 354 × SPV 839) × AKR 354} × SPV 839	BC ₂ F ₂	4.09	C	E	L
		3.77	C	C	L
		3.78	C	C	L
		3.95	C	C	L
		3.73	C	C	L
{(AKR 354 × SPV 839) × AKR 354} × AKR 354	BC ₂ F ₂	3.65	C	C	L
		3.79	C	C	L
		3.63	C	C	L
		3.72	C	C	L
		3.79	C	C	L
AKR 354 × RS 585	F ₃	3.62	C	C	L
		3.97	C	C	L
		3.75	C	C	L
		3.72	C	C	L
		3.77	C	C	L
AKR 354	Check	2.47	E	E	NL
	SE	0.02			

* Audilakshmi, personal communication.

Development of landrace pollinator-based hybrids: Postrainy season adapted landraces possess excellent grain quality traits and adaptive characteristics well suited to mitigate terminal drought and tolerance to shoot fly and charcoal rot. These can be utilized as restorers for crossing with *caudatum*-based A-lines derived from exotic germplasm to produce heterotic hybrids with acceptable grain quality traits that match those of M 35-1. It has been demonstrated at ICRISAT that landrace pollinator-based hybrids showed reasonably higher level of heterosis for both grain yield and fodder yields and were found superior to the cultivated landraces.

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Annexure 1. Pedigree and origin of rainy season adapted sorghum hybrids released at national level.

S.No	Hybrid	Year of release	Pedigree of the hybrid	Pedigree of the parental lines of the hybrid	Centre which developed the hybrid
1	CSH 1	1964	CK 60A × IS 84	CK 60A- <i>kafir</i> (USA) IS84-SA7529-55-1-1-1-1 from Texas <i>durra caudatum</i> race (USA)	NRCS
2	CSH 2	1965	CK 60A × IS 3691	CK 60A- <i>kafir</i> (USA) IS 3691- <i>durra caudatum</i> (USA) DD × EHEG	NRCS
3	CSH 3	1970	2219A × IS 3691	2219A-Selection from Rainy shallu IS 36914- <i>durra caudatum</i> (USA)	NRCS
4	CSH 4	1973	1036A × Swarna	1036A = CK 60B (<i>kafir</i> USA) × PJ&K (rainy Parbhani Jowar) Swarna-Selection from IS 3924. IS 3924- <i>kafir durra</i> (USA) SA 9804	NRCS
5	CSH 5	1975	2077A × CS3541	2077A-IS 2046 × 3677B IS2046 - <i>kafir</i> (Senegal) SA8339 CS 3541-IS 3675 × IS 3541; IS 3675- <i>kafir durra</i> (USA) Back 2931BKAFX8038B Yel. IS 3541-zerazera (Ethiopia)-NYITHIN	NRCS
6	CSH 6	1977	2219A × CS3541	2219A-Selection from Rainy shallu CS 3541-IS 3675× IS 3541; IS 3675- <i>kafir durra</i> (USA) Back 2931BKAFX8038B Yel. IS 3541-zerazera (Ethiopia)-NYITHIN	NRCS
7	CSH 9	1983	296A × CS3541	296A-IS 3922 × Karad local. IS 3922- <i>kafir durra</i> -yell end. Karad local-rainy local from Maharashtra	NRCS
8	CSH 10	1984	296A× SB 1085	296A-IS 3922 × Karad local. SB 1085- *	Dharwad
9	CSH 11	1986	296A× MR 750	296A-IS 3922 × Karad local. MR 750- *	ICRISAT
10	CSH 14	1992	AKMS14A × AKR 150	AKMS 14 A-(MR 760 × BT 632) × AKMS 2B AKR 150 = CS 3541× 900 *	Akola
11	CSH 13 K & R	1995	296A × RS 29	296A-IS 3922 × Karad local. RS 29-SC 108 × SPV126; SC 108 (Purdue University, USA) SPV 126-Tall mutant of CS 3541	NRCS

12	CSH 16	1997	27A × C43	27A-83B × 199B 83B-(CS 3687× CS 3922); 199B-(2219B × CS 3922) CS 3687- <i>durra caudatum</i> (USA); IS 3687-- <i>durra caudatum</i> (USA) CS 3922- <i>kafir durra</i> -yell end. Karad local- rainy local from Maharashtra C 43-CS 3541× IS 23549 IS 23549-guinea- <i>caudatum</i> (Ethiopia)	NRCS
13	CSH 17	1998	AKMS 14A× RS 673	AKMS 14A-(MR 760 × BT 632) × AKMS 2B RS 673-SPV 544 × K 24-1 SPV 544 = CS 3541 × CO 18 × CO 27 × 1022 K 24-1 = SG IRL-MR-1-midge resistant dwarf variety, Georgia, USA	NRCS
14	CSH 18	1999	IM 9A × Indore 12	IMS 9A× Indore 12 IMS 9A-2077A× (MA 9B × Vidisha 60-1)11-4-2-5-5A Vidisha 60-Sel. from a local (rainy) Indore 12- (SSV 53 × SPV 475)-7-1-1-1 * SPV 475-(IS 12622 × 555) × (IS 3621 × 2219B) × E 35-1 IS 12622- <i>durra bicolor</i> (Ethiopia). SA 2300 IS 3612- <i>caudatum</i> (Nigeria) BA 45 FARIA BOMKUM E 35-1-Ethiopian early line	Indore
15	CSH 21	2005	MLSA 848 × MLR 34	----	Mahendra
16	CSH 23	2005	MS 7A × RS 627	MS 7A- Selection from AKMS 14A RS 627- RS 71 × M 35-1	NRCS

* Released for Tamil Nadu and became popular in other states also.

Annexure 2. Pedigree and origin of rainy season adapted sorghum varieties released at national level.

S.No	Variety	Year of release	Pedigree of the variety	Pedigree of the parental lines of the variety	Name of the center which developed the variety
1	CSV 1	1968	Sel. from IS 3924	IS 3924- <i>kafir durra</i> (USA)	NRCS
2	CSV 2	1974	IS 3922 × Karad local	IS 3922- <i>kafir durra</i> (USA) Karad local-a rainy local from Maharashtra	NRCS
3	CSV 3	1974	IS 2954 × BP 53	IS 2954- <i>durra caudatum</i> (USA) BP 53 = IS 18432-India- <i>durra</i> , a Gujarat rainy local	NRCS
4	CSV 4	1974	IS 3675 × IS 3541	IS 3675- <i>kafir durra</i> (USA) Back 2931, BKAF × 8038B yel. IS 3541-zerazera(Ethiopia)	NRCS
5	CSV 5	1974	IS 3687 × Aispuri	IS 3687- <i>durra-caudatum</i> (USA) Aispuri (IS 1151)- <i>durra</i> (India), a Maharashtra rainy local	NRCS
6	CSV 6	1974	IS 3922 × Aispuri	IS 3922- <i>kafir durra</i> (USA) Aispuri (IS 1151)- <i>durra</i> (India), a Maharashtra rainy local	NRCS
7	CSV 7	1982	CS 3541 (Tall mutant)	CS 3541-IS 3675 × IS 3541 IS 3675- <i>kafir durra</i> (USA) Back 2931B KAF × 8038 B Yel. IS 3541-zerazera (Ethiopia)	NRCS
8	CSV 10	1983	SB 1066 × CS 3541	SB 1066-Sel from SC 108 (Purdue, USA) CS 3541-IS 3675 × IS 3541	Udaipur
9	CSV 11	1985	(SC 108-3 × CS 3541)-11-1	SC 108-3-Sel from SC 108 SC 108-Purdue, USA CS 3541-IS 3675 × IS 3541	ICRISAT
10	CO 26	1985	(IS 2947 × SPV232) × 1022	IS 2947- <i>caudatum</i> (USA) SPV 232-148 × 512 148 = IS 3687 × Aispuri IS 3687- <i>durra-caudatum</i> (USA) Aispuri (IS 1151)- <i>durra</i> (India) , a rainy local from Maharashtra 512-derivative of BP 53 (rainy local from Gujarat)	Coimbatore
11	CSV 13	1988	(IS 12622 × 555) × IS 3612 × E 35 -1-52	IS 12622- <i>durra bicolor</i> (Ethiopia) 555 - IS 3687 × Aispuri IS 3612- <i>caudatum</i> (Nigeria) E 35-1-Ethiopian early line	ICRISAT

12	CSV 15	1996	SPV 475 × SPV 462	IS12622- <i>durra bicolor</i> (Ethiopia) 555 - IS 3687 × Aispuri IS 3612- <i>caudatum</i> (Nigeria) E35-1-Ethiopian early line SPV 462- (IS2947 × SPV232) × 1022 IS 2947- <i>caudatum</i> (USA) SPV 232-148 × 512 148 = IS 3687 × Aispuri IS 3687- <i>durra-caudatum</i> (USA) Aispuri (IS1151)- <i>durra</i> (India), a rainy local from Maharashtra 512-derivative of BP 53 (rainy local from Gujarat)	NRCS
13	CSV 17	2002	SPV 946 × SPV 772	SPV 946- See CSV 15 SPV 772- SPV 233 × SPV 350 SPV 233-{(IC 419-1(2077A × 3541) × Merta local SPV 350-{(SC 108-3 × CS 3541)-3-1}	Udaipur

Annexure 3. Pedigree and origin of postrainy season adapted sorghum hybrids released at national level.

S. No.	Hybrid	Year of release	Pedigree of the hybrid	Pedigree of the parental lines of the hybrid	Centre which developed the hybrid
1	CSH 7R	1977	36A×168	36A = CK 60B × PJ8K CK 60B- <i>kafir</i> (USA) PJ8K-Parbhani jowar (rainy)	NRCS
2	CSH 8R	1977	36A × PD3-1-11	36A- CK 60B × PJ8K PD 3-1-11 = temperate US dwarf × BP 53 (Gujarat rainy local)	Parbhani
3	CSH 12R	1986	296A × M148-138	296A- IS 3922 × Karad local. IS 3922- <i>kafir durra</i> -yell end. Karad local- rainy local from Maharashtra M 148-138 = mutant of Maldandi	Dharwad
4	CSH 13R	1991	296A × RS29	RS 29-SC 108 × SPV 126 SC 108-Purdue (USA) SPV 126-tall mutant of CS 3541	NRCS
5	CSH 15R	1995	104A × RS 585	104A = 296B × Swati 296B = IS 3922 × Karad local. IS 3922- <i>kafir durra</i> -yell end. Karad local- rainy local from Maharashtra. Swati = SPV86 × M 35-1 RS 585 (CS 3541 × M 35-1) × Nandyal postrainy local	NRCS
6	CSH 19R	2000	104A× R354	104A- = 296B × Swati R 354-[(SPV-504 (20 KR) × (SPV 504 × R 263)] × R-67-4	Akola

Annexure 4. Pedigree and origin of postrainy season adapted sorghum varieties popular at national level.

S.No	Variety	Year of release	Pedigree of the variety	Pedigree of the parental lines of the variety	Centre which developed the variety
1	M 35-1	1969	Landrace sel. from local maldandi bulk	--	Mohal
2	CSV 7R	1974	IS 2950 × M35-1	IS 2950 = <i>Guinea durra</i> (USA)	NRCS
3	CSV 8R	1979	R24 × R16	R 24 = IS 3687 (<i>kafir</i> -USA) × M 35-1 R 16 = IS 2950 (<i>Guinea-durra</i> , USA) × M 35-1	NRCS
4	Swati	1984	SPV86 × M 35 -1	SPV 86 = R 24 × R 16 R 24 = IS 3687 (<i>kafir</i> - USA) × M 35-1 R 16 = IS 2950 (<i>Guinea-durra</i> , USA) × M 35-1	Rahuri
5	CSV 14R	1992	(M 35-1 × (CS 2947 × CS 2644) × M 35-1	CS 2947 = IS 2947 (<i>kafir</i> - USA) CS 2644 = IS 2644 (<i>durra</i> -India)	NRCS
6	Sel.3	1995	Sel.from Bidar Postrainy local	It is a reselection from Maldandi local	Rahuri
7	CSV 216R	2000	Landrace Sel. from postrainy germplasm Dhulia	Pure line Sel. from Tapi river valley RSLG 112-1-6	Rahuri
8	CSV 18	2005	A selection from cross (CR 4 × IS 18370)	A selection from cross (CR 4 × IS 18370)	Parbhani

Population improvement in sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth important cereal crop in the world after wheat, rice, maize and barley. Of late, it has emerged as 'fuel' crop in addition to its food, feed and fodder utilities. Sorghum is predominantly a self-pollinated crop and development of new varieties is a natural option for crop improvement. However, there is 5 to 15% outcrossing depending upon the nature of genotype and humidity (House 1985), which makes it possible to use population improvement and hybrid development methods to exploit the heterosis. Discovery of genetic male sterility (GMS) (Table 8) and cytoplasmic-nuclear male sterility (CMS) facilitated the application of recurrent selection procedures (population improvement) and hybrid cultivar development methods, respectively, in sorghum improvement programs.

Sorghum breeders have been successful in exploiting available vast genetic variability in sorghum by direct selection among landraces and/or by the use of conventional methods of handling segregating generations derived from carefully and deliberately effected crosses through pedigree and backcross breeding in the development of pure-line varieties and/or hybrid parents. These conventional breeding methods, used as a short-term strategy produce varieties with a relatively narrow genetic base, favor the accumulation of linkage blocks due to rapid fixation of genes, and limit recombination options because of continuous inbreeding. On the other hand, population improvement methods, besides offering greater opportunities for recombination to break linkages between desired and undesired traits, provides scope for increased utilization of biotic and abiotic stress resistant, but agronomically non-elite source germplasm lines. The population improvement provides long-term breeding strategy to derive diverse and broad genetic-based superior varieties/hybrid parents (Bola Nath 1982). Therefore, a comprehensive crop improvement strategy has to combine both short- and long-term programs for continuous improvement of economic traits.

What is population improvement?

A population is a group of plants sharing a common gene pool. Population improvement includes (1) the development of broad genetic-based gene pools and (2) its improvement through recurrent selection methods. Recurrent selection was first suggested by Jenkins (1940) and named by Hull (1945). Recurrent selection methods are most suitable for the improvement of those traits that are inherited in a quantitative manner and the essential features of these methods are i) the improvement of the mean performance of the population by increasing the frequency of the genes that effect trait/traits under selection, ii) appearance of new combination of genotypes that never existed in the base population and iii) maintenance of genetic variability by recombination of superior genotypes for further and continuous improvement.

The recurrent selection methods require extensive hybridization, which is tedious to follow in sorghum owing to its inbreeding system. However, the discovery of genetic male-sterility (GMS) and the advent of various mating systems and reciprocal recurrent selection methods in exploiting additive (A) and A×A and other epistatic genetic variation (Comstock and Robinson 1952, Eberhart 1972), led many breeders to adopt population improvement methods in sorghum in the 1960s (Maunder 1972; Doggett 1972a). Several sources of genetic male sterility have been reported from both India and USA, and in all cases it was shown that a recessive allele in homozygous condition at a number of loci with alleles designated as *ms*₁, *ms*₂, *ms*₃, *ms*₄, *ms*₅, *ms*₆, *ms*₇ and *al* confer male sterility (Table 8). Of these, only *ms*₃ and *ms*₇ alleles have been extensively used in population improvement as they are stable across locations and seasons (Reddy and Stenhouse 1994), although *al* (antherless) allele is also useful (House 1985).

Development of random-mating populations

Populations can be developed for different purposes: for improving a single trait; for selecting several traits simultaneously; and for generating fertility restorer and non-restorer (maintainer) populations for deriving hybrid parents. Whatever the purpose, the development of a population involves three steps: (a) selection of component parents (b) introgression of a GMS gene, and (c) random mating among parents.

Table 8. Genetic male sterility genes, their designated symbols and mechanism of sterility in sorghum. Source: Adapted from Rooney (2000).

Gene symbol	Mechanism	Reference
ms ₁	Normal pollen is dominant over aborted or empty pollen cells	Ayyangar and Ponnaiya (1937)
ms ₂	Normal pollen is dominant over aborted or empty pollen cells	Stephens (1937)
ms ₃	Normal pollen is dominant over aborted or empty pollen cells	Webster (1965)
ms ₄	Empty pollen cells	Ayyangar (1942)
ms ₅	Aborted pollen	Barabas (1962)
ms ₆	Micro-anthers without pollen	Barabas (1962)
ms ₇	Empty pollen cells	Andrews and Webster (1971)
al	Anther less stamens	Karper and Stephens (1936)

Population improvement methods

Once the broad genetic-based populations are developed, their genetic potential can be upgraded theoretically for any number of traits of interest through selection. Selection process is cyclic, with or without progeny testing and a recombination phase involving intercrossing among the selects. The process of selection and the intercrossing among the selects constitute one cycle and many such cycles are repeated and hence the whole process is referred to as recurrent selection. Various population improvement/selection methods have been developed and they could be classified based on the type of population (ie, intra-population and inter-population) and on the units of selection. In intra-population improvement, selection is practiced within a specific population for its improvement while in inter-population improvement; selection is based on the intercross performance between two populations. The unit of selection could be individual plants, half-sib families, full-sib families and selfed/inbred (S₁ and S₂) progenies. Selection based on the visual inspection of individual plant is referred to as phenotypic selection since its genetic worth cannot be known unless the trait under selection has high habitability. On the other hand, selection based on families/progenies is known as genotypic selection, as genetic worth of selected plant is assessed based on its progeny performance. Thus, several intra- and inter-population improvement selection methods are recognized and are described below.

Intra-population improvement methods

Mass selection. Mass selection is the simplest and easiest of all methods and requires the fewest resources and only one generation per cycle. It is effective, particularly if the trait of interest has high heritability. Jan-orn et al. (1976) predicted that mass selection would be effective in improving highly heritable traits like days to flower and plant height in sorghum. It is useful if the population is highly heterogeneous. Mass selection procedures in sorghum have been suggested by Doggett (1968). Mass selection involves selection and recombination of selected plants in a population. A population segregating for male-sterility is planted in isolation. Several superior male-sterile plants are selected based on visual assessment and harvested individually. Equal quantity of seeds from selected plants is bulked to constitute the population for next cycle of selection. Thus, it follows that the unit of selection is individual plant.

Doggett (1972a) has described modified mass selection with alternating male-sterile (female) and male-fertile (male) plants selection in successive generations, aimed at enhancing selection response by increased parental control. In one cycle, seed is harvested from only selected male-sterile plants. These seeds are bulked and sown to constitute the population for the next cycle of selection, wherein, male-fertile plants are selected and harvested seed from selected plants is bulked for selection of male-sterile plants. This procedure is continued. Mass selection should be used in the first few cycles of selection after synthesis of a population. This makes populations reasonably uniform for plant height and maturity before using more sophisticated methods of recurrent selection requiring family/progeny evaluation.

Half-sib family/progeny selection. Half-sib selection requires two generations per cycle since it involves progeny testing. Male-sterile plants are tagged at the time of flowering and are allowed for open-pollination. Each head is harvested and threshed separately. A part of the seeds from each head is sown in yield trial (evaluation phase) and the remaining is saved as remnant seed. The best entries are chosen from the yield trials, and the remnant seed from these entries is bulked to constitute the population for the recombination phase. Along with the replicated yield trial of half-sib families as well as other families (which will be described subsequently) for selection of best families to recombine, a separate nursery is planted simultaneously to identify male-sterile plants. Sib-mated male-sterile heads are harvested and bulked with remnant seed of families selected on the basis of grain yield and other selection criteria as appropriate in a yield trial. This bulk is sown to allow random mating in the next season. Again, male-sterile plants are tagged and harvested individually to form the next cycle of evaluation. This method of selection is known as “Half-sib family selection” because the unit of selection is half-sib families and a breeder has control over only one of the parents,

ie, male-sterile plants (females). Recombination is carried out in the off-season and evaluation in the main season. The method was used with low selection intensities to improve backup populations (eg, Downs Bulk, Brown Population, WABC, Bulk Y and RS5DX) at ICRISAT and progress was made for overall agronomic desirability, grain quality, and in increasing uniformity for plant height and maturity.

Full-sib family/progeny selection. Full-sib families can be developed by crossing selected male-fertile plants onto selected male-sterile plants. The full-sib families so generated are evaluated in a yield trial and the remnant seed of the selected families is then bulked and allowed to recombine. Crosses of male-fertile plants with male-sterile plants are then made and the cycle repeats. In this scheme of selection, the unit of selection is full-sib families and the breeder has the control over both the parents unlike in half-sib family selection.

S₁ family/progeny selection. It is one of the most effective selection schemes for sorghum (Gardner 1972). S₁ family selection requires three generations per cycle. Heads of male-fertile plants are bagged at flowering to ensure selfing, or they can be tagged to ensure that heads from male-fertile plants and not male-sterile plants are harvested at maturity. Selected plants are harvested and threshed separately, each head forming an S₁ family. These families are evaluated in yield trials. Remnant seed from the families selected or their sibbed families based on the yield trials is sown, and seed from male-sterile heads are selected to ensure recombination. Seeds from male-sterile heads are then bulked and sown. Male-fertile heads of good plants are identified for testing to begin next cycle. The units of selection and recombination are S₁ progenies. The basic concept behind selfed progeny selection is to expose deleterious recessive genes to facilitate their elimination during evaluation and to increase additive genetic variation. Doggett (1972b) reported the first evidence of success of this method and observed, on an average, 25% yield increase per cycle. After one cycle, the improved population produced a higher grain yield than the best varieties. Jan-orn et al. (1976) predicted that S₁ family/progeny testing and selection offered the greatest promise for improvement in NP3R, a population developed at the University of Nebraska, whether calculated on a cycle or on an annual basis. S₁ testing is very efficient if three generations can be grown a year. This is possible only with very early maturing populations.

S₂ family/progeny selection. In this scheme of selection process, heads of selected male-fertile plants are bagged at flowering to ensure selfing, or they can be tagged to ensure that heads from male-fertile plants and not male-sterile plants are harvested at maturity. The S₁ progenies are grown and plants in S₁ progenies rows are selected and again selfed. Selected selfed plants are harvested and threshed separately, each head forming an S₂ family. These S₂ families are evaluated in yield trials and handled exactly in a manner similar to that in S₁ progeny selection

and thus selfed/inbred progenies constitute units of selection in both the methods. S_2 progeny testing is expected to result in maximum gain per cycle and is most suitable when two growing seasons are available per year, thus permitting one cycle every two years. The method has several advantages over others: additive genetic variance is maximized in S_2 families; the families are sufficiently uniform to permit precise evaluation; two generations per year provide sufficient time between the generations for sending seed to test locations in a range of environments and analyzing the data for the selection of lines for recombination; selection for different traits can be done in various generations ranging from half-sib to S_2 according to the nature of their inheritance; and the lines evaluated are more homozygous and it is hence easier to extract pure lines. In addition, continuous selfing and evaluation is expected to improve the probability of deriving more vigorous inbred lines. The disadvantage of the scheme lies in the necessity to sib-mate S_2 lines to increase the frequency of male-sterile plants for reconstituting the next cycle of the population.

Testcross family/progeny selection. This selection method is a slight deviation from the concept of intra-population improvement in the sense that the targeted population is improved based on the evaluation of testcross progenies generated by crossing several selected plants of targeted population with a broad based tester population. Thus, the targeted population is not only improved for *per se* performance but also with respect to general combining ability (*gca*). It essentially involves three steps; (1) self-pollination of male-fertile plants and testcrossing them to a broad based tester, (2) evaluation of testcross progenies in a yield trial and (3) intercrossing of selected plants based on yield trial.

A number of male-fertile plants from the base population are selected and selfed and simultaneously crossed to a broad based tester. The resultant testcross progenies are evaluated in a yield trial to identify promising families, the units of selection. The remnant seed of selected testcross progenies is bulked and sown and allowed for open pollination with male-sterile plants. Seeds from male-sterile heads are then bulked and sown. Male-fertile heads of good plants are identified for testcrossing to begin next cycle. The cyclic nature of selection through testcrosses (with broad based tester) from selfed seed increases the frequency of genes conferring good *gca* and gradually improves the population *per se* performance as well. As the population is improved both for *per se* performance as well as *gca*, the probability of deriving vigorous lines with good *gca* is higher. The choice of the type of tester has been extensively debated. While broad based tester improves *gca*, a narrow genetic-based tester such as an inbred improves specific combining ability (*sca*). The use of low yielding testers, presumably with low frequency of favorable alleles at important loci, have been advocated because they are expected to increase variation in testcross progenies and thus allow better discrimination among the plants to be evaluated in testcrosses (Hallauer and Miranda 1981).

Inter-population improvement methods

Half-sib reciprocal recurrent selection. Comstock et al. (1949) first suggested the use of Reciprocal recurrent selection (RRS), which maximizes the genetic divergence between the two populations for loci with dominance and/or over-dominance effects. The main objective of this method is to develop the two populations simultaneously so that superior inbred lines would be extracted that combine well with each other. In this scheme of selection, each population provides a source material to advance/improve and also serves as a tester for the other population. Individual selected male-fertile plants (tag or bag these plants for identification at maturity) in one population, designated as 'A' will be crossed to several random male-sterile plants of the other population designated as 'B'. In a similar manner, several selected male-fertile plants of population 'B' are crossed onto several random male-sterile plants of population 'A'. The crosses thus generated are evaluated in a yield trial and seeds from selected male-fertile plants are bulked and grown in isolation. Incorporate heterozygous male-sterile plants into these populations. Allow for random pollination of male-sterile heads. Mark male-sterile plants at flowering. Harvest seed from male-sterile plants in each population and bulk to constitute the new populations from which male-fertile plants would be selected and crossed to male-sterile plants from the other population and the cycle repeats. This method is useful in sorghum since hybrids are commercially viable and large inter-population heterosis is observed. There are two types of RRS depending on the identity of the parents involved in the crosses; (1) half-sib RRS and (2) full-sib RRS. Half-sib RRS is the most promising in sorghum because it provides a better evaluation of males to be selected. In half-sib RRS method, random male-fertile plants (their identity is not maintained) of one population are crossed onto male-sterile plants of the other population to generate half-sib families for evaluation in yield trial and thus half-sib families form units of selection. The subsequent steps are as described above.

Full-sib RRS. In this method, only selected male-fertile plants of one population (their identity is maintained) are crossed onto several selected male-sterile plants of the other population to generate full-sib families for evaluation in yield trial and thus full-sib families become units of selection. Since identity of both female and male plants is known, this method is called full-sib RRS. The use of RRS in sorghum, especially full-sib RRS, is hampered by the sterility system used to enable random mating. All crosses (test and selection units) would be generated using the male-sterile as the female for which no selfed seed can be produced. Thus, from the selected full-sibs, only the male parents from each cross can be used as recombination units, effectively reducing selection intensity and failing to capture genes from those female parents producing superior crosses.

Reciprocal full-sib selection has been used to improve the sorghum populations KP9BC₀ and GTPP7R, a derivative of TP24. Nearly 200 reciprocal full-sibs from

each population (with the other population as male parent) were tested collaboratively by Kansas State University (KSU) at Garden City and the University of Nuevo Leon in Monterrey, Mexico. The top 15% full-sibs were selected from the best stress site, and remnant S_1 seed of the male parents of the selected full-sibs was used for recombination. Estimates of genetic variances, heritabilities, and intra-population predicted gains were reported by Chisi (1993). Estimates for the genetic variability and mean were found to be consistently higher in the TP24×KP9B (TP24 as female) than the KP9B×TP24 reciprocal crosses, suggesting that significant cytoplasmic effects may exist (Rattunde et al. 1997).

Population improvement programs at ICRISAT

Population improvement programs were followed extensively at ICRISAT during the initial periods of its inception. However, during later periods, it received less emphasis due to changes in funding patterns, donors' emphasis on short-term impact and the requirement of national programs. Population improvement programs at ICRISAT, Patancheru, Andhra Pradesh, India were initiated in 1973 with the introduction of a large number of populations (ICRISAT 1974) from Kansas State, Nebraska and Purdue Universities (USA), Serere (Uganda), Nigeria and from Australia using genetic male-sterility induced by ms_3 and ms_7 genes following recurrent selection procedures to breed for wider adaptability. New backup and advanced populations were synthesized by intercrossing selected progenies from populations of similar maturity, geographic origin, and restoration behavior (Bhola Nath 1977). Backup populations were selected under low selection intensity to maintain variability for a long time. However, the backup populations were later discontinued to reduce the size of the program. The advanced populations were subjected to rigorous selection, with the objective of producing superior varieties and hybrid parents. Initially when resources were abundant, half-sib/ s_1/s_2 family selection methods were followed to improve the populations. However, due to subsequent diminishing resources, simple mass selection alternated with recombination methods became corner stones of developing trait-specific broad genetic-based populations (Reddy et al. 2004). Over the years, an array of 19 populations (Fig. 25) for long-term improvement for key agronomic traits or trait combinations and resistance to major insect pests and diseases were developed using ms_3 and ms_7 genes and improved at ICRISAT, Patancheru (Reddy et al. 2005).

The populations developed by ICRISAT and NARS collaborators in the Southern African region target contrasting agro-ecological zones (ICRISAT 1989, Rattunde et al. 1997). *Guinea* and *caudatum* and *guinea*×*caudatum* populations have been developed in Mali (Rattunde et al. 1997). In Southern Africa, the four random mating populations developed jointly by ICRISAT and national agricultural research systems (NARS) using ms_7 gene provided broad genetic-based gene pools from

which national programs and South African Development Committee (SADC) could develop improved lines and varieties using recurrent selection (Obilana 1989).

Utilization of populations

The economic benefits of population improvement are ultimately realized when readily usable genetic variability for traits of interest from these populations is effectively exploited to develop pure-lines and/or hybrid parents. It is towards this goal that populations are improved by recurrent selection. It is assumed that as the mean performance of a population is improved, there will be a parallel improvement in the performance of its derived progenies (Eberhart 1972). The probability of deriving pure-lines with desired combination of genes is higher from an improved population with higher frequency of desirable alleles. Studies at ICRISAT have given good indications that it will work. A set of random S_1 progenies from different cycles of two populations were evaluated for grain yield in two trials (Prasit 1981). The contribution of the most advanced cycles in each population is the highest, followed by the previous cycle, indicating that as the average grain yield of populations increased, the grain yield of the derived lines also increased.



Fig. 25. A high-tillering forage population developed at ICRISAT, Patancheru.

Traditional pedigree selection methods used during the inbreeding produce pure-lines for direct use as varieties or hybrid parental lines or, more frequently, as improved parental lines for use in pedigree breeding activities. Purging of GMS gene/s from the derived lines is essential if it/they has/have been used for building the populations. Selection against the male-sterile gene can be easily handled by identifying sterile plants at flowering. The lines from the populations are produced by successive selfing of male-fertile plants at any stage until the progeny becomes uniform. Continued selection for male-fertile plants eliminates male sterility from the lines. This approach has been used in the development of lines from S₁ families originating in several populations at Purdue (Rattunde et al. 1997), and in the ongoing derivation of restorer lines and dual-purpose varieties out of the US/R (DP) population at ICRISAT.

At ICRISAT, the process of identifying superior lines began in the early stages of population development. For example, a line from the Diallel population has been released as Melkamash in Ethiopia (Table 9). A foliar resistant line A 2267-2 is derived from US/R population at ICRISAT and is extensively used as one of the parents in sorghum breeding programs in China, Ethiopia and Latin America. Several hundred pairs of cytoplasmic-nuclear male-sterility-based seed parents (A-) and their corresponding maintainer lines (B-) have been developed in an array of maturity and plant types. The male sterile line SPL 132A (renamed as 421A by China) developed from Diallel population at ICRISAT is directly used as female parent in development and release of five hybrids, Liao Za 4, 6, 7 and 10 and Jin Ja 94, in China (Table 9).

Table 9: Examples of successful utilization of populations at ICRISAT.

Variety	Population	Place of release	Released name	Year of release
Diallel Pop-7-682	Diallel	Ethiopia	Melkamash	1982
A 3681	FLR	China	Yuan 1-98	1982
A 3872	Bulk Y	China	Yuan 1-28	1982
A 3895	Bulk Y	China	Yuan 1-505	1982
A 6072	Indian Synthetic	China	Yuan 1-54	1982
SPL 132A (renamed as 421A in China)	Diallel	China	Liao Za -4, -6, -7 and -10 and Jin Ja 94	1988
M 36248	WABC	Myanmar	YEZIN 2 (Schwephyu 2)	1984

Source: Reddy et al. 2004.

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Genetic improvement of sorghum in the semi-arid tropics

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] – a major cereal of the world after wheat, rice, maize and barley, is a staple food for millions of the poorest and most food-insecure people in the Semi-Arid Tropics (SAT) of Africa and Asia. Being a C₄ species with higher photosynthetic ability, and greater nitrogen and water-use efficiency, sorghum is genetically suited to hot and dry agro-ecologies where it is difficult to grow other food crops. These are also the areas subjected to frequent droughts. In many of these agro-ecologies, sorghum is truly a dual-purpose crop; both grain and stover are highly valued products. In Africa, sorghum is predominantly grown for food purposes, while in USA, Australia, China, etc, it is grown for livestock feed and animal fodder purposes. Unlike in other parts of the world, sorghum is grown both in rainy and postrainy seasons in India. While the rainy season sorghum grain is used both for human consumption and livestock feed, postrainy season produce is used primarily for human consumption in India. Thus sorghum is the key for the sustenance of human and livestock populations in SAT areas of the world.

Production constraints

The yield and quality of sorghum is affected by a wide array of biotic (pests and diseases) and abiotic stresses (drought and problematic soils). These are shoot fly (India and Eastern Africa), stem borer (India and Africa), midge (Eastern Africa and Australia) and head bug [India and West and Central Africa (WCA)] among pests; grain mold (all regions), anthracnose (WCA and Northern India) among diseases and *Striga* (all regions in Africa); drought (all regions) and problematic soils – saline (some parts of India and Middle-East countries) and acidic (Latin America) – which together (except saline and acidic soils) cause an estimated total yield loss to the tune of US\$ 3032 million (www.agbiotechnet.com/pdfs/0851995640). The world sorghum productivity is dismally low (0.7 t ha⁻¹) because of these production constraints and the use of traditional cultivars (low-yielding) and traditional production practices during early 1970s.

Genetic improvement

Sorghum has not received wide attention in the scientific community especially in Africa and Asia in the past due to the fact that it is considered a coarse grain and much of its production is at subsistence level. Increased pressures of population growth on food supplies, enhanced utilization of animal products and depleting fossil-fuel reserves has driven attention towards utilizing the full potential of this crop as food, feed, fodder and fuel. Genetic improvement is the cost-effective means of enhancing sorghum productivity for different end-uses. Depending on the production environment and constraints and end-product utilization, the objectives of sorghum improvement programs have been different in different parts of the world. The purpose of this article is to review the sorghum improvement research efforts and its outcomes in different parts of the world.

Sorghum improvement in India

In India, unlike in other countries, sorghum is cultivated in two seasons - *kharif* (rainy) season (June/July-September/October) and *rabi* (postrainy) season (October-December/January). As production environment and production constraints are different, cultivar options are quite different for two seasons (Rana et al. 1997). Initially, development of pure-line varieties using specific adaptation approach was given importance. Several varieties were released within the states. After the discovery of stable and workable cytoplasmic-nuclear male sterility (CMS) system (Stephens and Holland 1954) and as a result of the efforts under the accelerated hybrid sorghum project initiated by the Indian Council of Agricultural Research (ICAR), the first commercial sorghum hybrid CSH 1 was released in 1964. Since then 22 hybrids have been released, some of them (6) with adaptability to postrainy season, which caters to the need of grain for human consumption. CSH 13, a dual-purpose hybrid with high biomass has global adaptability (Rao 1982). The program also released, 13 varieties for rainy season and 8 for postrainy season, but the varieties are not popular with farmers (Reddy and Stenhouse 1994). Considering the potential of sweet sorghum juice as a feedstock for bioethanol production following the Indian Government's initiatives for the production and use of biofuels, considerable progress has been made in the development of sweet sorghum cultivars. The sweet sorghum variety, SSV 84 and a hybrid CSH 22SS have been released for commercial cultivation. Biotechnology tools such as DNA-based markers and genetic transformation have been deployed to address the most intractable and major insect pests, shoot fly and stem borer, in both rainy and postrainy seasons. The Quantitative Trait Loci (QTLs) conferring shoot fly resistance have been identified. Of late, research on production of transgenic sorghums resistant to stem borer using *cry1Ab* gene has gained momentum. Meristem-specific promoters were isolated from sorghum in collaboration with National Research

Centre on Plant Biotechnology (NRCPB), New Delhi, which would help express genes of interest in the shoot meristem to control shoot fly. Intensified research efforts are underway on post-rainy season sorghum improvement for higher grain and fodder yields with acceptable grain quality in both the Indian national program and ICRISAT-Patancheru programs. Efforts are also being continued on the development of *kharif* hybrids and new male sterile seed parents with improved resistance to biotic stresses, and grain qualities through exploitation of diverse germplasm and alternative sources of CMS systems (mainly A₂ and A₄).

Sorghum improvement at ICRISAT

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was established in 1972 at Patancheru, Andhra Pradesh, India with sorghum as one of its five mandate crops. Improvement for yield potential and resistance to drought, *Striga*, grain mold, downy mildew, charcoal rot, shoot fly, stem borer, midge and head bug and wide adaptability received major attention up to 1980. Genetic male-sterility facilitated populations and pure-line varieties were the target cultivars during the initial years. In resistance breeding programs, emphasis was given to developing and standardizing screening techniques and identification and breeding of improved resistance sources. The initial emphasis on red grain types up to 1975 was gradually shifted to white grain types by the end of the 1970s.

During the 1980s, major emphasis was given to regional adaptation and breeding for resistance to biotic stresses (grain molds and insect pests only) in white grain background. Breeding for resistance to drought, downy mildew, charcoal rot and *Striga* was discontinued, while development and improvement of male sterile lines for grain yield and food quality traits were initiated.

Initially, several open-pollinated populations were introduced from the USA, West Africa and East African programs and were reconstituted with selection. A total of 19 improvised trait-specific open-pollinated populations are being maintained at ICRISAT and National Research Centre for Sorghum (NRCS), Hyderabad, India. Several of the hybrid seed parents derived from some of these populations are being extensively used for the development of high-yielding hybrids in China. Later on, several high yielding good grain inbred-lines and *zerazera* landraces (*caudatum*) were extensively involved in breeding at ICRISAT Asia Center (IAC), and *guinea* local landraces along with *caudatum* derived lines at ICRISAT West African center (Reddy and Stenhouse 1994). During 1985–89, major thrust was given for specific adaptation and trait based breeding for resistance traits. A total of 92 high-yielding A-/B-lines, including 17 early-maturity lines and 75 medium maturity lines were developed during this period. By late 1980s many national agricultural research systems (NARS) had enhanced crop improvement programs

aimed at specific adaptation. Thus, the global sorghum improvement program reoriented itself to develop materials suited for 12 productive systems (PS) in Asia, six in Western Africa, six in Eastern and Southern Africa and five in Latin America. As a result of this reorientation, at ICRISAT's Asia center (IAC), strategic research on the development of screening techniques, breeding concepts and methods and intermediate products for utilization in partnership with NARS programs was given emphasis during 1990–1994. A total of 567 trait based A-/B-lines (487 A₁, 51 A₂, 17 A₃ and 12 A₄ CMS systems-based) were developed during 1989–98. Besides these, 57 high yielding A-/B-lines were also developed. In addition, an extensive program of breeding new *Milo* cytoplasm male sterile lines for earliness, introgression with *durra* and *guinea* races, incorporating bold and lustrous grain characters, and resistance to *Striga*, shoot fly, stem borer, midge, head bug, grain mold, downy mildew, anthracnose, leaf blight and rust was carried out (ICRISAT 1993) (Fig. 26). A total of 39 new A-/B-lines (*durra* bold grain 23, *caudatum* 6 and *guinea* 10) based on A₁ cytoplasm and 46 new A-/B-lines (*durra* bold grain 28, *caudatum* 4, *guinea* 5 and *feterita* 9) based on A₂ cytoplasm were developed.



Fig. 26. A rainy season adapted B-line with stem borer resistance.

With a major objective of trait specific breeding, novel populations or trait-specific gene pools for bold grain and high productive tillering were developed. Test crosses involving postrainy season 'landraces' as pollinators were examined for their fertility restoration ability under cool nights and for productivity in postrainy season. Variability for restoration was quite significant indicating the possibility of selection within hybrids (Reddy and Stenhouse 1994).

At ICRISAT, Patancheru a set of 86 diverse sorghum lines involving parental lines of popular hybrids, varieties, yellow endosperm lines, germplasm lines, high digestible protein lines, high lysine lines and waxy lines were evaluated for grain Fe and Zn content.

Significant genetic differences for grain Fe and Zn content and anti-nutritional factors (tannin and phytate content) and agronomic and grain traits were observed. While the grain Fe content ranged from 20.1 ppm (ICSR 93031) to 37.0 ppm (ICSB 472 and 296B) with an average of 28 ppm, grain Zn content ranged from 13.4 ppm (JJ 1041) to 30.5 ppm (IS 1199) with an average of 19 ppm (Fig. 27). Nevertheless, it was evident that substantial genetic variability exists for grain Fe and Zn content and this variation did not appear to be significantly influenced by the environment as reflected from narrow differences between PCV and GCV and high heritabilities. The substantial variability coupled with higher heritability suggests that selection for high Fe and Zn and low tannin and phytate content is effective and hence offers better prospects of breeding Fe and Zn-dense sorghum cultivars in an anti-nutritional factor background.

Sweet sorghum research has been given major emphasis at ICRISAT and 10 new seed parents and 8 restorer lines were developed for use in hybrid development. The sweet sorghum based ethanol technology has become a reality with the establishment of Rusni Distilleries near Hyderabad, India in collaboration with ICRISAT's Agri Business Incubator (ABI). It has become a model for such distilleries all over the world. In collaboration with International Centre for Biosaline Agriculture (ICBA), Dubai and Agriculture Research Station (ARS), Gangavathi, Karnataka, India, ICRISAT has identified 18 lines that are promising under saline conditions. Similarly, improved lines have been developed for fodder quality and quantity and Al³⁺ tolerance. Efforts are underway to tag the QTLs associated with drought tolerance, grain mold resistance and shoot fly resistance.

From 1995 onwards, a partnership mode of conducting research to develop improved intermediate products at ICRISAT, Patancheru, India, and finished products (varieties and hybrids) at other



Fig. 27. ICRISAT-Indian NARS partnership dual purpose sorghum variety.

ICRISAT locations in Africa were being emphasized. Accordingly, the objectives of the program at present are breeding resistant (to biotic and abiotic stresses) seed parents and restorer lines, developing specific new gene pools and novel plant types. However, breeding programs in Africa will continue to develop high yielding cultivars (varieties and hybrids) with resistance to *Striga* and head bug appropriate in the region. At Patancheru center, ICRISAT is giving major emphasis to development of hybrid parents for sweet stalk traits, micronutrient density, salinity tolerance, bold grain types and multicut types.

ICRISAT, working in partnership with NARS, partners helped in release of 200 cultivars in several countries in Asia (53), Africa (112) and Latin America (35).

Sorghum breeding in China

Modern sorghum breeding in China began in the 1920s, and has progressed through three stages: (a) collection, classification and pedigree selection within the best local varieties; (b) cross breeding, using crosses between local varieties or local×exotic for variety production and (c) exploitation of heterosis through the development of single-cross hybrids from inbred parents (Zheng Yang 1997). Heterosis breeding has been the main method of breeding since 1965 in China. The major objectives of sorghum improvement research in China include: grain yield, multiple resistance to abiotic (low temperature and drought) and biotic (aphids and head smut) stresses, grain quality, grain feed and forages for livestock.

Breeding for high yield: Through a program of selecting the best introduced seed parents, and then evaluating Chinese bred pollinators, a series of hybrids have been developed starting in the 1970s. The increase in yield due to utilization of hybrids is estimated at 30–40% with the remaining improvement being due to better cultivation conditions (Zheng Yang 1997).

Breeding for multiple resistance to abiotic and biotic stresses

Low temperature: Low temperature is an important stress factor, especially for northeastern China in both the seedling and grain filling stages. Some cold-tolerant local varieties have been identified using low temperature seedling treatment. (Zheng Yang 1997).

Drought: Selection for rapid seedling emergence rates in water-limited areas was followed to develop cultivars resistant to drought at seedling stage. New male sterile lines and restorers with resistance to drought are being developed using this method of selection (Zheng Yang 1997).

Aphids: Most Chinese sorghum varieties lack genetic resistance to aphids. Several new male sterile lines with high degree of resistance have been developed by the Sorghum Research Institute (SRI), Liaoning Academy of Agricultural Sciences (LAAS) (Zheng Yang 1997).

Head smut: Sorghum head smut caused by the fungus, *Sphacelotheca reliana* is a serious disease. There are three different physiological races of the pathogen in China. Resistance to head smut is controlled by both major genes of 2 or 3 pairs and some minor genes (Yang Zhen and Yang Xiaoguang 1993). Among 10,083 germplasm accessions screened, 39 showed immunity to race 2, while 3 accessions have shown resistance to race 3 (Chen Yue and Shi Yuxue 1993). Based on these sources, resistance breeding is in progress (Zheng Yang 1997).

Breeding for quality

Chinese Kaolings are an excellent source of good grain quality types such as Xiang Yanai and Zhen Zhubai. However, they are not used directly in heterosis breeding due to low combining ability and poor restoration. Nutritive composition of hybrid sorghum grain is poor and the traditional fragrance of original local varieties needs to be recovered in high-yielding background (Zheng Yang 1997).

Grain for feed and forages for livestock

Since sorghum is the main raw material of compound feeds for livestock and poultry, breeding for feed quality has been one of the major objectives of sorghum breeding in China (Shi Yuxue et al. 1992). Most forage sorghums can be classified as dual purpose in China, with the grain for human consumption and stover for other purposes. At present, the focus is on breeding for high biomass coupled with good nutritional value and low Hydrocyanic acid (HCN) content (Zhen Yang 1997).

Breeding for sweet stalks

In China, sweet sorghum is primarily used for silage preparation. With the introduction of improved sweet sorghum varieties such as Rio, Roma, Ramada and Wray from several countries, a systematic sweet sorghum breeding program was initiated in LAAS in 1985. As a result, two sweet sorghum hybrids, Liaosiza No.1 and Liaosiza No.2 were developed and released during 1989 and 1995, respectively.

Sorghum breeding in Africa

The crop is grown as a rainfed crop in diverse environments across tropical and sub-tropical agroecologies in Africa; from extreme lowland arid and semi-arid zones (of Libya, Sahel of West Africa and Botswana) to the sub-humid and humid lowlands (of southern Guinea Savanna of West Africa) and the mid highlands (of Great Lakes Zone of East Africa). The semi-arid and sub-humid highlands are typified by highlands of Ethiopia, Eastern and Central Africa (ECA) and Lesotho (where sorghums are cultivated around Mokhotbog at an altitude of 2400 m). Sorghum breeding began in the late 1930s replacing traditional farmer selection activities. This led to the identification, selection and release of better landraces as “improved local selections”. At the same time, exotic germplasm lines were introduced, adapted and tested. Between 1948 and 1960, useful cultivars, local varieties and exotic germplasm lines were used in hybridization program and initiated pedigree and bulk breeding programs. Population development and its improvement through recurrent selection were possible with availability of genetic male-sterility (ms_3 has been extensively used). Greater prominence was given to wide adaptation and increased productivity. Between 1930 and 1950, a multilateral collaboration in Eastern Africa involving Kenya, Uganda and Tanzania began (Doggett 1988). In the late 1970s, a regional approach to sorghum breeding was initiated as a result of such collaborations. The first of such regional approaches to sorghum breeding was the Organization of African Unity/Scientific Technical and Research Commission (OAU/STRC) Joint Project 31 on Semi-Arid Food Grain Research and Development in Africa (SAFGRAD), which was initiated in 1976. Subsequently, regional sorghum breeding approach began at different periods in three regions - East and Central Africa (ECA), South African Development Community (SADC) and West and Central Africa (WCA). These regional breeding programs were set up with the objective of tackling different production constraints specific to different regions.

East and Central Africa

Sorghum improvement research in eastern Africa began with the collection and screening of local germplasm in Kenya, Uganda and Tanzania (1930–50) (Obilana 2004). Useful local selections were identified; the popular ones are: Dobbs (from western Kenya) and L 28 (from Uganda) (Doggett 1988). With sorghum gaining significance in Uganda and Tanzania, a program to breed for early maturing, white and brown grain ‘bird resistant’ varieties was initiated in Tanzania during 1948. The outcome of this program was the development of brown grain variety, SERENA. The variety was derived from the cross Swazi P1207×Dobbs through pedigree breeding in 1956/57. The sorghum-breeding program in these three countries (Kenya, Uganda and Tanzania) progressed into an East African regional sorghum

improvement program, which started in 1958 at Serere, Uganda. This regional program focused on managing the endemic weed, *Striga* in addition to bird damage in the next two decades (1958–1978). This phase resulted in the development of three varieties, two of which - SEREDO (Serena×CK60) with brown grains, and Lulu-D (SB77×Seredo) with white grain - are still popular in Kenya, Uganda and Tanzania.

Sorghum improvement research in Uganda is based at Serere Agricultural and Animal Production Research Institute (SAAPRI). In collaboration with ICRISAT and International Sorghum and Millet Collaborative Research Support Program (INTSORMIL), and a number of non-governmental organizations (NGOs) operating in the countries, several improved varieties have been released since 1969 such as Serena, Hijack, Himidi, Hibred, Lulu Tall, Lulu dwarf, Dobbs Bora, Seredo and 2Kx17/B/1.

ICRISAT came to the region in 1978 to assist in sorghum improvement; the focus was on the use of selected landraces as parents in hybridization to create variability, and adaptive testing of advanced lines derived from crosses. ICRISAT operated from India and Kenya under a project of the Tanzanian Government. This collaborative research expanded later into two successive regional networks during 1986–1993 - East Africa Regional Cereals and Legumes (EARCAL) network, and the East Africa Regional Sorghum and Millets (EARSAM) network. In 2002, the East and Central Africa Regional Sorghum and Millets (ECARSAM) network was set up. While EARCAL/EARSAM was funded by USAID through the SAFGRAD/ICRISAT collaboration, ECARSAM is funded by the European Union through ASARECA. Between 1993 and 1999, ICRISAT's involvement in East Africa was strengthened with inputs of improved varieties from the Southern Africa Development Community (SADC)/ICRISAT Sorghum and Millet Improvement Program (SMIP). Collaborative adaptive testing, both on-station and on-farm in Ethiopia during 1995–2000 has resulted in the release of five sorghum varieties for production in the western lowlands (ICSV 210, PP 290), central mid-highlands (IS 29415) and eastern lowland Wadi (89MW 5003, 89MW 5056). The varieties, Serena and Seredo are popularly used in mixtures with finger millet for making thin porridge, and with cassava flour for ugali, in the Great Lakes Region.

The two countries, Ethiopia and Sudan can be regarded as the strongest in the region for sorghum improvement. In line with the Ethiopian Government's policy guidelines, the Institute of Agricultural Research (IAR) organizes sorghum improvement research in a team approach in Ethiopia. For the purpose of sorghum improvement research in Ethiopia, four adaptation zones of sorghum are recognized. The zones are classified as: highlands (altitude of >1900 m) with about 800 mm rainfall; intermediate (altitude of 1600–1900 m), with more than 1000 mm rainfall; lowlands (altitude of <1600 m) with low rainfall, less than 600 mm; and low

land (altitude of <1600 m) with high rainfall (Gebrekidan 1981). Each of the four distinct zones of adaptation requires specific type of sorghum to match with agro-ecological conditions and which cater to the needs of the farmers and the end users. However, due to the shortage of trained human resources and the inadequate research infrastructure, only one coordinated national breeding program operating from Nazret/Melkassa Research Center is responsible for the identification and development of improved varieties/hybrids resistant to anthracnose, ergot, grain mold stalk borer (*Buseola fusca*), shoot fly in late-planted sorghum, *Striga* and frost before grain filling and improved management practices suitable to all the four adaptation zones in order to increase the productivity levels. To assist the Nazret/Melkassa Research Center, several technology testing centers representing each of the four adaptation zones have been set up. In addition to the Research Center at Nazret, Werer Research Center, low land irrigated center, serves as an off-season program site. The primary objective of this center is to seed increase of breeding lines selections and promising varieties and hybrids for the ensuing rainy season. Over the years, this center developed sorghum lines from indigenous germplasm lines and from introduced advanced breeding lines and recommended/released several cultivars (Debelo et al. 1995). EARSAM, the Ethiopian national program took the lead in developing large-scale field screening techniques for resistance to the major diseases such as ergot, anthracnose and grain mold and several resistant genotypes have been identified. These screening techniques and resistance sources have enhanced the pace of developing varieties and hybrids resistant to the major diseases.

The Ethiopian Sorghum Improvement Program (ESIP) started in full-scale in 1973. The program also served as home for the popular *zerazera* (*caudatum* race) type sorghums, which were extensively used as parents in ICRISAT sorghum improvement programs until the 1990s. Nationally, the ESIP made good progress with release of the varieties, Awash 1050, the popular ETS series, and Gambella 1107 (E 35-1) that has been widely used in ICRISAT breeding programs (Reddy et al. 2004a).

The sorghum research in Sudan dates back to early 1940s. Initially, crop improvement through breeding and crop husbandry research was given greater emphasis but later focus was shifted to more adaptive on-farm research. Sorghum research included genetic improvement for yields and grain quality and resistance to major production constraints such as *Striga*, and post-harvest handling and utilization. During early 1940s, sorghum research concentrated on collection and evaluation of local and exotic germplasm. The full-fledged sorghum improvement program was initiated in 1952 in the central rainfed research station at Tozi in Sudan (Ibrahim et al. 1995). A program for hybrid breeding was started by the Arid Lands Agricultural Development (ALAD) Project in collaboration with the

Agricultural Research Corporation (ARC) of Sudan in the 1970s. In 1977, the ICRISAT/Sudan Cooperative Sorghum/Millet Improvement Program was initiated. The most significant outcome of these collaborative research activities is the release of a commercial hybrid, Hageen Durra 1 (Tx623A×karper 1597) by ICRISAT and Sudan Agricultural Research Corporation (ARC) in 1983 (Doggett 1988, Ejeta 1986). Between mid 1970s and early 1990s, ARC has released many improved cultivars with yield advantages of 10% to 70% for commercial production in both irrigated and rainfed systems (Babiker et al. 1995).

South African Development Community (SADC)

Sorghum research in the Southern Africa Development Community (SADC) region, mainly in South Africa and Botswana, began before the Second World War with emphasis on selections within landraces, bird resistance, and resistance to *Striga* and drought. These activities spilled over into Zimbabwe and Zambia with diversified focus on hybrid development and production. As early as 1940, converted sorghum genotypes, especially combined *kafirs* and the white grain male-sterile lines were introduced into South Africa. The entry of private seed companies led to the commercialization of sorghum for industrial use such as in 'opaque beer' and malting for foods and drinks. In South Africa, selections from landraces included the then well known Red Swazi, which is still one of the earliest maturing (90–95 days) variety in the region and Framida, selected for *Striga* resistance from an introduced Chadian/Nigerian landrace. The male parent (Red Nyoni) of the most popular hybrid, DC 75 known for its opaque beer brewing quality is a landrace selection, which is popular in Zimbabwe and Zambia. Red Nyoni was selected from the improved landrace, Red Swazi in Zimbabwe (Doggett 1988). One of the most popular and widely grown sorghum varieties in Botswana and the rest of the Southern Africa region, Segolane, was also selected from landraces. Among the other varieties released earlier in Botswana and derived from the introduced *kafirs* from USA are 8D and 65D (Saunders 1942).

The cyclic occurrences of severe droughts in the late 70s in the region, led to the heads of States of SADCC (Southern Africa Development Conference Community) to deliberate on interventions to minimize the effect of drought. This led to the establishment of SADC/ICRISAT Sorghum and Millet Improvement Program (SMIP) in 1983/84. Sorghum improvement in SMIP has used a regional, collaborative, and multidisciplinary approach since its inception. In the 15-year period from 1983/84 to 1997/98, improved varieties and hybrids were developed, tested on-station and on-farm and released by the national agricultural research system (NARS) of the eight countries in the SADC region. The major objectives of sorghum improvement research in the region include: development of high yielding and early maturing dual purpose varieties with resistance to drought, downy mildew, leaf blight, sooty

stripe and *Striga*. Apart from these, the grains were also evaluated for food, malting and feed qualities.

More than 12,000 sorghum germplasm accessions were assembled from all over the world and made accessible to NARS for sorghum improvement. From these 10,075 improved breeding lines, 4634 improved varieties, 379 hybrid parents and 3436 experimental hybrids were developed and supplied to Angola (100), Botswana (2398), Lesotho (681), Malawi (1449), Mozambique (322), Namibia (139), South Africa (147), Swaziland (326), Tanzania (3702), Zambia (5330) and Zimbabwe (3930). A total of 27 varieties and hybrids were released in eight SADC countries: Botswana (three varieties and first white grain hybrid in the region), Malawi (two varieties), Mozambique (three varieties), Namibia (one variety), Swaziland (three varieties), Tanzania (two varieties), Zambia (three varieties and three hybrids), and Zimbabwe (five varieties and one hybrid). However, of these 27 released varieties and hybrids, only 9 (33%) are cultivated on about 20–30% of the sorghum area in six countries. Five sources of resistance to three *Striga* species were identified (Obilana et al. 1988, Obilana et al. 1991). Twenty-three drought-tolerant male parents (R-lines) and 36 female parents (A-lines) with their maintainer (B-lines) parents were developed and are presently being used by South Africa, Tanzania, Zambia and Zimbabwe in their hybrid development programs (Obilana 1998). The variety Macia proved most popular in the region, having been released in five SADC countries (latest was in Tanzania in 1999) and is increasing in hectareage.

West and Central Africa (WCA)

The West and Central Africa (WCA) region is the largest and most important sorghum production area in Africa. The sorghum crop in WCA is essentially rain-fed, and its cultivation extends from latitude 8°N to 14°N typified by varied agro-climatic zones of humid (Southern Guinea Savanna) and sub-humid (Northern Guinea Savanna) to semi-arid (Sudan Savanna) and arid (Sudano-Sahelian) conditions, from south to north. These agro-climatic zones are characterized by sharply varying rainfall, temperatures and soil conditions, ranging from high rainfall (600–1200 mm) in the Guinea Savannas to low rainfall (250–600 mm) and very high temperatures in the Sudan and Sudano-Sahelian zones. A combination of these with varying day length periods demands sorghum varieties with different maturity photoperiod sensitivities. Also, the production constraints and adaptations requirements vary with agro-climatic zone.

Before 1940, there was no account of sorghum breeding research work in WCA. However, by the early 1950s, local landraces were collected and selections were made in Burkina Faso, Cameroon, Mali, Niger and Nigeria. In Nigeria, the landraces were initially grouped into four main types-namely *Guinea*, Kaura (mostly yellow

endosperm types of *durra-caudatum* hybrid race), Farafara (white grain type of the race *durra*), and *caudatum* types (Curtis 1967). Several selections were made, most popular of which were the Warsha type sorghums, short Kaura and Janjare from Niger and Nigeria, and Muskwaris/Masakwa (transplanted sorghums in vertisols and hydromorphic soils) sorghum from Lake Chad and the inland delta of the river Niger in Mali. By 1966, exotic materials were introduced and tested, and pedigree-breeding programs began from the derivatives of local×local, local×exotic and exotic×exotic crosses. In the next 10–15 years, ie, by 1971–84, several improved pure line varieties and hybrids were developed, tested and released.

In Nigeria, before 1970s, the hybrids directly introduced from USA and India failed to make a dent to boost sorghum productivity due to their poor adaptation. Therefore, the exotic seed parents were crossed with local breeding lines to develop male-sterile lines from 1970 onwards. From 1977, testing of large number of hybrids involving three (RCFA, ISNIA and Kurgi A) of the four locally developed male sterile lines and improved and released varieties was intensified (Obilana 1982b). Of these, five hybrids (SSH 1, SSH 2, SSH 3, SSH 4, SSH 5) were identified as promising (Obilana 1982a). Similar efforts in Niger resulted in the development and production of the hybrid, NAD-1 by 1989.

ICRISAT's involvement in sorghum breeding in West and Central Africa began in 1979 with the establishment of centers at Kamboinse and Ougadougou in Burkina Faso; later shifted to Niamey in Niger, and now operating from Samanko in Mali since 1985. ICRISAT's genetic enhancement work in West Africa was preceded by Institut de Recherche Agronomiques Tropicales (IRAT's) involvement in francophone territories from 1964. Selection from segregating materials (derived from exotic×local crosses) and exotic germplasm introduction were the focus of both programs. ICRISAT was also involved in population improvement for grain food quality among *guinea* sorghums in Sotuba and Samanko, Mali. Breeding for *Striga* resistance was also initiated in 1979 in Burkina Faso by ICRISAT. One of the achievements from the joint presence of ICRISAT and IRAT in West Africa is the development of the variety, IRAT 204, derived from a IRAT 11×IS 12610 cross. IRAT 11 is a derivative from Senegal local (Hadien-kori)×Niger local (Mourmoure) cross. IS 12610 is an Ethiopian germplasm accession from ICRISAT genebank.

Improved varieties with good malt and brewing clear lager beer qualities were developed and released in Nigeria, during 1980s (Obilana 1985). Collaborative grain quality testing including malting quality and proximate analysis, between Institute for Agricultural Research (IAR), Ahmadu Bello University (ABU), Samaru, Zaria, and the Federal Institute for Industrial Research (FIIRO), Oshodi, Lagos (Obilana and Olaniyi 1983, FIIRO 1986) led to the identification of SK 5912, the best malting sorghum. These were followed by a series of pilot brewing and test

marketing of lager beer made of sorghum malt (barley malt was replaced in ratios of 25%, 50%, 75% and 100% by sorghum malt) in 1984 in collaboration with three breweries. Following positive outcomes from acceptability, quality testing and successful marketing of the 100% sorghum malt, the government of Nigeria banned import of barley malt in 1988, thus saving more than US\$100 million foreign exchange. The federal Nigerian government installed a brewing industry with a production capacity of 18 million hectoliters of beer in 1988 (Bogunjoko 1992). A spillover effect of this impact is the establishment of intermediate malt industries. This led to quantum increase of sorghum malt and sorghum malt syrup production by beverage industries producing malt drinks (maltina, malta and Amstel) by major breweries and beverage companies in Nigeria (eg, Cadbury Ltd., Lagos). Another spillover impact of breeding and selection of varieties suitable for brewing malt is the use of sorghum malt in composite flour with wheat and maize, as weaning foods (Murty et al. 1997). A white-grain sorghum variety ICSV 400 with good brewing qualities was identified by Nigerian and ICRISAT scientists (Murty et al. 1997). Using ICRISAT material, a total of 112 varieties have been released in different countries in Africa.

INTSORMIL

The International Sorghum and Millet (INTSORMIL) Collaborative Research Support Program (CRSP, pronounced “crisp”) located at the University of Nebraska, began in 1979 and includes the participation of seven US universities (University of Illinois, Kansas State University, Mississippi State University, University of Nebraska, Purdue University, Texas A&M University and West Texas A&M University) and the USDA/ARS, as well as research institutions in the US and collaborating countries. The INTSORMIL CRSP, or simply INTSORMIL, is a research organization focused on education, mentoring and collaboration with host country scientists in developing new technologies to improve sorghum and pearl millet production and utilization worldwide. The results of the research are of benefit to both the United States and collaborating countries. Drought tolerance and disease tolerance bred into US lines of sorghum developed by INTSORMIL researchers have been incorporated into lines of these crops in Africa and Latin America, improving crop production and fighting hunger in those areas.

Researchers of the Sorghum/Millet (INTSORMIL) CRSP have developed a rapid non-destructive bioassay for assessing *Striga* resistance, and new genes found to have stable *Striga* resistance are being bred into improved sorghum varieties. More than nine *Striga*-resistant varieties of sorghum have been tested on farms throughout the African continent, and multiplication of well-adapted varieties is in progress. A new discovery from INTSORMIL funded research at Purdue University is a sorghum grain type with high protein digestibility. More easily digestible sorghum

is expected to improve human nutrition, particularly in Africa and India, and has the potential to improve the nutrition of livestock, both in the US and elsewhere. Digestibility affects the value of sorghum as forage for livestock. The *brown-midrib* (*bmr*) trait in some lines of sorghum developed by INTSORMIL researchers provides greater digestibility than normal forage sorghums.

bmr Sorghum-Sudan grass forage: The first commercial production of NutriPlus *bmr* sorghum×Sudan grass forage hybrid, based on *bmr* sorghum mutants resulting from research by INTSORMIL scientists, was in 1996. This *bmr* hybrid has shown an 18.9% average increase in feed value, compared to normal sorghum×Sudan grass hybrids. Improved nutritional quality of sorghum for livestock forage is another benefit of INTSORMIL research, which through commercialization is providing value to the farmer.

bmr Sorghum forage: In Nebraska, research has shown that *bmr* forage sorghum outperformed standard sorghum hybrids and may be equal to some corn hybrids when used to feed dairy cows. *bmr* sorghum silage was shown to be 10% more digestible than regular sorghum silage. Palatability and feed intake of cows fed *bmr* silage were comparable to corn silage diets.

Latin America

In Latin America, sorghum is produced on intermediate to large farms except in some inland valleys and eroded mountain slopes of Central America utilizing hybrids imported from USA and cultivars developed in the regions. In Guatemala, El Salvador, Honduras, Nicaragua and Haiti, a large part of the production is on small subsistence holdings often less than one hectare size where farmers use photoperiod-sensitive landraces intercropped with maize and beans using traditional production practices. The damages due to downy mildew, anthracnose, grain mold, stem borer and midge among the biotic stresses and soil acidity and alkalinity, drought and cold temperature among the abiotic stresses are the major yield constraints apart from the lack of early-maturing, tropically adapted cultivars with high yield potential and tolerance to major stresses in the region. ICRISAT initiated the Latin America and Caribbean Program in 1976 by stationing its staff at International Wheat and Maize Improvement Center (CIMMYT), Mexico. The program was aimed at developing early, dwarf and bold grain varieties for fertile soils in both the highlands and lowlands of Central America. The program was later transformed as Latin American Sorghum Improvement Program (LASIP) in 1990. LASIP had a comparative advantage in the development of tropically adapted improved germplasm resistant/tolerant to major production constraints for food-grade cultivars in Latin America. Several varieties were released and adopted based on ICRISAT-bred improved germplasm. Due to funding constraints, LASIP was

discontinued in 1993. However, considering the interest shown by Latin American NARS, a program for improving sorghum for acid soil tolerance was initiated in 1996 with funding support from Inter American Development Bank (IADB).

The INTSORMIL program identified 20 acid soil tolerant lines in the 1980s (Gourley 1991), but they were susceptible to leaf diseases. At its centers in India and Africa, ICRISAT has developed diverse sets of high-yielding sorghum breeding lines useful as base materials for testing in acid soils of Latin America. Since 1996, ICRISAT, International Center for Tropical Agricultural (CIAT) and the national programs of Brazil, Colombia, Honduras and Venezuela have jointly implemented an IADB-funded project on “A research and network strategy for sustainable sorghum production systems for Latin America”. The major objectives of this project include: (1) to assemble, multiply and evaluate grain and forage sorghum breeding lines for tolerance to acid soils and resistance to foliar diseases, (2) to develop a research network of scientists working on this crop in the region and train them in sorghum research, and (3) to test the most promising genotypes in the target production systems.

A diverse set of 378 pairs of grain sorghum A-/B-lines, 784 grain sorghum R-lines/ varieties and 94 forage sorghum lines were introduced into Colombia in October 1995 from ICRISAT-Patancheru. In addition to these, male sterility inducing gene (ms_3)-based two-grain sorghum populations (ICSP LG-large grain and ICSP B-maintainer) and one forage sorghum population (ICSP HT-high tillering) developed at ICRISAT were introduced. These introductions were tested empirically for grain and forage under acid soil conditions and fifteen grain sorghum A-/B-lines were selected for high yield, resistance to leaf diseases and tolerance to acid soils and twenty-one R-lines (on A_1 cytoplasm) for high yield under acid soils (Reddy et al. 2004a). Besides these, four forage lines (IS 31496, IS 13868, ICSR 93024-1 and ICSR 93024-2) were selected for tolerance to acid soils.

In the back-up breeding program, ICSP LG-large grain and ICSP B-maintainer populations were merged and selected alternatively at CIAT farm under neutral pH, and at Matazul under acid soil conditions. Some of the selections (male-fertiles) were advanced through pedigree breeding. Several promising progenies were also selected from the segregating materials of the specific crosses made among the lines selected for acid-soil tolerance and less susceptible to foliar diseases. Nearly 200 hybrids involving selected A- and R-lines and INTSORMIL R-lines were evaluated at Matazul (60% Al^{3+} and 4.6% organic matter). These hybrids produced more than 5 t ha^{-1} grain yield while the Al^{3+} tolerant check Real 60 yielded 4 t ha^{-1} . These are less susceptible to leaf diseases, greener at maturity, and also taller than the check Real 60 (Reddy et al. 2004b).

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Genetic improvement of forage sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is grown extensively throughout the world owing to its wider adaptability to varied soil and environmental conditions and utility as food, feed, fodder and fuel. Sorghum, being a C₄ crop, is well suited to hot and dry agro-climates providing food and fodder security to the world's resource-poor people.

Mixed crop and livestock farming systems are mostly prevalent in India. Therefore, both grain and fodder are of equal importance, underscoring the interdependence of crops and livestock. Sorghum is an ideal forage crop due to its quick growth, high stalk yielding ability (35–40 t ha⁻¹ and 70–75 t ha⁻¹ of green fodder from single cut and multi-cut varieties/hybrids, respectively), high dry matter content (25–30%), better quality (crude protein 5–9%) and its suitability for various forms of utilization like green chop, silage and hay (Fig. 28). The stage of crop growth is the most important factor influencing the quality and quantity of forage produced. Maximum green fodder with highest nutritive value is obtained at 50% flowering stage.

The major objectives in forage sorghum breeding are to develop cultivars both for single cut and multi-cut with high tonnage, better quality, good seed yield and resistance to insect pests and diseases. Several morphological and biochemical traits have been determined for selection to improve adaptability, yield and quality of fodder as well as grain, and resistance to stresses. The effective sources of the desired characters for full expression in the suitable genetic background are also being identified.

Forage sorghums differ widely in chemical composition and nutritive value, both of which are genetically controlled. Some of the characters for improving the fodder yield and quality include: higher leaf/stem ratio (better digestibility), stalk sweetness (more cell solubles—nitrogen and sugars), brown midrib (high digestibility of the vegetative material due to low lignin), tan plant color (low polyphenol content and greater resistance to foliar diseases), bloomless (aid rumen degradation), glossiness (to reduce wax load on leaf surface), low tannin content (reduce interference with protein digestion) low HCN-p (minimize prussic acid poisoning)



Fig. 28. A forage variety developed at ICRISAT, Patancheru.

and dry stem (more pithy cortex and easier digestibility). Although most forage quality parameters are quantitatively inherited, several simply inherited qualitative characters have significant effect on forage quality such as brown midrib.

The main objectives of forage sorghum improvement are to develop high fodder yielding, disease and pest-resistant cultivars suited to single cut and multi-cut systems of fodder production under rainfed and irrigated environments. Breeding for high digestibility in forage sorghum would improve animal intake, growth rate and milk production.

Desired traits in different types of forage purpose sorghum

Multi-cut type varieties and hybrids

- Higher fodder yield
- High regeneration potential- tillering, thin stem
- Superior fodder quality- more protein, less lignin, sweet stalk
- Lower HCN potential
- Drought, insect and disease tolerance

Single-cut type varieties (harvested for fodder at 50% flowering)

- Higher fodder yield
- Fast growth (more fodder yield/day)
- Superior fodder quality- more protein, less lignin, sweet stalk
- Lower HCN potential
- Insect and disease tolerance

Dual-purpose varieties and hybrids (harvested at grain maturity or later)

- High biomass–higher fodder and grain yield
- Superior quality of grain and stover- more protein and starch in grain; more protein and less lignin in stover
- Drought, insect and disease tolerance

Major constraints for forage sorghum cultivation

- Drought stress – pre-anthesis and post-anthesis stress
- Insect pests – shoot fly, stem borer, aphids and headbug
- Foliar diseases – anthracnose, downy mildew, zonate leaf spot, grey leaf spot, sooty stripe and rust
- Other diseases – grain mold, ergot and smut
- Birds, small animals

Early forage sorghum varieties like Haryana Chari, SC 136 (single-cut) and Meethi Sudan SSG 59-3 (multi-cut) were developed at Hisar Centre. The multi-cut variety, Meethi Sudan, with a potential of four cuts, became popular due to its high green foliage, yield potential, regeneration and excellent forage attributes. Subsequently, another ten varieties, namely MP Chari, UP Chari 1, UP Chari 2, RC 1, RC 2, PC 9, PC 23, HC 171, HC 260 and HC 308 were developed for all India cultivation and a few other varieties released at State-level provided the base for varietal transformation in forage sorghum. The latest varieties possess improvement in terms of resistance to leaf spot diseases and stem borer and seed yield. Seed yield improved up to 14 to 15 q/ha under North Indian conditions. These varieties also exhibited higher per day productivity and improved in vitro dry matter digestibility (IVDMD), dry matter digestibility (DMD) and total soluble sugars (TSS) with comparable protein content. Release of dual purpose sweet (TSS 16%) and stay green variety CSV 15 for green forage production in North West India is expected to ensure better seed supply and nutrition to livestock.

Forage sorghum hybrids

Development of multi-cut forage sorghum hybrid is still to have an impact on forage production, though intensive work is underway at a few centers. A good amount

of heterosis has been reported for almost all the traits contributing to grain as well as fodder yield. However, sorghum hybrids with high forage yield are yet to be developed. Some multi-cut forage sorghum hybrids from both public and private sectors have been released for cultivation, which exhibited 15–20% superiority over the multicut check variety.

Future breeding strategies for the development of forage sorghum hybrids

Development of tall hybrids which will satisfy both feed and fodder needs to be addressed through the identification of suitable restorers that will give tall hybrids with resistance to lodging. Identification of male sterile lines with synchronous flowering and restorers with wider adaptability will eliminate the problem of staggered sowing for hybrid seed production. The following approaches are needed to achieve the set objectives:

- Evaluation of forage value of elite cultivars, male sterile lines and dual-purpose breeding lines
- Development of male-sterile lines with diverse cytoplasmic background suitable for forage hybrid production
- Breeding Sudan grass and sorghum restorer lines with profuse tillering, better regeneration and faster growth
- Synchronous flowering and high seed production potential of parents
- Breeding high yielding and better quality forage hybrids having resistance to pests and diseases
- Developing early maturing hybrids to escape terminal drought

Keeping in view the extent of heterosis and nature of genetic component for forage yield and quality and availability of diverse sources of male-sterile lines and pollinators, developing forage hybrids would help in achieving quantum jump in production per unit area and per unit production of quality fodder. Salient features of different types of forage sorghum varieties and hybrids released for commercial cultivation in India are furnished in Tables 10, 11 and 12.

Table 10: Single-cut varieties released.

S. No.	Variety	Fodder yield (q ha ⁻¹)		Characteristics
		Green	Dry	
1	Pusa Chari-6	440	165	It flowers in 85-90 days. Stem is medium thick, non-sweet, pithy and non-juicy. Leaves are medium long and broad with white midrib. Panicle is semi-compact. Seeds are medium bold and white. It matures for seed in 135-140 days and gives 8-9 q ha ⁻¹ seed.
2	HC-136	550	175	It is tall, sweet, juicy and medium thick stemmed. The leaves are very broad and long with green midrib and remain green up to maturity. It has high protein, low toxic constituents and better digestibility. It is also good in regeneration and yields 800-850 q green fodder and 260-280 q dry matter per ha in two cuts. It matures for seed in 140 days.
3	Jawahar Chari- 6 (JC-6)	412	120	Tall stem, thick juicy and non-sweet. Leaves are broad and long with white midrib. It is tolerant to leaf spot diseases. Panicle is semi-lax. It flowers in 80 days and matures in 130-135 days. Grains are bold, pearly white with reddish brown glumes.
4	UP Chari-1	330	90	It flowers in 75-80 days and thus becomes ready for fodder. Its stem is medium thick, sweet and juicy. Leaves are dark green, long and broad with green midrib. Panicle is medium in size, flat and chalky white. It matures in 115-120 days for seed and yields 8-10 q ha ⁻¹ seed.
5	UP Chari - 2	380	125	It flowers in 75-80 days. It is medium thick stemmed, tan colored and juicy. Leaves are very broad and long having light green color. Panicle is oval-shaped, medium in size and compact. Grains are medium bold, round and pearly white in color. It matures for seed in 110 days and gives 10-12 q ha ⁻¹ seed yield.

Contd.

Table 10: Single-cut varieties released.

S. No. Variety	Fodder yield (q ha ⁻¹)		Characteristics
	Green	Dry	
6 Pusa Chari -9	425	135	It flowers in 80–85 days. It is medium thick stemmed, non-sweet and pithy. Leaves are long and medium broad with midrib. Panicles are semi-loose, straight but sometimes goose necked. It matures in 120 days and yields 8 q ha ⁻¹ .
7 Rajasthan Chari-1 (RC-1)	450	125	It flowers in 80–85 days. Its stem is medium thick, leaves long and dark green with white midrib. Earheads are oblong, fully exerted from the flag leaf. Grains are bold and chalky white. It matures for seed in 110–115 days.
8 Rajasthan Chari - 2 (RC-2)	330	100	It flowers in 60–70 days. Its stem is medium thick, leaves light green having 60–70 cm length and 5–6 cm breadth with white midrib. Plants have 11–13 leaves that droop. Earheads are fully exerted from the leaf. Grains are bold, flat and chalky white. It matures for seed in 100–150 days.
9 HC 171	410	122	Its stem is sweet and juicy and leaves have green midrib. Panicles are semi-compact with small and creamy white seeds. It is highly resistant to most of the foliar diseases being tan pigmented. It is highly resistant to mites.
10 HC 260	450	140	It becomes ready for fodder in 55–60 days and matures for seed in about 85–90 days. It is non-sweet and juicy. Leaves are medium broad and long with white midrib. Its panicles are semi-compact with white seeds. It is resistant to foliar diseases. It gives good seed.
11 Haryana Chari-6 (HC-308)	415	128	Tall, leafy and medium maturity, stem is sweet and juicy, highly resistant to all foliar diseases, midrib green, panicle semi-compact.

Table 11: Multi-cut hybrids and varieties released.

S. No.	Variety/ hybrid	Fodder yield (q ha ⁻¹)		Characteristics
		Green	Dry	
1	MP Chari (variety)	300	95	Tall, thin stemmed, leaves medium long and narrow with white midrib. Non-sweet, non-tan, non-juicy and pithy. Takes 65-70 days to become ready for fodder. Panicles are erect and lax with purple or black glumes. Grains are brown colored. Better regeneration ability up to two cuttings. Crop matures for seed in about 110 days. Leaf spot disease susceptible.
2	Meethi Sudan SSG 59-3 (variety)	570	138	A popular multi-cut Sudan grass, suitable for 4 cuts for green fodder during the crop season. It is thin stemmed, sweet with profuse tillering early flower in 55-60 days. Tolerant to drought and water logging. Panicle is lax with profuse lateral spikes. Seeds purple red colored with glumes adhered to them. Matures in 95-100 days and yields 10-12 q ha ⁻¹ seed.
3	Jawahar Chari - 69 (JC-69) (variety)	500	165	A multi-cut Sudan type having high regeneration, and faster growth and multi-cut ability. Its stem is thin and non-sweet and leaves are narrow and long with white midrib. Its panicle is highly lax with small seeds covered with black or brown glumes.
4	Pusa Chari 23 (variety)	550	160	A multi-cut variety with thin stem, Sudan type non-sweet and non-juicy. Leaves narrow with white midrib. Plants have narrow leaves with white midrib. Plants have lateral branches. Seed yield 14 q ha ⁻¹ under North Indian conditions.
5	Hara Sona (hybrid)	630	147	Multi-cut forage hybrid, 3 cuts, 5-6 tillers, days to flower 60-65, 9-10 leaves, quick in regeneration, thin stem, non-juicy, non-tan and palatable. It is recommended for Punjab state.
6	Punjab Sudex (hybrid)	592	170	Multi-cut hybrid 3-4 cuts, 8-10 tillers under irrigation, takes 60-64 days for flowering, 10 leaves, profuse tillering habit, quick regeneration, thin stem, non-juicy, non-tan and palatable. It is recommended for Punjab state.
7	PCH 106 (hybrid)	640	180	Multi-cut forage hybrid (Pedigree 2219A×PC 23), suitable for 3-4 cuts, profuse tillering, quick regeneration, thin stem, non-tan, flowers in 60-63 days. Recommended for all sorghum growing areas in the country.
8	CSH 20 MF	742	198	Tan, dark green heavy foliage with green midrib. Medium thick juicy stem, resistant to foliar diseases. Suitable for all sorghum growing areas in India.

Table 12: Dual-purpose hybrids and varieties released.

S. No.	Variety/hybrid	Fodder yield (q ha ⁻¹)		Characteristics
		Green	Dry	
1	CSV 15 (as fodder variety)	440	128	It is a dual-purpose variety but can be grown as single cut forage variety in North Western India. It is tall, juicy with sweet stem, flowering in 68–70 days, tan, stay green, resistant to leaf spot diseases. Tolerant to grain mold and drought. Its seed can be multiplied in Bundelkhand and Northern MP as a dual-purpose variety and sold as fodder.
2	CSH 13 (as fodder hybrid)	480	145	Green forage yield as single cut, 10% higher than HC 6, tan, resistant to leaf spot diseases, juicy stem, early vigor, stay green as multi-cut, gives more yield in first cut, flowering in 68–70 days.

Alternative cytoplasmic male sterility systems in sorghum and their utilization

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Introduction

In sorghum, Stephen and Holland (1954) discovered cytoplasmic male sterility (CMS) designated as *milo* cytoplasm in the progenies of a cross between two cultivars, *milo* and combine *kafir*, with *milo* as the female and *kafir* as the male. Male-sterile plants to the extent of 25% were observed in the F₂ generation of the above cross when *milo* was used as female and not as male. The male-sterile segregants from this cross produced male-sterile hybrids when crossed with the *kafir* parent and fully fertile hybrids when crossed with the *milo* parent. Thus, it was recognized that *kafir* could be used as a maintainer of this source of CMS. Since the progeny received the cytoplasm from the female, it was hypothesized that the *milo* parent had a male sterility-inducing cytoplasm and dominant genes for pollen fertility, whereas the combine *kafir* parent contained a normal (fertile) cytoplasm but the recessive male-sterile genes. All progenies of the *milo*×combine *kafir* cross contained *milo* (sterility-inducing) cytoplasm, but those that also inherited the homozygous recessive genes from the *kafir* parent were male-sterile. The male-sterile plants in the *milo*×combine *kafir* cross were used as females in repeated backcrossing with *kafir* as the male parent. At the end of seven backcrosses, the entire genome of *kafir* was transferred into the *milo* cytoplasm. This resulted in two morphologically similar versions of the combine *kafir* (CK 60) parent: a male-sterile combine *kafir* (CK 60A) and a male-fertile combine *kafir* (CK 60B). The male-sterile lines are designated as A-lines and their maintainer lines as B-lines. Subsequently, several sources of CMS systems (A₁, A₂, A₃, A₄, A₅ and A₆) (Table 13) were discovered.

Inheritance of fertility restoration in CMS systems

The inheritance of fertility restoration is dependent on the specific combinations of cytoplasm and nuclear genes. Fertility restoration is controlled by a single gene in

some combinations (eg, A_1) but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm (Schertz 1994).

Segregating progenies with A_1 cytoplasm in F_2 generation showed that a single gene was responsible for fertility restoration of A_1 male-sterile cytoplasm (Murthy 1986; Murthy and Gangadhar 1990). Other studies on A_1 cytoplasm have concluded that 1 or 2 genes (Qian 1990) or even 1 to 3 genes (Lonkar and Borikar 1994) are involved in controlling fertility restoration. Murthy (1986) reported that at least three genes control the fertility restoration of A_2 cytoplasm. In another study, F_2 progenies with A_2 cytoplasm showed a 9:7 ratio indicating that two complementary genes (both Msc_1 and Msc_2) are necessary for fertility restoration in A_2 (Murthy and Gangadhar 1990). Lonkar and Borikar (1994) indicated that 2 to 4 genes are necessary, but three genes were more optimal for the fertility restoration in A_2 cytoplasm in backcross generations. Research at ICRISAT showed that the frequency of recovery of fertile plants were least on A_3 than A_4 and A_2 and A_1 indicating that more number of genes are involved in controlling fertility restoration on A_3 than the other systems (Reddy and Prasad Rao 1992). El'konin et al. (1996) concluded that the fertility restoration in sorghum is controlled by an interaction of two complementary dominant genes in 9E cytoplasm. These studies clearly suggest the involvement of at least two genes for fertility restoration on A_1 and three on A_2 cytoplasm.

CMS diversity assessment

The *milo* CMS system has been extensively used in developing the hybrids for commercial cultivation in America, China, Australia and India. Nearly all the hybrids released so far, and widely grown have *milo* (A_1) cytoplasm (Reddy and Stenhouse 1994). Cytoplasm diversity can be assessed through restoration pattern in testcrosses and anther morphology (classical method) and through biotechnological tools (molecular markers).

Classical method. Schertz and Pring (1982) summarized various cytoplasm sources with respect to restoration pattern of 42 lines from India, 24 from USA, and one from Africa. Some of the cytoplasm sources were similar in reaction considering their restoration pattern. For example, Schertz and Pring (1982) indicated that cytoplasm sources of G_1 (G_1 -S, ms G_1 , G_1 -G, G_1 A) are analogous to IS 1112C of USA. A more comprehensive classification of cytoplasm sources is provided in Table 13.

Over the years, many of these cytoplasm sources were either lost or not widely available. The most commonly available ones include: A_1 (*milo* source), A_2 (IS 12662C or TAM 428), and A_3 (IS 1112C) of USA origin, A_4 (Guntur, VZM and Maldandi) of Indian origin, and 9E (a selection made in 9E) from Ghana. These cytoplasm sources were grouped on the basis of fertility restoration patterns. Reddy and

Stenhouse (1994) reported the identification of minimum differential testers for A_1 to A_4 cytoplasm as:

- TAM 428B (A_2) gives fertile F_1 s only on A_1 cytoplasm,
- IS 84B (A_4 -Maldandi) gives fertile F_1 s on A_1 and A_2 cytoplasm,
- IS 5767R (A_4 -Maldandi) gives fertile F_1 s on all cytoplasm, except A_3 , and
- CK 60B (A_1) gives male-sterile F_1 s on all cytoplasm.

Based on pollen development and anther morphology, these A_1 to A_4 (Guntur, VZM, Maldandi) and 9E cytoplasm were further subdivided into two distinct groups: (i) those with small anthers but without fertile pollen which degenerates during microsporogenesis (A_1 , A_2 , A_5 and A_6) and (ii) those with large non-dehiscent anthers that may contain some viable pollen (A_3 , A_4 and 9E) (Schertz et al. 1997). A_1 to A_4 CMS cytoplasm are being maintained at Patancheru (Andhra Pradesh, India) by ICRISAT (Fig. 29).

The lack of differential restoration patterns, however, does not provide conclusive evidence that the CMS sources involved are necessarily similar as it is possible that the pollinator parents used in developing the testcrosses were not adequate in number and diverse enough to pick up the CMS differences. It is also important in such field studies that testcrosses to be evaluated are made on isonuclear A-lines to ensure that genotypic differences of the female parents are not confounded with their cytoplasmic differences in determining fertility restoration of testcrosses.

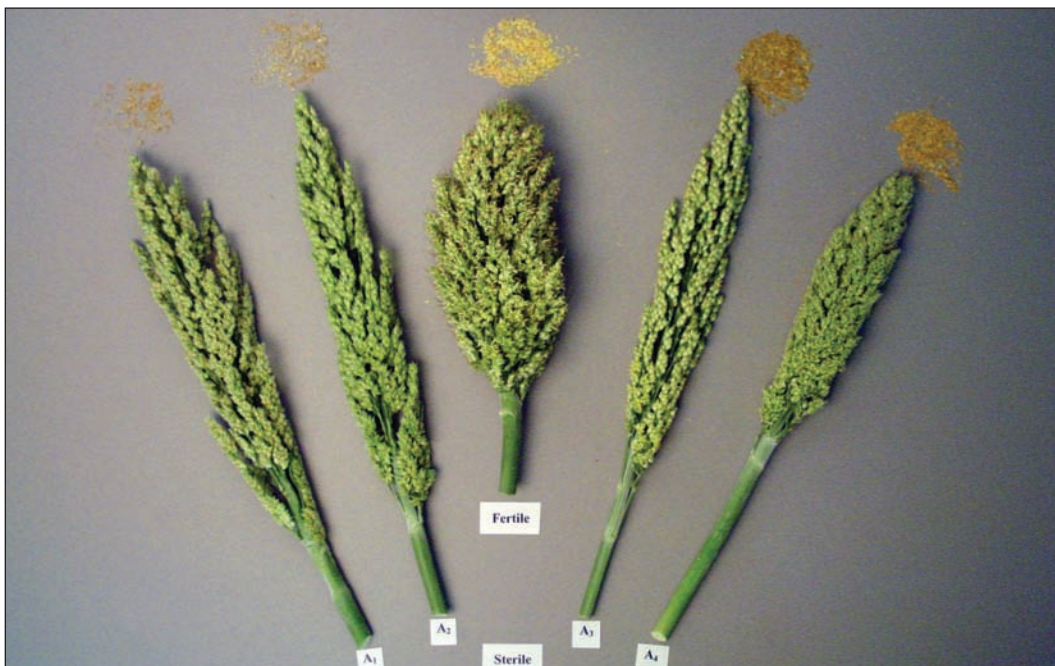


Fig. 29. Milo (A_1) and non-milo (A_2 , A_3 & A_4) CMS systems in sorghum along with a fertile panicle.

Table 13. Sources of CMS systems in sorghum.

Cytoplasm		Source	
fertility group	Identity	Race ^z	Origin
A ₁ ^y	Milo	D	-
	IS 6771C	G-C	India
	IS 2266C	D	Sudan
	IS 6705C	G	Burkina Faso
	IS 7502C	G	Nigeria
	IS 3579C	C	Sudan
	IS 8232C	(K-C)-C	India
	IS 1116C	G	India
A ₂ ^z	IS 7007C	G	Sudan
	IS 12662C	G	Nigeria
	IS 2573C	C	Sudan
A ₃ ^z	IS 2816C	C	Zimbabwe
	IS 1112C	D- (DB)	India
	IS 12565C	C	Sudan
A ₄ ^z	IS 6882C	K-C	USA
	IS 7920C	G	Nigeria
9E ^z	IS 7218		Nigeria
	IS 112603C	G	Nigeria
A ₅ ^z	IS 7506C	B	Nigeria
A ₆ ^z	IS 1056C	D	India
	IS 2801C	D	Zimbabwe
	IS 3063C	D	Ethiopia

^zD = *Durra*, G = *Guinea*, C = *Caudatum*, B = *Bicolor*, K = *Kafir*

^yType member for each fertility group

Source: Adapted from Schertz (1994).

Molecular markers. In conventional breeding, as indicated earlier, cytoplasm in various female parents are differentiated through the pattern of male sterility or restoration response in the testcrosses of various female lines. Other approaches to determine diversity among cytoplasm include restriction fragment length polymorphism (RFLP) as molecular markers (Schertz et al. 1997). Cytoplasmic factors associated with male sterility have been shown to be encoded by mitochondrial genome (Hanson and Conde 1985). As indicated earlier, using maize and pearl millet mitochondrial (mt) DNA specific probes, the comparison of RFLPs of mtDNA showed the difference between cytoplasm of A₁ to A₆ (Sivaramakrishnan et al. 1997). A₄ and 9E have been distinguished by RFLP analysis (Xu et al. 1995), and their cytoplasm were found to include an abnormal form of the mitochondrial gene

Cox 1 (Bailey-Serres et al. 1986a, Bailey-Serres et al. 1986b, Pring et al. 1995). Moreover, these cytoplasms also share several mtDNA RFLPs that distinguish them from all other Indian and US cytoplasms examined to-date, including polymorphism of the gene *atp9* (Schertz et al. 1997). Similarly, the restriction analysis of mtDNA from six Kansas State male-sterile lines using several endonucleases revealed two subgroups, with the patterns of KS 34, KS 38 and KS 39 corresponded to that of *milo*, as represented by CK 60 male-sterile; and KS 35, KS 36 and KS 37 differed both from *milo* and the other male-sterile lines (Schertz and Pring 1982).

Molecular characterization of CMS systems

As a consequence of the 1970 epidemic of southern corn leaf blight CMS-T cytoplasm is no longer widely used in commercial maize hybrids (Kishan and Borikar 1988, Wise et al. 1999). Therefore diversification of cytoplasm base of the cultivars is important. It is relatively easy to study diversity through the molecular markers. Now, it is well known that changes in the mitochondrial genome are responsible for male sterility (Sivaramakrishnan et al. 1997). Mitochondrial DNA of CMS line, IS 1112C in sorghum exhibits several unusual configurations—two forms of the *atp6* open reading frames (*orf 107* and *orf 265*) and chloroplast DNA insertions (Pring et al. 1998). RNA editing of transcripts of mitochondrial *atp6* was strongly reduced in anthers of A3Tx 398 male-sterile line of sorghum, suggesting that loss of *atp6* RNA editing contributes or causes CMS (Howad and Kempken 1997). The incompatibility in nuclear cytoplasmic interactions leading to aberrant microgametogenesis in sorghum may be explained in terms of incompatible subunits being synthesized by the mitochondria and nucleus for a multi-subunit complex of the mitochondrial membrane such as ATP synthase (Sane et al. 1994). Further, aberrant microgametogenesis in sorghum CMS line IS 1112C occur very late in pollen maturation and restoration of pollen fertility is conferred by two genes designated as Rf3 and Rf4. Rf3 is tightly linked to Rf4 or represents a single gene that regulates a transcriptional processing activity that cleaves transcripts of *orf 107*, a chimeric mitochondrial open reading frame specific to IS 1112C and results in fertility restoration (Tang et al. 1998, Pring et al. 1998, Tang et al. 1999).

Factors influencing the use of CMS systems

Although numerous CMS sources have been found, all have not been commercially useful. There are various factors that determine CMS options. These include stability of male sterility, effect of male sterile cytoplasm on agronomic traits, restorer gene frequency in germplasm and the availability of commercially viable heterosis.

Stability of CMS systems

Instability of male sterility in A-lines increases the problem of rouging of pollen shedders in seed production plots, which results in increased seed production cost. Such an unstable CMS system also reduces breeding efficiency as the backcross progenies found fully sterile initially may not be necessarily so during the subsequent generations, leading to their rejection. Stability of male sterility also has a direct bearing on the cost and quality of hybrid seed production. Ideally, a commercial male sterile line should neither shed pollen nor should set seed when selfed, regardless of the location and the season. This however is seldom possible. For instance, several A-lines based on A_1 CMS systems in sorghum have been extensively used to breed hybrids, which are planted in millions of hectares in India alone. Yet, most of these A-lines produce, albeit low frequency (less than 1%) of pollen shedders, depending on the environment (Reddy et al. 2003). Thus, stability of male sterility across environments is an important criterion in the utilization of CMS systems for commercial production of hybrids. Several workers reported the role of temperature on the expression of male sterility and restoration in sorghum (Downes and Marshall 1971, Li et al. 1981). It affects some cytoplasm more than others (Schertz et al. 1997). The work at ICRISAT showed that restoration is poor when night temperature falls below 10°C, just before flowering, during postrainy season, and that the male sterility in CMS lines breaks down when the day temperature rises above 42°C, before flowering (Reddy and Stenhouse 1994). This evidently increases the need to screen the CMS lines in areas where the temperature rises above 42°C before flowering for the absence of seed setting under bag to ensure stability of male sterility. The hybrids need to be screened in areas where night temperatures are low (below 10°C) for seed setting under bags to identify stable fertility restorers.

On comparison of A_1 , A_2 , A_3 and A_4 male-sterile lines for seed setting upon selfing during summer (when temperature goes beyond 42°C) at Bhavanisagar, Tamil Nadu, India, it was found that A_1 is more stable in maintaining male sterility than A_2 , A_3 and A_4 (Maldandi); A_3 than A_2 and A_4 ; and A_2 than A_4 (Reddy and Stenhouse 1994 and 1996). Tapetum was intact and pollen was sterile in A_2 male-sterile lines in winter (below 10°C temperature), while there was partial or complete degeneration of tapetum and pollen grains were fertile in summer (beyond 42°C temperature), indicating the unstable nature of male sterility in A_2 CMS system (Devi and Murthy 1993). In the sorghum breeding program at ICRISAT, the frequency of maintainer lines observed in A_1 and A_2 CMS systems was higher in postrainy season (below 10°C temperature) than in rainy season (Reddy et al. 2003). However, Indian researchers have reported higher fertility restoration in A_2 CMS system in postrainy season than in rainy season (Murty UR, personal communication). Thus, stability of the expression of male sterility varies with the temperature as well as type of

cytoplasm. Research involving same CMS lines in both the seasons would give a clear picture of the stability of different CMS systems in different seasons.

Effect of CMS systems on economic traits

The observed frequency of segregation for tall and dwarfs confirmed the known theory that height is controlled by four recessive non-linked genes in crosses of two dwarf isocyttoplasmic lines carrying A_1 cytoplasm and two tall tropical landraces (IS 2317 and IS 35613) (Murthy 1986). While in the crosses of dwarf isocyttoplasmic lines of A_2 cytoplasm and two landraces, the segregation pattern of dwarf and tall deviated significantly from the four gene theory indicating the effect of A_2 cytoplasm on plant height.

Considerable variation was observed at ICRISAT sorghum breeding program between the available male-sterile lines and maintainer lines in A_1 CMS system for flowering. In the early group, a few A-lines tend to be late by a day or two but not of much significance. But in the medium and late maturity groups, A-lines tend to be significantly late in flowering and there is a tendency of increased delay in flowering in A-lines with the increased maturity period. B-lines have more open panicles than those of their A-lines.

Sharma et al. (1994) and Sharma (2001) reported that the spikelet damage and adult emergence of midges was significantly lower on midge resistant B-lines (PM 7061 and PM 7068) than their corresponding A-lines, and *vice versa* in the midge susceptible parental lines (296A and ICSA 42). At Patancheru, the maintainer lines (B) flower early by one or two days and has more open panicles than those of their A-lines. Further, A_1 cytoplasm was more susceptible to shoot fly than the maintainer line cytoplasm, while the reverse was true for stem borer resistance (Reddy et al. 2003). This finding has significance in developing shoot fly and stem borer resistant hybrids. In a different study using five pairs of sorghum isonuclear A_1 and A_2 CMS lines in Mexico, it was revealed that CMS system did not have any effect on days to flowering (Williams-Alanis and Rodriguez-Herrera 1992).

Evaluation of five pairs of sorghum isonuclear A_1 and A_2 CMS lines in four locations of Tamaulipas, Mexico, viz., Rio Bravo (irrigated), El Tapo (drought), El canelo (drought), Guelatao (drought), during fall summer season of 1992 indicated significant differences between A_1 and A_2 CMS lines for grain yield only in drought conditions (Rodriguez-Herrera et al. 1993). However, no significant differences were found between A_1 and A_2 CMS lines for plant height, panicle length and panicle exertion. In yet another study using 32 isonuclear A_1 and A_2 CMS lines-based hybrids evaluated in ten environments in Northern Mexico during fall winter season of 1990, 1991 and 1993 under irrigated and dry conditions, Williams-Alanis et al.

(1993) reported absence of significant differences between A_1 and A_2 CMS lines-based hybrids for grain yield, plant height, panicle length and panicle exertion.

Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines with three common dual restorers at Patancheru during postrainy season of 2001 and rainy season of 2002 indicated absence of significant differences between A_1 and A_2 CMS systems for mean performance for traits such as days to 50% flowering, plant height and grain yield, lodging resistance and aphid resistance. Although hybrids based on A_2 cytoplasm showed agronomically superior performance, A_1 based hybrids excelled in seed set under open pollination as well as selfing. A comparative evaluation of A_1 and A_3 cytoplasm-based isonuclear sorghum-Sudan grass hybrids at the University of Nebraska field laboratory, Ithaca during 1990 and 1991 by Pedersen and Toy (1997) revealed that cytoplasm had no effect on days to 50% flowering, plant height, dry matter of forage yield, *in vitro* dry matter digestibility and protein content. However, while fertility restoration was equivalent in A_1 - and A_3 -based hybrids, it was significantly lower in a few A_3 -based hybrids. Recently, by evaluating a set of 12 isonuclear hybrids each in A_1 , A_2 and A_3 cytoplasmic background at Weslaco and Texas Agricultural Experimental Station farm located near college station, TX during 1998 and 1999, Moran and Rooney (2003) have reported that A_1 , A_2 and A_3 cytoplasm had no effects on plant height and had minimal practical effect on days to anthesis. However, grain yield in A_3 cytoplasmic background was significantly reduced as compared with A_1 and A_2 cytoplasm-based hybrids. Although specific reason for the reduced yield of A_3 hybrids is not known, seed set data indicated that it was not associated with fertility restoration.

Restorer gene frequency

The availability of restorers determines the extent of the use of various CMS systems in hybrid seed production. Scheuring and Miller (1978) reported a frequency of 0.62 restorers and 0.23 maintainers on milo (A_1) cytoplasm in the world collection of 3507 sorghum accessions. The work carried out at ICRISAT showed a restoration frequency of 0.9 on A_1 , 0.5 on A_2 , 0.1 on A_3 , and 0.3 on A_4 , when 48 germplasm lines were test crossed onto A_1 , A_2 , A_3 and A_4 CMS systems (Reddy et al. 2003). Senthil et al. (1998) found that the frequency of restoration was 0.15 on A_1 , 0.04 on A_2 , 0.01 on A_3 and 0.03 on A_4 CMS systems. This suggests that the restorer frequency is high on A_1 and low on A_3 system. Hence, considering the restoration frequency, A_1 CMS system provides the widest possible choice in selecting restorers.

Cytoplasm effects on heterosis for economic traits

Even if all the requirements are met in a CMS system, the existence of commercially viable heterosis ultimately determines the use of a CMS system. In sorghum, as indicated earlier, A_1 cytoplasm is more stable than other alternative cytoplasm, and restorer frequency with A_1 CMS is higher than with others. The heterosis estimates reported for grain yield using A_1 CMS system vary. For example, results from the Indian National Program Testing showed that the superiority over check with A_1 CMS system for grain yield ranged from 18 to 31% in the rainy season, and from 19 to 29% in the postrainy season in the years 1999 and 2000. The heterobeltiosis estimates for the same period ranged from 15 to 26% in the rainy season and 1.5 to 11% in the postrainy season (Reddy et al. 2003). Siddiq et al. (1993) reported that heterobeltiosis was 38% for grain yield in rainy season. Similar studies with alternate CMS systems are limited. Senthil et al. (1998) also reported that the A_1 CMS system produced higher number of heterotic combinations than A_2 , A_3 or A_4 system. Kishan and Borikar (1989a) observed that A_2 -based hybrids had larger grains and higher yields than A_1 - and A_4 -based hybrids. Based on testing of 15 hybrids derived from three isonuclear male-sterile lines and five common restorers, the A_4 -based hybrids were inferior to others for grain yield in the rainy season. However, another report indicated that A_4 -based hybrids had higher grain yield and larger grain size than A_1 hybrids during the postrainy season study (Kishan and Borikar 1989b).

Keeping in view the importance of diversifying cytoplasm in sorghum hybrids, ICRISAT, Patancheru has developed as many as 46 A_2 based CMS lines in different racial backgrounds; 17 A_3 based CMS lines and 12 A_4 based CMS lines. Evaluation of two sets of 36 hybrids obtained by crossing two different sets of six A_1 and A_2 isonuclear CMS lines with three common dual restorers at ICRISAT-Patancheru during postrainy season of 2001 and rainy season of 2002 indicated absence of significant differences between A_1 and A_2 CMS systems for mean heterosis (%) for any of the traits indicating that A_2 cytoplasm can be used in commercial hybrid seed production.

Efforts at NRCS, Hyderabad in the diversification of CMS lines resulted in the development of nine A_2 based CMS lines with high yield and grain mold resistance (GMR) and a unique hybrid SPH 1225 based on A_2 cytoplasm promising for grain yield and fodder yield in both irrigated and deep soil conditions in the postrainy season.

Considering the restoration frequency, development of high yielding male-sterile lines using A_2 restorers, and hybrid performance, it is advantageous to use A_2 CMS system among the alternate cytoplasm available. Based on A_2 CMS systems, the hybrid, Zinza No.2, was released in China for commercial cultivation. This hybrid is

now grown in an area of 200,000 ha accounting for one sixth of the total sorghum area in China (Liu Qing Shan et al. 2000). Extensive research is underway at ICRISAT and the Indian national program for the development of A₂ cytoplasm based hybrids.

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Inter-specific hybridization in sorghum genetic improvement

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Introduction

Plant breeding is the art and science of improvement of crops through exploitation of natural and induced genetic variation. Methods involve crossing of genotypes belonging to same species or to other cross-compatible species, leading to new variation, followed by selection. Very often, crop improvement programs have encountered ceiling in improvement of yield and other traits of interest such as resistance to economically important biotic and abiotic stresses. It has been established that uncultivated and wild crop species are potential sources of economically important traits especially plant defense traits. Exploitation of genetic variation created by inter-specific hybridization can circumvent the problem yield ceiling in crop improvement programs. However, transfer of useful genes from uncultivated and/or wild species is constrained by reproductive barriers.

The various reproductive barriers normally encountered in inter-specific hybridization are classified pre-zygotic and post-zygotic depending on whether barriers are observed before or after the formation of zygotes.

Reproductive isolation and wide hybridization in the genus *Sorghum*

The greatest variability in both the cultivated and the wild sorghums is found in the northeast part of Africa. There are different views regarding the origin of sorghum, which include Indian (Linnaeus), Abyssinian (Vavilov) and the tropical West Africa. Though *S. halepensis* crosses easily with *Sorghum bicolor*, it is not considered as its ancestor due to its perennial and rhizomatous nature. Many consider that *S. sudanense*, *S. arundinaceum* and *S. verticilliflorum* are likely ancestors. *Sorghum* belongs to the tribe Andropogoneae and group Sorghastrae and contains six subgenera. The subgenus *Sorghum* (formerly *Eu-sorghum*) has *Halepensis* with $2n=40$ and *Arundinacea* with $2n=20$. It may be noted that many of the wild relatives have excellent resistance to insect pests and diseases and tolerant to abiotic stresses.

The genus *Sorghum* has 25 species forming two lineages: one with $2n=10$ having relatively large genome species and polyploid relatives (subgenera *Parasorghum* and *Stiposorghum*); the other with $2n=20$ and $2n=40$ species having smaller genomes (Subgenera *Eu-sorghum*, *Chaetosorghum*, *Heterosorghum*). Strong reproductive isolation barriers have prevented successful hybridization of *S. bicolor* with any other *sorghum* species classified in subgenera other than *Eu-sorghum*. Pollen-pistil incompatibility is the primary reason for unsuccessful inter-specific hybridization. There is a reduction of alien pollen germination and tube growth on *Sorghum bicolor* stigmas. This incompatibility mechanism can be overcome by using a *S. bicolor* genotype that allows growth of pollen tubes of alien *Sorghum* species. Hybrids have been recovered after dusting the stigmas of *S. bicolor* with pollen from diverse species such as *S. angustum*, *S. nitidum* and *S. macrospermum*. Yet another technique employed was utilization of CMS line to act as a female. Though it offers a viable option, there is a possibility of inheriting a host of nuclear – cytoplasmic male sterility genes to inter-specific hybrid derivatives. Hybrids between sorghum and sugarcane/maize/rice were produced in this way. Candidate species which are useful for purposes of transferring useful genes include *S. matanrankense*, *S. australiense*, *S. intrans* and *S. versicolor*, although they might pose some initial problems of genome imbalance. These are highly resistant to shoot pests and *S. versicolor* is resistant to *Striga* as well. Mention may be made that the new tools such as somatic hybridization by protoplast fusion, embryo rescue technique and combining alien pollen and anti allergic chemicals to assist inter-specific hybridization can be employed to overcome inter-specific hybridization barriers. Successful hybridization between *S. bicolor* and sugarcane and between *S. bicolor* and rice has been achieved. *S. bicolor* pollen induces haploids/maternal diploids with maize, wheat and bamboo.

Management of seed genebank

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Introduction

The seed genebank standards was first set by then International Board on Plant Genetic Resources (IBPGR) and International Plant Genetic Resources Institute (IPGRI), now Bioversity International on the basis of a report from a FAO/IBPGR Expert Consultation Group which met in Rome, Italy from 26–29 May 1992. The most widely used technique for conserving plant genetic resources is seed banking. Seeds are dried to low moisture content and stored at subzero temperatures in cold stores or deep freezers. According to FAO, this technique accounts for 90% of the 6 million accessions conserved *ex situ* globally. However, this technique is only possible for species with seeds that can tolerate desiccation and low temperatures. Many species have seeds that cannot survive under such conditions. For species with so-called ‘recalcitrant’ seeds or species that are vegetatively propagated, such as root tubers and aroids, different conservation techniques are used.

Genebanks conserve genetic resources. The most fundamental activity in a genebank is to treat a new sample in a way that will prolong its viability as long as possible while ensuring its quality. The samples (or accessions as they are called) are monitored to ensure that they do not lose viability. A cornerstone of genebank operations is the reproduction—called regeneration—of its plant material. Plant samples must periodically be grown out, regenerated, and new seed harvested because, even under the best of conservation conditions, samples will eventually die (Bioversity International 2006). There are almost 1500 genebanks in the world housing some 6.5 million samples of plant germplasm, of which some 1–2 million are thought to be distinct (GCD Trust 2006). The Indian subcontinent is one of the 12 mega biodiversity centers (Zeven and Zhukovsky 1975) and represents two of the eight Vavilovian centres of origin and diversity of crop plants (Harlan 1971).

National Genebank

National Bureau of Plant Genetic Resources (NBPGR) is the lead institute for national network on plant genetic resources conservation. The import and export of seeds, plants, products and planting materials in India are regulated by the rules

and regulations under the DIP Act of 1914. NBPGR has been authorized to issue Import Permit and import/export of agricultural, horticultural and agri-silvicultural plants in small quantities for research purpose for public and private sector research use. NBPGR houses the national genebank, primarily responsible for conservation of seed collections on long term basis for posterity. Long term conservation is achieved by conserving the seeds in modules maintained at -20°C and through cryopreservation of ex-plants, organs and/or cells at -196°C. The viability of the seeds is maintained at -20°C for 25–50 years and at -196°C for an infinite period. The base collections are not meant to be distributed, except in an emergency, on loss of an accession, for regeneration of active collections and facilitate the activity of community banks. The materials introduced from other countries are assigned Exotic Collection (EC) numbers while the indigenous Collections (IC) are assigned IC numbers.

National Active Germplasm Sites (NAGS)

Under the National Network on conservation of plant genetic resources (PGR), 40 crop specific National Active Germplasm Sites (NAGS) hold active/working germplasm collections in cold storage modules for sustainable use. Active collections are for use in present day research and crop improvement efforts. NAGS are responsible for multiplication, evaluation and distribution of germplasm to the bonafide users. They are situated at various Indian Council of Agricultural Research (ICAR) institutes, State Agricultural Universities (SAUs) and other research organizations, which hold 2.0 lakh accessions. Apart from these, NBPGR holds 1.1 lakh active collections in its 10 Regional Stations.

Seed genebank

Seed genebanks are set with the objective of minimizing the loss of viability and genetic integrity of seed material during storage and regeneration. Seed genebanks are for orthodox seed species that can be dried to very low seed moisture. There are three types of seed bank storage, namely, short-term, medium-term and long-term storages.

Short-term storage (STS): Seeds are generally maintained at a temperature of 15–20°C and relative humidity (RH) of 30–40 percent.

Medium-term storage (MTS): The temperature of 0–10°C and relative humidity (RH) of 25–30% and seed moisture of 6–8% are adequate for medium-term storage modules. It can maintain seed longevity for a period of 25–35 years. These storage modules are meant to maintain active collection. The accession size comprises of as large a quantity as possible to avoid frequent regeneration. RH is controlled

through a de-humidifier. Since RH is controlled, the seeds in MTS can be kept in paper bags, cloth bags or any other moisture pervious containers. The viability of the seeds is monitored in the interval of five years.

Long-term storage (LTS): The temperature of 10 to -20°C is maintained in the long-term storage with no control of RH. Only the base collections are maintained in these conditions and it can maintain the seed for about 50–100 years. The base collections with a minimum of 2000 seeds for self-pollinated crops and 4000 seeds for cross-pollinated crops are generally preferred for storage. In case of wild species, 500 seeds must be stored. The seeds are dried to a moisture content of 3–7 percent and they are stored by hermetically sealing the container.

Standards for seed genebank

Seed drying: Seeds should be dried prior to their placing in the storage bags/containers. Seeds are dried using sun drying/shade drying/natural air drying/hot air drying. Desiccants in sealed containers are generally used for drying of seeds. Longevity of the seed prolonged using the dehumidifier or drying the seeds at 10–25°C and 10–15 percent RH.

Seed cleaning: The seeds to be stored should be free from weeds, pests and diseases.

Storage containers: The storage containers are different for the three types of storage purposes. Generally, paper bags or plastic bags are used in the short-term storage, plastic containers and plastic pouches are used in the medium-term storage. In the long-term storage modules, aluminum containers, or multi-layered pouches made of polyethylene are used.

Monitoring of seed viability: The seed germination of 65–85% is the minimum acceptable limit for storage in genebanks. Seed germination will be assessed through germination test. A minimum of 100–200 seeds are used for germination at the start of the seed storage. The period of viability monitoring is 10 years for base collections and 5 years for active collections.

Regeneration: Regeneration should be undertaken when the seed viability falls below 85%. Seeds used for regeneration should be genetically as close as possible to the original sample.

Information management: PGRI database is very important at national, international, regional and global levels for conservation of rapidly disappearing genetic stocks for future use in crop improvement programs. The following databases are important for the management of seed genebanks.

Germplasm Passport Database: Refers to data pertaining to the identity and history of an accession, collected at the time of collection.

Germplasm Characterization Database: Refers to the database of the individual accessions on those characters that are highly heritable, can be seen by the naked eye, and are expressed in all environments.

Germplasm Evaluation Database: Refers to the database of individual accessions on those characters that are important from the point of value of an accession.

Genebank Conservation Database: Refers to the database with the information on genebank storage (accession number, form of material, form of storage, location, storage date, seed quantity), viability monitoring and regeneration of the material.

Germplasm exchange: The seeds should be supplied in suitable containers with adequate information of the material for effective use. Seed lot should have high viability level. Plant quarantine regulations are strictly followed. The supply of the material should accompany Material Transfer Agreement (MTA) as per national requirement.

Maintenance of module: A voltage stabilizer to protect from voltage fluctuations, a built-in electric control panel with complete operation information and indicator lights to display operating conditions is important in seed storage. Spares such as compressor unit, thermostat, fan motor, expansion valves, compressor contractor, overload relays and fuses, etc, for immediate replacements are very essential to run the module without any major interruption

Safety and security of module: Uninterrupted power supply to the module especially in summer season, fire safety measures, alarm, security to the personnel by providing them protective clothes and material are some of the safety precautions that need to be considered. Refrigeration standards and construction and installation should be followed as per the Design of Seed Store Facility (DSSF).

Sorghum seed Genebank at NRCS

National Research Centre for Sorghum (NRCS) is one of the NAGS to act as a national repository for sorghum germplasm in India. The objectives of the sorghum genebank are collection, conservation, characterization, evaluation, documentation of sorghum genetic resources and distribution to the bonafide users of the country.

Germplasm collection: A total of 639 accessions have been collected by NRCS during 2002-2006; 393 accessions were supplied by NBPGR (Regional Stations); 356 accessions of exotic collections were supplied by NBPGR (head quarters),

New Delhi ; and 4474 accessions of International Sorghum (IS lines) were received from ICRISAT during 2001–2006.

Germplasm characterization: A total of 3012 accessions maintained at NRCS have been characterized since 2001. The post-rainy season (*rabi*) adapted germplasm materials are characterized at NRCS-Hyderabad and Solapur; and the rainy season adapted (*kharif*) germplasm materials at NRCS-Hyderabad, All India Coordinated Sorghum Improvement Project (AICSIP) centers-Indore, Udaipur and Mauranipur. In addition, 326 accessions of exotic collections supplied by Germplasm Exchange Division, NBPGR, and 203 accessions received from USA, 110 accessions received from Brazil, 8 accessions received from Sri Lanka, 3 accessions received from Mali and two accessions received from Eritrea have been characterized at NRCS-Hyderabad during 2002–2006.

Germplasm evaluation: A total of 6136 accessions have been evaluated for various agro-morphological traits, biotic and abiotic stresses.

Germplasm multiplication: A total of 8245 accessions have been multiplied during the period 2001–2006.

Germplasm conservation: A total of 20,812 accessions have been conserved at medium-term storage along with 1456 accessions as duplicates. During the period between 2000 and 2006, a total of 9908 accessions have been submitted to the National Genebank (NGB), NBPGR, for long-term storage.

Germplasm documentation

Database: On-line database on “Status of Sorghum Genetic resources” at NRCS, along with passport data, has been prepared and connected to the desktop computers of the scientists of NRCS. Database on the status of 9984 accessions of sorghum genetic resources maintained at AICSIP centers along with varieties developed from 1975–76 to 2005–2006 with complete passport details were compiled. Similarly, database on sorghum hybrids (SPH 1 to SPH 1578) developed from 1975–76 to 2005–2006 have been compiled.

GIS mapping: With the help of DIVA Geographical Information System (GIS) software developed by the International Potato Research Center under the umbrella of the Consultative Group on International Agricultural Research (CGIAR), a sorghum diversity map has been prepared for the NRCS collections to identify unexplored areas for future exploration.

Material Transfer Agreement: Standard Material Transfer Agreement (MTA) for exchange of sorghum genetic resources to the bonafide users of the country for research purposes has been developed and documented since 2002.

Germplasm distribution: Since 2001, a total of 1604 germplasm materials were distributed to the sorghum researchers of the country.

Germplasm for trials: A total of 3752 accessions were supplied for trials at NRCS and AICSIP centers.

Germplasm utilization: Since 2001, a total of 952 germplasm accessions collected from different states are being utilized by the sorghum researchers of the country.

Summary

History stands testimony to the fact that human beings conserved the seeds for food and later seeds for multiplication and livelihood security. The world today is fighting for rights and ownership. Hence, there is a need to conserve the diversity in the seed banks to prevent bio-piracy.

Suggested reading

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Sorghum germplasm: diversity and utilization

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Introduction

Plant genetic resources can be defined as the “Genetic material of plants that is of value as a resource for the present and future generations of people” (IPGRI 1993). The importance of genetic resources was recognized at the inter-governmental platform under the umbrella of Food and Agricultural Organization (FAO) of the United Nations as “common heritage of mankind” that should be made available without restriction (FAO 1983). The genetic resources have evolved as a product of domestication, intensification, diversification and improvement through conscious and unconscious selection by countless generations of farmers, man-guided diversity in the form of landraces and improved cultivars that provide basic and strategic raw materials for crop improvement the world over in present and future generations.

The amount of genetic variability available in sorghum (*Sorghum bicolor* L. Moench) is immense. Much of the genetic variability is available in areas of the first domestication of the crop (Africa) and regions of early introduction (Asia). In Africa, the genetic variability is available in both cultivated species and wild progenitors of the crop (Gebrekidan 1982). DeWet and Harlan (1971) reported on the distribution of both wild relatives and the major cultivated races of the crop in Africa. However, this natural genetic diversity is subjected to a range of threats from natural selection and destruction of habitats and often merely expedient agricultural practices of mankind. Landraces and wild relatives of cultivated sorghum from the centers of diversity have been rich sources of resistance to new pathogens, insect pests and other stresses such as high temperature and drought, as well as sources of traits to improve food and fodder quality, animal feed and industrial products. Preventing the vulnerability of landraces and wild relatives of cultivated sorghum from extinction, following the release of varieties and hybrids, collection and conservation of sorghum germplasm was accelerated about four decades ago. Since then, germplasm collection and conservation has become an

integral component of several crop improvement programs at both national and international levels (Rosenow and Dalhberg 2000).

Status of genetic resources

Sorghum germplasm collections vary in number and kind in various parts of the world. However, status of germplasm maintained at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, USA, Africa and China are worth discussing considering the size of the crop improvement programs (Rosenow and Dalhberg 2000) in these centers.

ICRISAT, Patancheru, India: The first major effort to assemble a world collection of sorghum was made in the 1960s by the Rockefeller Foundation in the Indian Agricultural Research Program (House 1985, Murty et al. 1967). A total of 16,138 accessions were assembled from different countries, and International Sorghum (IS) numbers were assigned to them. In 1976, ICRISAT was given the responsibility to add sorghum germplasm to the world collection in accordance with the recommendations made by the Advisory Committee on Sorghum and Millet Germplasm sponsored by the International Board for Plant Genetic Resources (IBPGR) [now the Bioversity International] (IBPGR 1976, Prasada Rao et al. 1989).

At present, ICRISAT is a major repository for the world sorghum germplasm collection with a total of 36,774 accessions from 90 countries. The existing collections of sorghum germplasm conserved at ICRISAT have been estimated to represent about 80% of the variability present in the crop (Eberhart et al. 1997). About 90% of these collections have come from developing countries in the semi-arid tropics. About 60% of these collections have come from six countries: India, Ethiopia, Sudan, Cameroon, Swaziland and Yemen. The largest collection is from India. It is interesting to note that about 84% of the total collections are contributed by landraces in contrast to wild species, which contributed only one percent to the total collection. Based on the geographical origin, the accessions are further classified. About 63% of the total number of accessions are from African countries and about 30% are from Asian countries. In addition to this, the germplasm maintained at ICRISAT, India, are classified into five races: *bicolor*, *guinea*, *caudatum*, *kafir* and *durra* and their derivative (Gopal Reddy et al. 2002). The collection is predominantly represented by three basic races: *durra* (21.8%), *caudatum* (20.9%) and *guinea* (13.4%). Among the intermediate races, *durra-caudatum* (12.1%), *guinea-caudatum* (9.5%) and *durra-bicolor* (6.6%) are common. The country-wise distribution of basic and their intermediate races revealed that three countries, India, Uganda and Zimbabwe have all the five basic and ten intermediate races (Gopal Reddy et al. 2002).

Ethiopia: Ethiopia, one of the rich centers of diversity, began a centralized collection of sorghum at the Jimma Agricultural Technical School between 1958 and 1960 (Rosenow and Dalhberg 2000). In the early 1970s, the Ethiopian Sorghum Improvement Project (ESIP) began the collection, evaluation, documentation and conservation of germplasm. Through the early 1980s ESIP had amassed a collection of approximately 5500 accessions (Doggett 1988). It is estimated that through continued research efforts, the germplasm has grown to roughly 8000 collections (Rosenow and Dalhberg 2000). The distinct types of sorghum from Ethiopia are (1) *zera zeras*, (2) *durras* and (3) *durra-bicolor* derivatives. The *zera zeras* have been extremely useful in providing germplasm for the improvement of food type sorghums.

Sudan: A large collection of Sudanese landraces was assembled in Sudan in the 1950s. These were maintained at Tozi Research Station and the entire collection was provided to the Rockefeller Foundation Project in India (Rosenow and Dahlberg 2000). The *caudatum* race dominates in Sudan and Sudanese sorghums have been very useful as sources of drought resistance (Rosenow et al. 1999).

United States of America: Around 1905, the United States Department of Agriculture (USDA) undertook collection and distribution of sorghum and Texas was selected as the first research station to work on sorghum in collaboration with USDA and Texas Agricultural Experimental Station (Quinby 1974). A total of 42,221 germplasm accessions have been maintained at National Plant Germplasm System (NPGS) in the United States of America (Dahlberg and Spinks 1995).

China: Through extensive and planned collections of landraces throughout the country, 12,836 accessions have been assembled in China and 10,414 of these have been registered as genetic resources and are currently being preserved in National Genetic Germplasm Resources Bank (Qingshan and Dahlberg 2001). These accessions include 9652 local varieties, improved varieties and strains that originated from 28 provinces, municipal and autonomous regions. These were further classified according to use: 9859 sorghum accessions were classified as food types, 394 accessions were classified as varieties for fodder and 125 varieties were classified for use in sugar production. Only a limited number of local Chinese cultivars are in the sorghum collections at ICRISAT, India or US National Collection (Qingshan and Dahlberg 2001).

At the global level, sorghum germplasm collections consist of approximately 168,500 accessions; one of the largest collections (21% of global total) is held at ICRISAT, Patancheru, India. The total accessions consists of 18% landraces/old cultivars, 21% advanced cultivars/breeding lines, 60% of mixed categories of unknown material, while very few are wild relatives (Chandel and Paroda 2000).

NRCS, India: A total of 20,812 accessions are conserved at medium-term storage along with 1456 accessions as duplicates. The maximum accessions are a repatriation material (11,113 acc.) followed by other IS lines (3442 acc.). Local germplasm (3560 acc.) and exotic collections (494 acc.) are other important materials. 9984 accessions of sorghum genetic resources are held at AICSIP centers. 2373 accessions of specific sorghum germplasm are held at 7 AICSIP centers.

Maintenance of genetic resources

Genebanks conserve genetic resources. The most fundamental activity in a genebank is to treat a new sample in a way that will prolong its viability as long as possible while ensuring its quality. The samples (or accessions as they are called) are monitored to ensure that they are not losing viability. A cornerstone of genebank operations is the reproduction—called regeneration—of its plant material. Plant samples must periodically be grown out, regenerated, and new seed harvested because, even under the best of conservation conditions, samples will eventually die (Bioversity International 2006).

Sites for preservation and maintenance of the largest sorghum accessions are located at ICRISAT, India; the National Seed Storage Laboratory, Fort Collins, Colorado, USA; and the USDA-ARS Plant Genetic Resources Conservation Unit (PGRCU), Griffin, Georgia, USA. Several countries also maintain their own collections within their national collections. Major grow-outs and regeneration takes place at ICRISAT, India and at the USDA-ARS Tropical Agriculture Research Station, Mayagüez, Puerto Rico (Rosenow and Dahlberg 2000). At ICRISAT, India, all collections are maintained in the post-rainy season by selfing about 20 representative panicles from each line. Seeds harvested in equal quantities from these panicles are mixed and a bulk of about 500 g is preserved in aluminum cans in the medium-term storage facility (4°C and 20% relative humidity). Freshly rejuvenated accessions with 100% viability and 5±1% seed moisture content are being stored in long-term storage (-20°C).

Core collection

Although several subsets of total base collection were developed for utilization by sorghum scientists, they turned out to be location-specific and did not give a fair representation of the base collection (Prasada Rao et al. 1995). Therefore, the concept of “core collection” (Brown 1989) was used to set up a “core collection” at ICRISAT. A core collection consists of a limited set of accessions derived from an existing germplasm collection, chosen to represent the genetic and geographical spectrum of the whole collection. The rationale behind a core collection is the

maintenance of as much genetic diversity as possible within a smaller, more manageable representative “core collection” (Dahlberg and Spinks 1995). Following this concept, a core collection was set up at ICRISAT-Patancheru, India by stratifying the total base collection geographically and taxonomically into subgroups within regions. Accessions in each subgroup were further clustered into closely related groups based on the principal component analysis (PCA) using agronomic data. Representative accessions from each cluster were drawn in proportion to the total number of accessions present in that subgroup. Thus, the core collection of 3475 set up at ICRISAT, India represents approximately 10% of the total world collection (Prasada Rao and Ramanatha Rao 1995). The core collection is an economical, practical and effective method for conservation, maintenance and utilization of the germplasm (Eberhart et al. 1997).

In the USA, a core collection containing over 200 out of 42,221 accessions was developed by a sorghum curator located at USDA in Mayagüez, Puerto Rico, representing genes for plant height, maturity, drought resistance, pericarp color, and greenbug, aphid, downy mildew resistance (Dahlberg and Spinks 1995).

Characterization, evaluation and documentation of genetic resources

Characterization: Characterization of each sample involves a careful description of the special characteristics that are inherited, easy to score, and expressed consistently in all environments. Since most of the traits recorded during characterization are those that can be seen, the person responsible for managing the germplasm material is best placed to carry out the work of documenting these characteristics. Many of the characteristics that are recorded on individual accessions can serve as diagnostic descriptors for the accessions. Such diagnostic characters help genebank curators keep track of an accession and check for the genetic integrity over a number of years of conservation. Again, descriptors lists are a vital tool for ensuring that those who are documenting the characteristics of conserved species are using the same language and standards.

Evaluation: Germplasm is evaluated for growth traits, agronomic performance, yield and responses to biotic and abiotic stresses using visual observations and measurement on standard scales. Molecular markers (such as isozymes and DNA markers), which are neutral to environment variation may also be used for evaluation. Evaluation is primarily carried out by germplasm users, in multidisciplinary teams that include breeders, entomologists, pathologists and agronomists. The potential value of the germplasm depends on the efficiency of the techniques designed to differentiate accessions. Since farmers possess valuable traditional knowledge and are the ultimate users of the finished products of crop improvement programs,

it is advantageous to involve farmers and consider their views and expectations during germplasm evaluation.

Documentation: The proper documentation of plant genetic diversity is an indispensable part of making diversity useful to farmers, breeders and researchers. Before we can use what we've got, we've got to know what we have. In order to increase international exchange of material, a certain amount of uniformity in data collection, recording, storage and retrieval is critical. Developing standards for documentation and protocols for exchanging information is essential for ensuring that bridges can be built between myriad information sources. Coping with the vast amount of data on crop species and varieties and making it available requires adequate database design and information management systems (Bioversity International 2006).

As a prerequisite for efficient utilization of the germplasm, it must be properly evaluated, characterized and documented with a workable retrieval system so that any group of entries carrying any desired characteristics could be easily retrieved and used in breeding programs (Gebrekidan 1982). A total of 29,180 accessions have been characterized for 23 important morpho-agronomic characters based on "list of sorghum descriptors" (IBPGR/ICRISAT 1980) at ICRISAT, Patancheru, India, during rainy and postrainy seasons. The range of variability available in cultivated races and their wild relatives is extensive, and the extreme types are so different as to appear to be separate species (Prasada Rao et al. 1995).

The characterization data with passport information have been documented using ICRISAT Data Management Retrieval System (IDMRS) program and have been converted to the System 1032 (a Relational Database Management Software), for rapid and more efficient management (Prasada Rao et al. 1995). A number of trait-specific promising genetic stocks are maintained at ICRISAT-Patancheru, India. These are: stocks resistant to insect pests such as shoot fly, stem borer, midge and head bug; resistant to diseases like grain mold, anthracnose, rust and downy mildew; resistant to parasitic weed, *Striga*, and stocks with important traits like glossiness, pop, sweet stalk and scented types. Approximately 50% of the total US germplasm accessions have been characterized based on 39 agronomic descriptors (Dahlberg and Spinks 1995) and 21,661 accessions located at USDA-ARS S-9 site in Griffin, Georgia, USA. In addition to these initial descriptors, many accessions have been screened in nurseries for further evaluation and various accessions have been identified as having resistance to aluminum toxicity, shoot fly, stem borer, *Striga*, midge, rust and downy mildew (Dahlberg and Spinks 1995). The details of the passport data and characterization of US sorghum germplasm collection have been documented in the Germplasm Resources Information Network (GRIN) and are also available through the sorghum curator located in Mayagüez, Puerto Rico.

A total of 3012 accessions with NRCS have been characterized since 2001. An online database on status of sorghum genetic resources at NRCS, along with passport data and evaluation data, has been prepared for easy retrieval of the information on the required germplasm line. Database on status of sorghum genetic resources at All India Coordinated Sorghum Improvement Project (AICSIP) centers with 9984 accessions of sorghum genetic resources held at AICSIP centers has been prepared. A Geographic Information Systems (GIS) map for the sorghum germplasm collections held at NRCS has been prepared.

Utilization of genetic resources

It is obvious that only a small fraction of the total available collection could be fully utilized by breeders at any given time. Crop improvement programs are often interested in portions of the collection that carry special desirable characters that are highly important at any particular point of time (Gebrekidan 1982). Early work on utilization of sorghum germplasm was confined to pure line selection within cultivated landrace populations in Africa and India that resulted in improved cultivars, some of which continue to be widely grown. Selection within dwarf populations was taken up, followed by exploitation of cytoplasmic male-sterility, which permitted the production of commercial hybrids (Dahlberg et al. 1997). Crossing and/or backcrossing between adapted introductions and local germplasm has been used to derive improved pure-line varieties and parental lines (Prasada Rao et al. 1989). Useful traits, such as increased seed number, larger panicles, greater total plant biomass, drought tolerance, disease resistance, greater plant height, longer maturity, greater leaf area indices, increased green leaf retention and greater partitioning of dry matter have contributed to increased yield (Miller and Kebede 1984).

Utilization has been primarily limited to agronomically important and in some cases, wild sources of germplasm. For example, use of *zera zera* sorghum has become widespread in the development of new, superior hybrids because of superior yield potential and grain quality (Duncan et al. 1991). The classic example of germplasm utilization in sorghum has been the Texas A&M-USDA Sorghum Conversion Program. Till date 633 converted lines have been released globally; 50 are listed in Rosenow et al. (1995); and 40 new lines have been released (TAES and USDA-ARS 1996).

The successful introgression of resistance to midge and downy mildew has greatly stabilized sorghum production in Australia and Argentina. Considerable opportunities remain for exploiting the collections to improve sorghum production globally. For example, over 340 accessions of the genus sorghum belonging to sections *Chaetosorghum*, *Heterosorghum*, *Stiposorghum*, *Parasorghum* and *Eu-*

Sorghum have been evaluated for resistance to shoot fly at ICRISAT, Patancheru, India. Seven accessions with high levels of resistance and in some cases close to immunity were found (Nwanze et al. 1995). Transfer of this high level of resistance to cultivated sorghum could greatly improve productivity of late sown crops in Africa and Asia, where shoot fly is a major production constraint (Dahlberg et al. 1997).

Since its establishment in 1972, ICRISAT has made efforts to (1) diversify the germplasm base to enhance yield levels and (2) to identify resistance sources and use them to develop varieties and seed parents. The major germplasm sources utilized so far in varietal improvement include temperate lines from US, *zera zera* lines from Ethiopia and Sudan, and some lines of Indian origin. The male-sterile gene sources used were mainly CK 60, 172, 2219, 3675, 3667 and 2947. These were further diversified by using parents such as CS 3541, BTx 623, population derivatives (Bulk Y, Indian Synthetic, FLR, RS/B, US/B, Serere, Diallel and WAE), IS 6248, IS 2225, IS 3443, IS 12611, IS 10927, IS 12645, IS 517, IS 1037, IS 19614, E 12-5, ET 2039, E 35-1, Lulu 5, M 35-1 and Safra. In the development of restorer parents and varieties, the basic germplasm sources used were: IS 84, IS 3691, IS 3687, IS 3922, IS 3924, IS 3541, IS 6928, ET 2039, Safra, E 12-5, E 35-1, E 36-1, IS 1054, IS 1055, IS 1122, IS 1082, IS 517, IS 19652, Karper 1593, IS 10927, IS 12645, IS 12622, IS 19652, IS 18961, GPR 168 and IS 1151.

The stable resistant sources for shoot fly and stem borer, such as IS 1082, IS 2205, IS 5604, IS 5470, IS 5480, M 35-1 (IS 1054), BP 53 (IS 18432), Karad Local (IS 18417), Aispuri (IS 18425) in India, IS 18577, IS 18554 in Nigeria, IS 2312 in Sudan, IS 18551 in Ethiopia, IS 2122, IS 2134 and IS 2146 in US have been used both in Indian and ICRISAT programs to impart resistance.

The midge resistant lines DJ 6514 and IS 3443 were used at ICRISAT to develop an improved midge-resistant variety, ICSV 197 (SPV 694).

The multiple disease resistant sources like ICSV 1, ICSV 120, ICSV 138, IS 2058, IS 18758 and SPV 387 (anthracnose and rust); IS 3547 (grain mold, downy mildew, anthracnose and rust); IS 14332 (grain mold, downy mildew and rust); IS 17141 (grain mold and anthracnose); IS 2333 and IS 14387 (grain mold and downy mildew); and IS 3413, IS 14390 and IS 21454 (grain mold and rust) are currently being used in breeding programs.

Some of the *Striga* resistant germplasm lines used in *Striga* resistance breeding are IS 18331 (N 13), IS 87441 (Framida), IS 2221, IS 4202, IS 5106, IS 7471, IS 9830 and IS 9951. Some of the breeding lines like 555, 168, SPV 221 and SPV 103 proved to be useful resistance sources. The *Striga*-resistant variety SAR 1 developed at ICRISAT from the cross 555×168 was released for cultivation in *Striga*-endemic areas.

Nearly 1300 germplasm lines and 332 breeding lines were screened for early- and mid-season drought stresses. The most promising of these are: early season and terminal drought tolerant: E 36-1, DJ 1195, DKV 17, DKV 3, DKV 4, IS 12611, IS 69628 and DKV 18; mid-season drought tolerant: DKV 1, DKV 3, DKV 7, DJ 1195, ICSV 378, ICSV 572, ICSV 272, ICSV 273 and ICSV 295.

The high-lysine sorghum lines, IS 11167 and IS 11758 from Ethiopia were used in the breeding program for transferring the gene to a desirable agronomic background. Some promising high-lysine derivatives with shriveled and plump grain have been obtained.

Several sweet-stalked sorghum lines like IS 20963, IS 15428, IS 3572, IS 2266, IS 9890, IS 9639, IS 14970, IS 21100, IS 8157 and IS 15448 have been promising and the sweet stalked trait is being incorporated into elite agronomic background.

Many lines with desirable forage attributes have been identified: IS 1044, IS 12308, IS 13200, IS 18577, IS 18578 and IS 18580. Regarding quality parameters, IS 1059, IS 2944, IS 3247, IS 4776 and IS 6090 were selected for low HCN, and IS 3247 and PJ 7R for low tannin content (Vidyabhushanam et al. 1989).

A total of 86 trait-based hybrid parents (B- and R-lines), advanced breeding lines and germplasm accessions evaluated in the initial stage at ICRISAT, Patancheru to find out the grain Fe and Zn content indicated low levels of micronutrient density. The grain Fe content ranged from 20.1 ppm (ICSR 93031) to 37.0 ppm (ICSB 472 and 296B) with an average of 28 ppm and the grain Zn content ranged from 13.4 ppm (JJ 1041) to 30.5 ppm (IS 1199) with an average of 19 ppm.

Studies on 2262 sorghum core germplasm accessions at ICRISAT, Patancheru indicated that a large variability for grain Fe (7.7 ppm to 192.3 ppm) and Zn (13.7 ppm to 91.3 ppm) content exists among and is much higher than that exists among hybrid parents of released/ marketed hybrids and popular varieties. As many as 17 accessions recorded grain Fe content over 90 ppm (range 96–192 ppm) and 11 accessions recorded grain Zn content over 58 ppm (range 58–91 ppm). Accessions from USA recorded the highest Fe content that belonged to *bicolor* race.

Over the years, the sorghum improvement program at ICRISAT developed 758 A-/B-lines and 922 R-lines for various traits. Evaluation of 222 designated B-lines indicated that the grain Fe content ranged from 22.4 ppm to 51.3 ppm and the grain Zn content ranged from 15.1 ppm to 39.6 ppm. As many as 20 B-lines recorded Fe content over 45 ppm and 13 B-lines recorded Zn content over 32 ppm. Two most promising B-lines, ICSB 406 with high grain Fe (51 ppm) and Zn (40 ppm) and ICSB 311 with high grain Fe (47 ppm) and Zn (36 ppm) were identified, which can be used in development of new hybrids with high Fe and Zn content.

For salinity tolerance IS 164, IS 237, IS 707, IS 1045, IS 1049, IS 1052, IS 1069, IS 1087, IS 1178, IS 1232, IS 1243, IS 1261, IS 1263, IS 1328, IS 1366, IS 1568, IS 19604 and IS 297891 were found to be good based on two years of testing at three salinity levels (5, 10 and 15 dS/m).

In India, the varietal improvement program was initiated in the 1930s. The locals were tall, late maturing, flowering after the rainfall ceased, generally photosensitive and characterized by localized adoption and low harvest index. Their response to improved management in terms of the increased yield was very poor. Most of the improved varieties were the result of pure line selection practiced in principal local varieties. Local × local hybridization followed by selection resulted in varieties with marginal increase in grain yield. Notable among these varieties developed during the early period and still under cultivation are the Co-series in Tamil Nadu; the PJ *kharif* and *rabi* selections, Saoner, Ramkel, Aispuri, the Maldandi, Guntur and Anakapalle series of Andhra Pradesh; the bilichigan, fulgar white, fulgar yellow, kauvi, Nandyal, hagari, yanigar varieties of the erstwhile Mysore state (Rao 1972).

With the discovery of workable cytoplasmic-nuclear male-sterility and initiation of the accelerated sorghum project (which later became the All India Coordinated Sorghum Improvement Project) in 1962, hybrid breeding was given due emphasis. Initially, the germplasm from the USA (*kafir milo* cytoplasm and other germplasm) and hybrid combinations by making temperate×temperate crosses were tested. During 1962–1969, out of temperate×temperate and temperate × tropical crosses, three hybrids, ie, CSH 1, CSH 2 and CSH 3 were released.

Introduction of CSH 1 in farmers' fields during 1960s resulted in a quantum jump in productivity and production as the hybrid responded well to improved management practices as compared to old varieties. It became popular with farmers as it had high yield potential, was suited to light soils and low rainfall areas. The second hybrid CSH 2, was based on the same male sterile, CK 60A as that of CSH 1 and a new R-line IS 3691, which was a yellow endosperm selection of *Hegari* from USA. Later on, a new male-sterile line, 2219B was identified from germplasm line *kafir shallu* and a hybrid CSH 3, was developed by using 2219 A and IS 3691 (R-line of CSH 2). CSH 2 and CSH 3 did not become popular due to inherent seed production problems.

During the next decade (1970–1979), three hybrids, CSH 4, CSH 5 and CSH 6 were released. Hybrid CSH 4, based on ms 1036A had better fodder yield. The female line, ms 1036A was developed from a cross of CK 60B and PJ 8K (a local variety from Maharashtra) and the R-line being a selection from IS 3924. Though this hybrid was good for grain yield, the hybrid was not popular among the farmers as its grain quality was not better than CSH 1.

In general, grain yield of improved cultivars was tripled by utilization of exotic breeding material in hybrid program, however, these cultivars especially hybrids were not well received by the farmers because of their increased susceptibility to major pests and diseases and inferior grain quality compared to locals.

Keeping the above factors in mind, new male-sterile lines were developed with different genetic backgrounds. The ms lines and R-lines were developed from derivatives of crosses between temperate × tropical crosses. The ms line of CSH 5, (2077B) was developed from IS 2046, a germplasm line from Senegal. The hybrid CSH 5, based on converted lines ms 2077A and CS 3541, contributed not only to substantial yield improvement but revolutionized sorghum seed industry. The hybrids were tolerant to mites and aphids also. This improvement was achieved by introduction of genes from tropical material IS 3541. An early maturing hybrid CSH 6 developed from early MS line 2219A and CS 3541 became very popular as hybrid suited for inter-cultivation with pigeonpea.

Further increase in grain yield was achieved by development of hybrids like CSH 9, CSH 10 and CSH 11 based on new MS line 296A during 1980-1989. Indian germplasm line *karad local* was crossed with American material IS 3922 to develop the MS line 296A, which was the best combiner. This ms line has a very compact panicle with more number of primary branches. CSH 9, a medium duration hybrid is widely adapted and extensively grown. The hybrids CSH 10 and CSH 11 developed from the same ms line, 296B, showed marginal superiority for grain yield.

During 1990s, most of the hybrids tested in all-India trials were based on 296A with various restorers but could not make any remarkable dent for grain yield over CSH 9. Though there was no significant grain yield improvement, useful diversification for early maturity and higher fodder yield has been achieved with the release of CSH 13 and CSH 14. The fodder yield of CSH 13 is 40% more than that of CSH 9 although its grain yield is marginally improved. The R-line of this hybrid, RS 29 that contributes to heterosis for fodder yield is developed from SC 108, an American elite line and SPV 126 (a tall mutant of CS 3541). Another hybrid, CSH 14, is about 10 days earlier than CSH 9 maintaining the same level of grain yield. The need for diversification of female parent was felt in view of seed production problems and stagnating yield level. Another high yielding hybrid, CSH 16 was developed from new ms line 27A, and R-line C 43. This hybrid showed further improvement in grain mold tolerance as the new genes from Ethiopian germplasm line IS 23549 was introduced into its R-line. The R-line has very compact panicle. In contrast to 296B, ms line 27A of this hybrid is long and has loose panicles with bold and round seed like that of post-rainy season genotype that has consumer preference. 27B has been developed from multiple crosses using germplasm lines such as IS 3687, IS 3922 and 2219B. Recently two hybrids CSH 17 and CSH 18 were released.

Though the yield levels are on par with CSH 16, these were diversified for early maturity and high fodder yield, respectively. By utilizing local variety Vidisha 60-1 at Indore center, the ms line of CSH 18 (MS IMS 9A) was developed. The local variety, Vidisha 60-1 not only contributes to high stover yield but also to improved grain quality. The R-line of this hybrid, Indore 12 is developed from multiple crosses of SSV 53 and SPV 475 germplasm lines.

Whenever there was a change in the male sterile line, the yield benefit was obvious. Hence, there is a need for developing new male sterile lines having better combining ability in comparison to that of available ms lines. So far, several germplasm lines of different botanical races have been utilized in development of parental lines. The grain yield levels of rainy season hybrids have reached the plateau and there is a need to exploit unused germplasm and landraces to diversify the genetic base.

Simultaneously, varietal improvement was achieved by introducing temperate and tropical material. The first variety, CSV 1 is a direct introduction of American line IS 3924. By crossing temperate and tropical germplasm, subsequent varieties CSV 2 and CSV 3 were developed. CSV 4, which was used as restorer of three most popular hybrids, CSH 5, CSH 6 and CSH 9, became a very popular variety. The variety is a converted line of an African germplasm line IS 3541, and developed by crossing it with a US germplasm line, IS 3675. CSV 5, another variety developed from Indian local and US line IS 3687 has *Striga* resistance. CSV 10, which became popular for high fodder value was developed from a cross between American elite variety SC 108 and Indian elite variety CS 3541.

Another variety which became very popular, SPV 462 (from Coimbatore), was developed from multiple cross involving IS 2947 and IS 3687 from USA and IS 1151 and BP 53, locals of Maharashtra and Gujarat in India, respectively. The variety is high yielding for grain and fodder with good grain quality. CSV 13, yet another variety developed from multiple cross having exotic and local genetic base is high grain yielding with medium height. The latest variety, CSV 15 developed from SPV 462 and CSV 13, is a dual purpose variety having grain yield equal to that of hybrid CSH 5 and fodder yield equal to CSH 10.

Germplasm has been used for trait based breeding in rainy season sorghums, especially for resistance to major diseases and pests. Though present day varieties and the restorer lines possess moderate level of resistance, the male sterile lines are highly susceptible. Genetic stocks such as IS 14387, IS 14374, and IS 25017 were extensively used for incorporation of grain mold resistance into male sterile lines. Recently, grain mold tolerant ms line 219 was developed by crossing AKMS 14B (ms line of hybrid CSH 14) with grain mold resistant line IS 14387. Similarly, many grain mold tolerant lines, GMRP and SR, were developed at Parbhani and Surat centers. Important resistant lines are GMRP 9, GMRP 13,

SR 839 and SR 384. Similarly, shoot fly resistance material is developed at Rahuri and Akola centers of AICSIP by using germplasm lines. Seed of varieties bred for rainy season is not as remunerative as that of *rabi* varieties because the seed is not bold and lustrous. Now, efforts are being made to breed varieties with bold and lustrous seeds by using *guinea caudatum* and *durra caudatum*. Some of the germplasm lines contributing bold grain size are IS 51, IS 3142, 9742, IS 17600, IS 19305, IS 31690, etc.

The attempt to develop heterotic hybrids for postrainy season with whole grain quality matching that of local variety Maldandi has met with limited success; Maldandi has a unique feature that combines good grain quality with drought and shoot fly resistance as required for *rabi* season cultivation. Hybrids like CSH 7R, CSH 8R, CSH 12R were based on ms lines developed from rainy season lines, and R-lines were developed from local×US lines that did not show much superiority over M 35-1 for yield and quality. Another *rabi* hybrid, CSH 13 is superior to the *rabi* local for grain yield but inferior to M 35-1 for grain quality. Recently released hybrid CSH 15R, is based on *rabi* adapted MS line having good grain quality and shoot fly tolerance. The R-line of this hybrid is developed from *rabi* locals and CS 3541, an elite *kharif* variety.

Postrainy season varieties were developed by crossing Indian locals, M 35-1 and IS 2644 with American germplasm lines. Marginal improvement was achieved for grain yield over the most popular local variety M 35-1. Recently released variety, CSV 216R (CSV 16) is a landrace selection from *rabi* germplasm from Maharashtra.

The need for further critical evaluation of germplasm and its utilization in grain and forage sorghum improvements is keenly felt. Sorghum collection missions may be further arranged on the specific targeted germplasm availability areas based on the usage, such as sweet sorghum, pop sorghum, dual-purpose sorghum and sorghums resistant to pests and diseases, etc. Landraces are known to possess local adaptation and stability and may offer opportunities for direct utilization. On the other hand, improved varieties with diverse genetic base are likely to show wider adaptation and enhanced performance. Judicious combination of both in recombination breeding programs can further lead to upgradation of yield potential. As discussed earlier, so far, *caudatum* and *durra* types have been exploited. Inclusion of *guinea* germplasm may bring further increase in yield potential, tolerance to grain mold and lodging resistance.

Traditionally, sorghum is used as whole meal flour and milled fractions. The whole meal flour has been used for making '*rotis*' (*bhakri*) and gruel. To a limited extent, sorghums are also used for puffing and making some special dishes. Fortified foods, a blended food product containing grain sorghum and quality protein sources such as soybean have been developed in countries like the USA; these have more

relevance to India but are yet to become popular here. Sorghum malt is used as food in many parts of the world. The *kafir* beer of Africa is a traditional drink of *Bantu* people. Modern malting techniques used in the malting of barley have been successfully applied to grain sorghums.

Sorghum is a multipurpose and bioenergy crop; the grain, stem and glumes are the useful parts. The grain is used as human food (bread, baby food, popped, parched, flakes); livestock feed for poultry, farm animals (rabbits, ducks and pigs); alcohol production (potable and industrial), fuel; malt (malt syrup, beer, beverages; glucose liquid/powder), and other industrial productions: starch, dextrin, dextrose, glue, liquid glucose, alcohols, plastics, textiles, paper board, 'U' – foam industries; stem is used in the production of syrup, jaggery, alcohols and sugar; bagasse obtained as byproduct is used as fodder and in the manufacture of fuel, paper, particle and corrugated boards. Natural color is extracted from glumes (Rana 2000). Sorghum is considered as an industrial and high-energy crop with its diverse uses. Sorghum alcohol, syrup, liquor, beer and malt play an important role for small industries. Commercialization of sorghum lies in proper utilization of its diverse economic importance (Elangovan 2005).

Concurrent with continued conservation, evaluation and documentation of germplasm, efforts to fully exploit the greater genetic diversity of the world collection of sorghum must be escalated. In sorghum, as in other crop species, the need to investigate alternate methods for evaluation and enhancement of exotic germplasm has reached a critical stage. Quantitative traits governing adaptability, such as yield and stress tolerance, will be extremely important in future improvement projects. Although height and maturity conversion of exotic germplasm allows for some evaluation and utilization for these traits, other alternatives must be considered. The evaluation of an exotic source could be conducted prior to conversion in the native environment, where the source is better adapted or another location where information from the collection site is utilized. This evaluation phase will be critical to the increased use of either partially converted lines or the germplasm source *per se*. Increased use of sorghum germplasm in future also may depend on the practical application of molecular techniques. DNA marker techniques to identify diverse segments of the chromosomes controlling inheritance of quantitative traits will become necessary in germplasm enhancement programs. Genetic transformation with exotic genes also plays a major role in sorghum improvement since many desirable genes exist in the World Collection. The challenge to sorghum improvement will be to concentrate on utilization of desirable traits that may aid in evolving superior improved lines aiming to surpass the present productivity plateau combined with better drought, disease and pest resistance and improved grain quality.

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Prospects for biological control of insect pests in sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important cereal crops grown for food, feed, forages and fuel in the semi-arid tropics. In India, the major states growing sorghum are Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh, Rajasthan and Tamil Nadu. Sorghum production in all parts of the world is constrained by damage due to several insect pests - shoot fly, stem borer, midge, headbug, aphids, etc. The use of insect resistant varieties is a proven, effective, economical and safe method of pest control ideally suited to Integrated Pest Management (IPM). However, most of the insect resistant varieties are low or moderate yielders. Theoretically, IPM optimizes low cost tactics and utilizes insecticides only when other strategies fall short or need additional support. Insecticides are generally not economical in subsistence agriculture, making it particularly important for marginal farmers to take advantages of all low input methods for pre-harvest crop protection. Thus biological control is often described as special assurance for subsistence agriculture. It involves importing, conserving or augmenting useful natural enemies for managing selected pests of sorghum especially in the developing countries.

Historical background

Biological control in general has a similar historical background in nearly all countries. It was used with much success since the beginning of the 20th century. It began in Latin American countries, such as in Peru, Argentina and Bolivia in the 1930s and 40s with the introduction of the coccinellid *Rodolia cardinalis* Mulsant [= *Vedalia cardinalis*] to control cottony cushion scale, *Icerya purchasi* Maskell (Homoptera., Margarodidae), in citrus, and the parasitoids *Aphelinus mali* (Haldeman) (Hym., Aphelinidae) and *Apanteles thurberiae* Muesebeck (Hym., Braconidae) to control woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera., Aphididae), and the cotton pest *Sacadoses pyralis* Dyar (Lep., Noctuidae), respectively. However, its effectiveness has been relatively slow if applied alone (Table 14).

Table 14: Key pests of sorghum and the prospects for their biological control.

Pest/Scientific name	Geographical Distribution ¹	Pest status	Biological control		
			Status/ Requisites ²	Prospects ³	Type ⁴
			1 2 3 4 5 6		
Shoot fly, <i>Atherigona</i> spp	AF, AS	Key	+ ? + ? ? ?	?	?
Stem borer					
<i>Chilo partellus</i>	AF, AS	Key	+ ? + ? ? ?	E	I,C
<i>Diatraea grandiosella</i>	NW	Occasional	+ ? + ? ? ?	E	I,C
<i>Diatraea saccharalis</i>	NW	Occasional	+ ? + ? ? ?	E	I,C
<i>Eldana saccharina</i>	AF	Occasional	? ? + ? ? ?	E	I,C
<i>Busseola fusca</i>	AF	Occasional	? ? + ? ? ?	E	I,C
<i>Sesamia cretica</i>	EE	Occasional	? ? + ? ? ?	E	I,C
Aphids					
<i>Schizaphis graminum</i>	COS	Key	+ + + + - -	E	I,C
<i>Sipha flava</i>	NW	Occasional	? ? ? ? ? ?	?	I,C
<i>Aphis sacchari</i>	AF, AS	Occasional	? ? ? ? ? ?	E	I,C
Sorghum midge, <i>Contarinia sorghicola</i>	COS	Key	+ ? + ? ? ?	P	I,C
Head bug					
<i>Calocoris angustatus</i>	AS	Key	? ? ? ? ? ?	?	?
<i>Nysius raphanus</i>	NW	Occasional	? ? ? ? ? ?	?	?
<i>Pentatomidae</i>	COS	Occasional	? ? ? ? ? ?	P	?

Source: Young and Teetes (1977).

1. COS = Cosmopolitan, AF = Africa, EE = Eastern Europe, NW = New world, AS = Asia, O = Oceania.
2. Requisites + = studies are complete and results were positive, - = study complete and results were negative ? = studies not yet done.
3. E = excellent; P = possible.
4. I = importation, C = conservation, A = augmentation, and ? = unknown.

Natural enemies on some key pests of sorghum

Shoot fly (*Atherigona soccata* Rond.)

Most of the information available is related to the occurrence of natural enemies of *A. soccata*. However, in a few cases, little information is reported on the extent of parasitism/predation. A number of natural enemies has been recorded and summarized by Pradhan (1971), Pont (1972), Jotwani (1978), Reddy and Davies (1979), Sharma (1985), Zongo et al. (1993a,b) and Singh and Sharma (2002). Three natural enemies, ie, *Oxybelus* sp. (Sphecidae), *Neotrichophoroides nyemitawus* (Rohwer) (Eulophidae) and *Opius* sp. (Braconidae) were also identified from the shoot fly (Sileshi et al. 1996). *Trichogramma* spp is predominantly reported as an egg parasitoid. A total of 24 larval parasitoids and six larval and pupal parasitoids have been recorded and most of these were recorded from India, Kenya, Burkina Faso and Nigeria. *Abrolophus* sp, *Tetrastichus nyemitawus*, *Trichogramma* spp and *Trichogrammatoidea* sp. are important natural enemies of sorghum shoot fly (Sharma 2001).

Stem borer (*Chilo partellus*)

The spotted stalk borer, *Chilo partellus* (Swinh.) (Lep: pyralidae) is a serious pest of sorghum and other graminaceous crops. A very few number of natural enemies are reported on spotted stem borer, *C. partellus*. The key natural enemies recorded are: *Trichogramma chilonis*, *Cotesia flavipes* Cam. *Sturmiopsis inferens* and *Xanthopimpla stemmator*. However, their potential efficacy has not been demonstrated on a large scale.

A classical example from the surveys of International Centre of Insect Physiology and Ecology (ICIPE) in 1993 revealed that *Cotesia flavipes* (Hymenoptera: Braconidae) imported from Pakistan in 1991 recorded 30% parasitism on stem borer in Kenya. Collaborative biological control activities are now being initiated in Mozambique, Uganda, Somalia, Eritrea, Ethiopia, Zanzibar, Zambia and Malawi. Host range studies demonstrated that *C. flavipes* would attack not only its old association host, *C. partellus*, but also two native African stem borers, *Chilo orichalcociliellus* and *Sesamia calamistis* (Lepidoptera: Noctuidae).

C. flavipes was released in the coastal area of Kenya in 1993, and sampling during the season of release revealed that the parasitoid had colonized maize and sorghum fields (Banat 1996). Biological control by importation of exotic parasites is an ideal prospect for managing at least some of the stem borers (Gilstrap 1980).

Sugarcane aphids (*Melanaphis sacchari*, Zehntner)

About 50 species of natural enemies have been recorded on sugarcane aphids throughout the world. In sorghum, the key enemies maintain the sugarcane aphid population below the economic threshold levels (van Rensburg 1973b; Anonymous, 1978; Chang 1981a,b; Meksongsee and Chawanapong 1985). There are a few notable reports on natural enemies that cause reduction in aphid populations. *Aphelinus maidis*, a parasite, recorded on sugarcane aphid in Hawaii (Zimmerman 1948), a parasitoid *Enrischia comperei* Ashm. in Australia and *Exochonus concavus* (Fursch), *Leucopus* spp. and *Liodalia flavomaculata* (DeGeer) in South Africa have been recorded by van Rensburg (1973b). The aphid parasite, *Lysiphlebus testaceipes* (Cresson) was recorded in Hawaii by Zimmerman (1948) and indigenous *L. dehliensis* Zehntner was recorded on aphid in India by Varma et al. (1978). High mortality of aphids have been noticed when predators such as lady bird beetles (Coleoptera: Coccinellidae), lacewings (Neuroptera: Chrysomelidae and Hemerobiidae), and hoverflies (Diptera: Syrphidae) attack the aphids, and these have been witnessed in South Africa by van Rensburg (1973a,b), and in India by Reddy and Devies (1979). The control of aphids by natural enemies is greater early in the season since the population build up is slow as compared to late season where aphid population builds up rapidly (Singh et al. 2004).

Sorghum midge (*Contarinia sorghicola*, Coquillett)

The sorghum midge *Contarinia sorghicola* (Coquillett) is a cosmopolitan pest and limited information is available about its natural enemies. A few natural enemies such as *Apanteles* sp. (Reddy and Devies 1979), *Aprostocetus* sp. (Jotwani 1978) and *Daryhelea* sp. as a predator have been reported.

Some classical examples of biological control

In 1991, an IPM program was launched in sorghum in El Tigre (Anzoátegui State, USA). A total of 18,820,000 *Telenomus remus* were released along with 89.375 square inches of *Trichogramma* sp. wasps (one square inch is equivalent to 2500 to 3000 *Trichogramma* wasps). The release led to reduction in pest control costs by the farmers to an extent of US\$ 23 ha⁻¹ (at that time US\$1 = Bolívares 171) over the budgeted cost for non-IPM management of close to US\$72 ha⁻¹ (12,300 bolívares). Some farmers saved US\$ 50 ha⁻¹.

A survey has been carried out by ICRISAT on the incidence of spotted stem borer and midge in farmers' fields in order to assess the extent of parasitism in sorghum growing states such as Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu. *Aprostocetus gala* was the most predominant parasitoid species (85–90%)

found during the survey. The larval parasitism was significantly higher in Andhra Pradesh (>50%) than in Maharashtra (>15%), while pupal parasitism was greater in Maharashtra (34%) than in Andhra Pradesh (18%). The tachinid *Sturmiopsis inferens* was the predominant larval parasitoids in Andhra Pradesh; whereas in Maharashtra, it was the braconid *Cotesia flavipes*. *Xanthopimpla stemmator* was the predominant pupal parasitoids in Andhra Pradesh and Maharashtra. The findings from the survey indicate that natural enemies are closely associated with sorghum insect pests in farmers' fields. The complexity and predominance of parasitoids species varies considerably across the region. Farmers in Maharashtra predominantly (90%) cultivate high yielding sorghum hybrids. In Andhra Pradesh, the situation is the reverse where >75% of the fields are sown to varieties and landraces which are relatively more tolerant or moderately resistant to these pests than hybrids. Although the incidence of stem borer in both the states is similar, it can be inferred that the difference in parasitoids compositions and predominance may be associated, at least to a certain extent, with differences in sorghum genotypes cultivated in these states (Duale and Nwanze 1997).

Conclusions

- The demand for IPM services in a number of crops is on the rise because of the realization of the problems associated with chemical control. A majority of industrialized countries demand agricultural products free from pollutants. Therefore, IPM services are becoming essential. However, the reality shows that little preparation has been made to cope with this change in strategy. In view of the scattered resources in India to tackle the problem, technology exchange and improved communications are vital.
- In sorghum, the scope for biological control appears limited because the cropping period is short and there is no crop continuity to sustain the natural enemies and their hosts.
- Successful use of natural enemies for insect control depends on the biology and ecology of both the pests and the beneficial organisms operating on it, hence research on biological control should be strengthened.
- The most significant obstacle to biological control of sorghum pests is lack of availability of trained human resources.
- The key approach to popularize biological control options for insect management is through demonstrations of beneficial effects of biological control on environment and human health and economic cost-benefit ratio.

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Prospects of biological control of sorghum diseases

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Introduction

Sorghum is a dryland crop grown in rainy (*kharif*) and post-rainy (*rabi*) seasons in India. Among the biotic stresses, diseases cause severe losses to the crop. Of these, grain molds during *kharif* and charcoal rot in *rabi* are economically important. Host plant resistance (HPR) is an important component in Integrated Disease Management (IDM). However, effectiveness of control of these diseases through HPR has been limited as many pathogens are involved in grain molds and virulence differences are observed in the charcoal rot causing soil-borne fungus *Macrophomina phaseolina* isolates.

Biological control is a novel method that reduces the pathogen multiplication or reduces its inoculum density and activity of the pathogen. Besides HPR, introduction of bioagents is another viable option in disease management. Fungal bioagent *Pseudomonas fluorescence* has been reported as a promising natural enemy of grain molds. Biological control has a multifold advantage over other management strategies as detailed below:

- Do not require repeated application, hence cost effective.
- Soil, air and water pollution is avoided.
- Residual toxicity in the crop product is avoided.
- Research costs on the development of pest-resistant strains are reduced.
- Adverse effect on beneficial microbes is avoided.
- Soil-borne diseases can be managed, where even chemical methods fail.

Mechanism of biological control

The mechanism of biological control is by Induced Systemic Resistance (ISR). Systemic Acquired Resistance (SAR) can be induced by host inoculation with necrosis-forming pathogen (specific plant-associated bacteria can be used as an alternative to pathogens for inducing resistance). Another alternative mechanism

of biological control includes production of various secondary metabolites that inhibit the pathogen, including antibiotics, siderophores, Hydrocyanic acid (HCN) and cell wall degrading enzymes.

Biological control of grain mold

Management of sorghum grain mold caused by 2 to 3 predominant fungal pathogens was attempted through biological control. Raju et al. (1999) have reported the efficiency of biological control of grain mold pathogen *Fusarium moniliforme* by *P. fluorescence* and *Trichoderma harzianum*. Indira et al. (2006) compared biological control of grain mold by four well-known species of *Trichoderma* with chemical control through seed treatment (Thiram). Of the tested five bioagents (*T. viride*, *T. harzeanum*, *T. koeningi*, *T. hamatum* and *P. fluorescence*), *P. fluorescence* was found more effective in checking the grain mold pathogens and enhanced germination and seedling vigor (Table 15), proving that the biocontrol agents have an edge over the chemical seed treatment to ward off seed borne diseases.

Table 15. Effect of biological control agents on seed germination and seedling vigor.

Treatments	Germination (%)	Vigor index
<i>P. fluorescence</i> @ 4g/kg	88.0	2635
<i>T. viride</i> @ 4g/kg	86.7	2478
Thiram @ 3g/kg	70.6	2055
Control	46.6	587
SEm±	4.70	72
CD (0.05)	9.88	152

The results from the field studies on biological control of grain mold are similar to those of in vitro studies when formulations of *P. fluorescence* were applied individually. Among the several biological agents, the biological control of grain mold through bacterial bioagents is more effective compared to fungal bioagents against all the test pathogens.

Biological control of foliar diseases

The efficiency of various neem based products on sorghum foliar diseases management has been studied. The neem-based products such as Neemazal, Nimbecidine and Neemark were highly inhibitory to the conidial germination of the pathogens causing leaf blight, grain mold, sugary disease, anthracnose, leaf rust, and loose smut (Rajasab 2000) (Table 16).

Table 16: Causal organisms of sorghum and maize diseases.

Disease	Causal organism	Crop
Leaf blight	<i>Helminthosporium turcicum</i>	Maize and sorghum
Grain molds	<i>Alternaria alternata</i>	Sorghum
Sugary disease	<i>Gleocercospora sorghi</i>	Sorghum
Anthraxnose	<i>Colletotrichum sublineolum</i>	Sorghum
Leaf rust	<i>Puccinia sorghi</i>	Maize and sorghum
Loose smut	<i>Sphacelotheca cruenta</i>	Sorghum

Biological control of charcoal rot

Charcoal rot caused by a soil-borne pathogen *M. phaseolina* is more prevalent under terminal drought situations. Use of cultivars with juicy stems and stay greenness coupled with maintenance of good plant health are the factors contributing to charcoal rot resistance. Charcoal rot can be managed by the biocontrol agents *Pseudomonas*, *Bacillus*, *Trichoderma* and Vascular Arbuscular Mycorrhiza (VAM) (Hindumathi et al. 2000) as in other crops such as maize, pearl millet, oilseeds, fruits and vegetable crops. Biological control is a key component in IDM strategies for crops like Mungbean (*Rhizoctonia bataticola*), safflower (*F. oxysporum*), pigeonpea (*F. udum*), and sesame (*M. phaseolina*).

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DNA markers and marker-assisted selection – Application of theory

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Introduction

Over the past 30 years there has been a revolution in the development and application of molecular genetic tools for marker-assisted breeding of crops, livestock and microbes. Although the vast majority of research programs in this area has focused on human medicine and model systems, and on higher-value crops of favorable production environments such as rice and maize, there have also been useful advances for staple food, fodder, fiber and fuel crops of stress-prone production environments, including sorghum.

Where they are available, molecular markers can now provide appropriate complements to conventional crop breeding methods. They are effective and sometimes appropriate tools for crop improvement research addressing biological components in agricultural production systems. Molecular markers offer specific advantages in the assessment of genetic diversity and in trait-specific crop improvement.

This paper provides an introduction to the most broadly applicable types of molecular markers and their use in applied plant breeding. It will first describe some of these markers, then outline the steps required to map genes controlling a trait of interest in sorghum and use this information to improve this trait in an elite hybrid parental line. While there is a growing body of literature about the use of DNA markers for genetic diversity assessment, this aspect of marker-assisted breeding will not be covered in this presentation.

DNA markers

Genetic markers can be thought of as nothing more complex than flags along the strands of genomic material that allow geneticists to track segregation of various portions of the genome across generations. Variation at individual genetic marker loci is inherited in a simple Mendelian fashion, and tracking of segregation at large numbers of such simply inherited loci allows construction of dense genetic linkage maps that are used to identify genomic regions contributing to the genetic control of more complexly inherited “quantitative traits”.

DNA markers are the result of heritable sequence variation in the deoxyribonucleic acid molecules that form the core of chromosomes. Compared to morphological markers (such as Mendelian genes controlling grain color variation, foliage color, and semi-dwarf plant height), and protein markers (such as seed storage proteins and enzyme variants differing in mobility in an electronic field), DNA markers have many advantages. First, there are many more of them and they are distributed widely throughout the genome. Second, large numbers of DNA markers can be detected by a common set of laboratory procedures. Third, many of them appear to be the result of neutral DNA sequence variations that have no apparent deleterious effects on crop adaptation or crop quality. Because of these advantages it is possible to use DNA markers to cost-effectively track segregation of particular genomic regions associated with genetic variation in one or more traits of interest.

Types of markers preferred for plant breeding applications

For most marker-assisted breeding applications there is a strong preference for co-dominant markers, especially those such as sequence-tagged microsatellite site (STMS) markers [also referred to as simple sequence repeat (SSR) markers, variable tandem repeat (VTM) markers and several other names] and amplified fragment length polymorphism (AFLP) markers that are compatible with the polymerase chain reaction (PCR) and thus require only small amounts of fair quality DNA from the individual plants being genotyped. For PCR-compatible marker systems the current standard for simple, low-cost detection of allelic variants is electrophoretic separation of PCR products on a gel followed by staining (typically silver staining) to visualize banding patterns. Lower-cost alternatives are coming, albeit slowly. For high-throughput (HTP) applications and applications where the ability to detect differences of less than 5 base pairs in PCR product length are required, the standard is separation of fluorescent dye-labeled PCR products by gel or capillary electrophoresis, with automated detection of the passage of the fluorescing PCR products. For sorghum there are now reasonably large numbers of SSR (several hundred) and AFLP (several thousand) markers that have been mapped.

The main other category of co-dominant markers and that upon which the revolution in DNA marker-based genetic mapping was initiated, is restriction fragment length polymorphism (RFLP) markers. These are the result of variation in the lengths of fragments following digestion of DNA samples with “restriction” enzymes that cut the DNA at sites having specific base sequences. However, RFLPs are hybridization-based markers and although robust, reasonably polymorphic and historically the basis for comparative mapping, they are normally considered to be too slow, expensive and low-throughput to be considered for use in applied plant breeding – in large part because they require large amounts of good quality DNA.

Two dominant marker types that promise to become more important in future are single nucleotide polymorphism (SNP) markers, which detect insertion/deletion mutations and even single base pair changes in the DNA sequence using primer sequences similar to those for STMS markers and diversity arrays technology (DArT) markers. SNPs are a PCR-compatible marker system that requires DNA sequence information for primer development, but ways of exploiting sequence information from model species for developing SNP markers for orphan species are advancing rapidly (Feltus et al. 2006). DArT markers are a HTP hybridization-based system that appears to have great potential. DArT is a microarray-based method that allows simultaneous scoring of hundreds of restriction site-based polymorphisms between genotypes and does not require DNA sequence information or site-specific primers. Several hundred mapped DArT markers are now available for sorghum, and many more are being developed.

Marker-assisted breeding

Marker-assisted breeding for any target trait is comprised of two complementary groups of activities. The first of these is related to mapping genomic regions contributing to genetic variation in the target trait and identification of sources of favorable alleles in these genomic regions. The second group of activities is associated with incorporating one or more of these favorable variants into the genetic background of agronomically elite, economically important hybrid parents or varieties adapted to the target region.

Genetic mapping involves a number of important steps:

- parental line selection,
- Development of mapping population,
- phenotyping the mapping population progenies,
- genotyping the mapping population progenies,
- linkage map construction, and
- quantitative trait locus (QTL) analysis.

Parental line selection: Selection of an appropriate pair of parental lines is critically important to the success of a QTL mapping project. The ideal parental pair differs greatly both for phenotypic values for the trait(s) of interest as well as for marker genotype at many loci distributed across the nuclear genome. Compromises are often necessary. However, for the purposes of QTL mapping it is generally better to favor crosses that offer the greatest marker polymorphism. Mapping multiple traits from a single mapping population has the potential to substantially increase the return on investment in marker data costs—provided that the costs of the additional

phenotyping are not unreasonably high and that there is a reasonable chance for applied use of the additional information.

Development of mapping population: There are several different types of populations that can be used for QTL mapping. These vary in the time that is required to generate them, whether they are for short-term use or can serve as tools for long-term study, the amount of marker data that is required for effective mapping, and the amount of information that is provided by each genotyped individual. F_2 , BCF_1 , and random inbred line (RIL) populations can all provide the individuals to be genotyped and phenotyped. In each of these cases, QTL mapping is limited to a comparison of the effects of the two alleles segregating in the population at any trait loci that are linked to polymorphic loci. An F_2 population provides twice as much information per genotyped individual as a BCF_1 population, but both of these types of mapping populations are ephemeral. Most QTL mapping is done with RIL populations as these are potentially immortal and greatly facilitate replicated assessment of phenotypic traits. However, RIL populations do not permit assessment of dominance. Doubled-haploid (DH) populations are equivalent to RIL populations for the purposes of QTL mapping. Random-mating of an F_2 population prior to deriving the RILs doubles the frequency of recombination events, and so allows more refined mapping with a given amount of marker information. Finally, recent increases in computational power and reductions in marker data point costs in some cases permit mapping using populations of RILs derived from complex crosses (4, 8 or 16 parents). Similar advances also allow exploitation of linkage disequilibrium in germplasm collections and breeding programs by means of association mapping, but this will not be covered here as the amount of marker data required (polymorphic loci at 1–2 cM intervals across the genome for a diverse set of sorghum germplasm accessions) is prohibitively expensive at present.

A mapping population suitable for conventional QTL mapping with the most commonly available software packages can only be segregating for two alleles at any locus. Further, it is strongly preferred that there is no segregation distortion in the population. Therefore, the mapping population progenies are typically advanced by repeated selfing, without selection, in the progeny of a single F_1 plant (preferably derived from a plant×plant cross from which selfed seed of both parental plants is available). A reasonably large population size is required to avoid random drift, but the costs of genotyping and phenotyping a large mapping population must also be considered. Although the first published QTL mapping study in crop plants was based on a population of just 44 F_2 plants, a population derived from 200 to 400 F_2 individuals is much preferred. The F_2 individuals are each advanced at least to F_6 , without selection, by modified single-seed descent with selfing each generation. The resulting population of F_6 RILs is then used for genotyping and phenotyping, with the mapping population parents serving as control entries. Phenotyping can

make use of the RILs themselves or testcross hybrids of the RILs when this is more appropriate.

Phenotyping: Phenotyping of the mapping population progenies and their parents is done to quantitatively assess variation for the target trait and its components or related factors. The goal of this phenotyping is to accurately rank the mapping population progenies for each target trait. Replication is generally required to accurately rank the progenies in a given test environment (site, year, season, pathogen isolate, ...). Spatial analysis of data sets may be required to obtain unbiased estimates of relative performance of the progenies in each test environment. Finally, testing across multiple environments is generally required to characterize the importance of genotype×environment (G×E) interaction(s) associated with each trait (and each QTL). For example, in case of controlled greenhouse screening of mapping population progeny sets for pearl millet downy mildew resistance, we typically use one or two pots of approximately 40 seedlings per progeny per time replication, with three or four time replications per downy mildew isolate, treating downy mildew isolate as the environment for the purpose of G×E interaction assessment. We are most interested in resistance QTLs that have relatively large effects across the range of pathogen isolates that we consider most relevant, and where the allele from one parent confers a greater degree of resistance in all cases where a significant effect is detected. Further, we would have greater confidence in such a QTL if each of the phenotyping experiments upon which it was based exhibited high operational heritabilities (eg, broad-sense heritabilities greater than 0.50). Thus for a QTL mapping study, the investment in phenotyping—both in raw data generation and in subsequent statistical analysis to obtain either treatment means or best linear unbiased predictors (BLUPs)—should be at least as great as in a conventional quantitative genetics study. In fact, quality of phenotyping data is now more often a problem for QTL analysis than is marker data quality. Sloppy phenotyping will not allow QTL detection with enough precision to be of much use.

Genotyping: Marker genotyping is done by extracting a DNA sample from each progeny in the mapping population (F_2 individual or F_2 -derived RIL) and using these samples to assess DNA sequence variation at 15–20 cM intervals across the nuclear genome of each mapping population progeny. The resulting “coarse” map allows detection of genomic regions most strongly associated with segregating variation in the target trait, and these portions of the genome can then be genotyped with additional markers to identify marker loci that are more tightly linked to individual genes or gene blocks controlling the target trait.

Linkage map construction: Linkage map construction makes use of computer software to perform “simple Mendelian linkage analysis” on a large scale—following

the segregation of many marker loci in moderately large populations of mapping progenies. There are several software packages that can be used for this, ranging from the very powerful (but quite expensive) commercially marketed products like “JoinMap” to freeware such as MapMaker/Exp and GMendel. Each of these software packages has specific formatting requirements for the marker data sets, but these are usually compatible with text or spreadsheet files. With any of these packages, linkage map construction should be treated as an iterative process, in which the marker data set is refined to eliminate “buggy” data points in order to obtain a robust, reliable linkage map. While doing this, it is often preferable to discard data for marker loci that exhibit distorted segregation or for which one is not absolutely certain that they have been scored correctly, rather than retain such data as the latter can result in an incorrect genetic linkage map that will ultimately provide incorrect QTL mapping results.

QTL mapping: QTL analysis is a process of assessing the probabilities of association between phenotypic values and genotypic scores at each marker locus, across the set of mapping population progenies. Again, there are relatively simple approaches that are possible (such as single marker regression, which can be done with any statistical software package or even simple spreadsheet software) and more complex approaches. Single marker regression does not even require a linkage map, but with this method the degree of association between the marker locus and the gene (or gene block) controlling the trait is confounded with the strength of the effect of the gene (or gene block) on the trait. Simple interval mapping (SIM) as implemented in MapMaker/QTL uses logarithm of odds (LOD) scores to identify marker intervals (and positions within those intervals) having the greatest probability of association with a particular trait. A LOD score is nothing more than the negative log₁₀ of the probability of an association due to chance alone. Thus a LOD score of 2.0 indicates a 1% probability of an association due to chance—and for many QTL mapping experiments this is not a high enough value to be considered worth pursuing. For sorghum shoot fly resistance, a QTL of large effect, mapping to a well-saturated portion of the linkage map, can have a LOD score greater than 7, indicating less than 1 in 10,000,000 chance of a spurious association with the flanking marker loci. More complex procedures are variations on the theme of composite interval mapping (CIM), which with sufficiently large populations considers marker variation not only in a particular interval, but at other unlinked genomic regions associated with variation in the particular trait of interest and so can be used to adjust for epistatic interactions between various portions of the genome. Further, in its most complex form CIM can adjust for the effects of one trait on another. QTL Cartographer and PlabQTL are two publicly available QTL mapping software packages in which CIM is implemented. PlabQTL implements CIM using a regression approach and is far faster to run than is QTL Cartographer.

Interestingly, the signs of QTL effects from these two software packages are exactly opposite. This means that whether a favorable allele for seedling leaf blade trichome density (an important component of shoot fly resistance) has a negative or positive sign depends on the software package used to identify it — therefore you need to read the instructions to be able to correctly interpret the QTL mapping output.

The product of QTL analysis is information on the genomic location and genetic effects of gene blocks that appear to contribute to the phenotypic variation that has been observed in the mapping population progenies (or their testcross hybrids). These are “putative quantitative trait loci”. They represent working hypotheses that need to be tested before they can be recommended for use in an applied plant breeding program. There are several different ways in which they can be tested. The first is to test an independent sample of the same segregating population for the presence of the putative QTL. The second is to test for the presence of the QTL in an independent segregating population based on the cross of a different parental line pair. The third, and perhaps the most obvious, is to attempt to change phenotype of one of the parental lines of the mapping population by marker-assisted introgression of the QTL allele from the other parent, using polymorphic markers flanking the putative QTL. This latter approach has the advantage that it may directly provide a useful breeding product if the more favorable allele is introgressed into an elite background and the putative QTL is not an artifact of the analysis. Demonstration of an economically significant phenotypic response to marker-assisted selection, along lines predicted by the QTL analysis, can provide sufficient support to justify marker-assisted selection for that particular QTL allele in an applied breeding program.

Thus genetic mapping, or tagging, of genomic regions contributing to the control of a phenotypic trait of interest can be a long and expensive process involving selection of appropriate parents, production of a mapping population (or mapping populations), phenotyping and genotyping the mapping population progenies, genetic linkage analysis of the marker data set, and combining the linkage map and phenotyping data sets in QTL analysis. The quality of the output of QTL analysis can be adversely affected by problems at any stage of the process, so short cuts often end up being expensive in the long run. It is usually necessary to strike some balance between cost (in time, and in human and operational resources) of the QTL mapping project and the confidence that you expect to have in the results from that program, but it is better to not invest at all in a QTL mapping project than to seriously under-invest in one (Fig. 30).

Marker-assisted selection (MAS)

Where these are available, molecular markers can now provide appropriate complements to conventional breeding methods in some crops, including sorghum. They are effective, and sometimes appropriate tools for crop improvement research addressing biological components in agricultural production systems. Molecular markers offer specific advantages in the assessment of genetic diversity and in trait-specific crop improvement. Marker-assisted selection in backcross and pedigree breeding programs is, or will be, cost-effective for introgression of some traits. Marker-assisted selection will help in rapidly diversifying the range of genotypes in which a rare trait (eg, high seedling leaf blade trichome density) is available. It will also be very helpful for pyramiding genes that would conventionally require testcross progeny evaluation to detect desirable segregants (eg, adding seedling glossiness and high trichome density to the genetic background of an elite hybrid parental line to improve the shoot fly resistance of its hybrids). It is exceptionally helpful for manipulation of resistances against pathogens, or pathogen strains, for which there are phytosanitary restrictions on pathogen movement. Finally, marker-assisted selection should be useful for traits, such as drought tolerance or improved grain and stover yield in hybrid combination, which are difficult or expensive to assess reliably.

Marker-assisted selection involves a number of important steps:

- choice of appropriate elite parents and donor parents,
- marker polymorphism assessment,
- breeding population development,
- DNA sampling,
- Foreground selection (genotyping individual segregants at marker loci to ensure donor trait allele introgression),
- marker-aided selection of desirable segregants,
- Background selection (optional genotyping of desirable segregants at marker loci to speed recovery of eliteness followed by selection of most desirable segregants and,
- rapid generation advance.

Choice of parents: Molecular marker information, complemented by good quality phenotyping, can greatly facilitate the appropriate choice of trait donor parents and elite parents to be used in crosses in a marker-assisted breeding program. The donor parent will be contributing to the progeny its allele(s) at previously mapped QTL(s). The donor parent should be genetically distant from the elite parent, at least in genomic regions carrying QTLs of interest. Thus the best options for donor

parents are typically a mapping population parental line, a mapping population progeny, or the product of an earlier cycle of marker-assisted breeding. The elite parent for a marker-assisted pedigree or backcross selection program should be an economically important genotype that is weak for a particular economically important target trait for which QTL mapping information is available. In the simplest case it is the susceptible parent of a mapping population. Finally, the elite parent must exhibit marked marker polymorphism compared with the donor parent, at least for the genomic regions associated with the QTL(s) targeted for marker-assisted selection.

Marker polymorphism assessment: The purpose of marker polymorphism assessment of the elite and donor parents is to determine (or confirm) the extent of marker polymorphism between the candidate donor parents and candidate elite parents. Such marker polymorphism is absolutely essential in the regions of target QTLs, as without it there is no basis for marker-assisted selection. In non-target regions of the genome, marker polymorphism can permit more speedy recovery of elite recurrent parent genotype in a backcrossing program (by “background selection”), but is likely to be associated with undesirable traits that may require additional cycles of crossing or backcrossing to eliminate from product lines.

Breeding population development: The breeding population size (F_2 , $BCnF_1$, or $BCnF_2$) must be large enough to ensure a reasonable probability of obtaining desirable segregants given the number of target QTLs and the length (in recombination units) of the genomic regions associated with each of these. Simple tables and prediction equations (eg, Sedcole 1977), and software packages are available for estimating the population size that you require, but a simple rule of thumb is that three times the minimum perfect population will give about 5% probability of failure, and five times this number of plants will give less than 1% probability of failure to obtain at least one desirable segregant (Table 17). The strategy that we typically follow here at ICRISAT is to advance two independent populations per target QTL (or combination of target QTLs), each with a population size approximately four times the minimum perfect population. Of course, all of this means that with the same amount of resources you can move many more QTLs in parallel programs than you can move simultaneously in a single population. This in turn means that the pathway to pyramiding a number of target QTLs in a single genetic background can get quite complex (eg, Fig. 4).

DNA sampling: For marker-assisted selection in a segregating population, DNA sampling has very different requirements than for other marker-related activities. You need only a very small sample of “reasonable” quality from each individual in the breeding population. This sample should be good enough for 10–20 PCR reactions per sampled individual. The important thing is to collect, and keep track of, samples from each of the individuals in the segregating population that are being

considered for generation advance. For PCR-compatible markers, tissue sampling should be completed early—perhaps 10–15 days after seedling emergence. Each plant needs to be labeled at the time of tissue sampling, and the corresponding plant number should be unique to avoid confusing samples being prepared for various purposes. Tissue samples are either chilled (collected on ice) or fixed immediately (eg, by placing them in absolute ethanol) to prevent DNA degradation. Each tissue sample is then disrupted to release DNA into solution, using a mortar and pestle with dry ice or liquid nitrogen if sample numbers are small, or using a semi-automated technique if the lab doing the work has high throughput capabilities. DNA is extracted from the solution using various commercially available kits or ‘mini-prep’ protocols. The protocol used in the MS Swaminathan Applied Genomics Laboratory here at ICRISAT-Patancheru (Mace et al. 2003) uses CTAB buffer. The quality and concentration of the DNA samples is then assessed by spectroscopy and/or agarose gel electrophoresis, and the samples having adequate quality are diluted to a standard concentration for use as template in PCR reactions with various primer pairs. DNA samples that do not have acceptable quality are discarded and fresh tissue samples are collected and processed to replace the discarded samples.

Table 17. How many plants and progenies does one need to genotype?

Generation	Target genes	Minimum perfect population	Plants needed for a given probability of failure to obtain at least n desirable individuals						
			5% failure			1% failure			
			$n=1$	$n=3$	$n=5$	$n=1$	$n=3$	$n=5$	
F_1	1	1	1	1	1	1	1	1	1
F_2		4	11	23	34	17	31	43	
BC_1F_1		2	5	11	16	7	14	19	
F_1	2	1	1	1	1	1	1	1	1
F_2		16	47	99	144	72	132	182	
BC_1F_1		4	11	23	34	17	31	43	
F_1	3	1	1	1	1	1	1	1	1
F_2		64	191	401	584	293	535	739	
BC_1F_1		8	23	49	71	35	64	89	

After Sedcole (1977).

Foreground selection: DNA marker data is generated for three to five loci flanking the genomic region associated with each QTL being targeted for selection in a particular segregating population. The use of at least a pair of flanking markers is generally preferred to use of a single “perfect” marker (which is only rarely available), and use of the third marker reduces the potential for inadvertent loss of the favorable allele at the target QTL due to multiple recombination events. For marker-assisted selection to be effective, it is more important that the order of these foreground marker loci is known with certainty than it is that they are all very close to the target QTL. In fact, three markers distributed across a 15–20 cM interval containing the target QTL are sufficient for an effective marker-assisted selection program—provided that the trait donor parent does not also carry seriously deleterious alleles at other loci in this interval. Foreground marker genotyping in the next generation of marker-assisted selection will provide an opportunity to identify mistakes that have been made in foreground marker genotyping for the current generation, so using a large number of markers in the target region is not the normal practice.

In order to avoid delaying the marker-assisted breeding program, this foreground marker data generation needs to be completed by the time the first individuals in the segregating population come to flower—unless all of the plants in the population can be selfed and/or backcrossed to the recurrent parent, in which case the foreground marker data must be produced prior to harvest of the pairs of selfs and backcrosses made on individuals having the desirable foreground marker genotype. This means that the foreground genotyping work must be completed relatively quickly in sorghum (which has relatively low tillering ability and for which controlled crossing required tedious manual emasculations); whereas in pearl millet (where growth cycle is very short, most genotypes can be induced to tiller, and controlled crosses are easy to make), it is operationally simplest to plan on selfing and backcrossing all plants in the segregating population while generating the required foreground marker data.

Operational costs of foreground genotyping can be reduced somewhat by selective genotyping of individuals in the segregating population—for example, one might only genotype individuals at the flanking marker locus immediately below the target QTL that had already been shown to have the donor parent allele at the flanking marker locus immediately above the target QTL. However, the juggling of DNA samples that is required for this may slow the marker genotyping process (especially with “slow” marker genotyping methods such as RFLP), or make the marker data generation process sufficiently complicated that data tracking mistakes jeopardize the validity of its results. Therefore, it is probably safest to genotype the entire segregating population for at least three markers in the target region for

the particular QTL of interest, and then to perform selective background marker genotyping (see below) on DNA samples from individual segregants that have the desirable allelic complement at the foreground marker loci.

Marker-aided selection: At least one individual from the segregating population is identified having donor parent alleles (heterozygous or homozygous, depending upon the breeding scheme and generation) at the three central loci (one on either side of the QTL and one as close to it as possible) associated with a particular QTL. Such individuals are highly likely to have the favorable allele at the target QTL. Self and/or backcross progenies of such individuals are used for generation advance. It is operationally safer to advance several progenies for each target QTL and allow for some mistakes in the marker genotype data — unless one is willing to take the risk of the breeding program being set back a generation when such genotyping mistakes (or other types of data tracking or seed packet tracking mistakes) occur.

Background selection: When more than one individual in the segregating population is identified as having the desired marker genotype at loci flanking the target QTL, there are opportunities to select between these for agronomic eliteness. This can be done on the basis of the phenotypic appearance of the plants themselves or on the basis of marker allele composition across genomic regions expected to segregate independently of the target QTL. The latter is referred to as background selection, and preference is given for plants having the highest degree of recovery of the marker genotype of the more elite parent in genomic regions distant from the target QTL. The number of markers to be used in this background genotyping will depend upon the number of individuals that are available with favorable foreground genotype and the number of individuals that can be advanced to the next generation. The highest priority for background genotyping is often given to identifying recombination events flanking the QTL target region (Fig. 30) that will minimize the size of the introgressed region from the donor parent. A complementary alternative is to use one marker on each chromosome arm, perhaps with another for each centromeric region, for linkage groups other than that on which the target QTL is located. Because sorghum has a small number of chromosome pairs, and appears to have limited recombination in centromeric regions, this means that 20-30 background markers is probably the most for which use can be justified in a given generation. Of course, in the next generation of marker-assisted selection, background genotyping does not need to consider loci for which the elite parent allele has already been demonstrably recovered in homozygous form, and additional background marker loci can be considered.

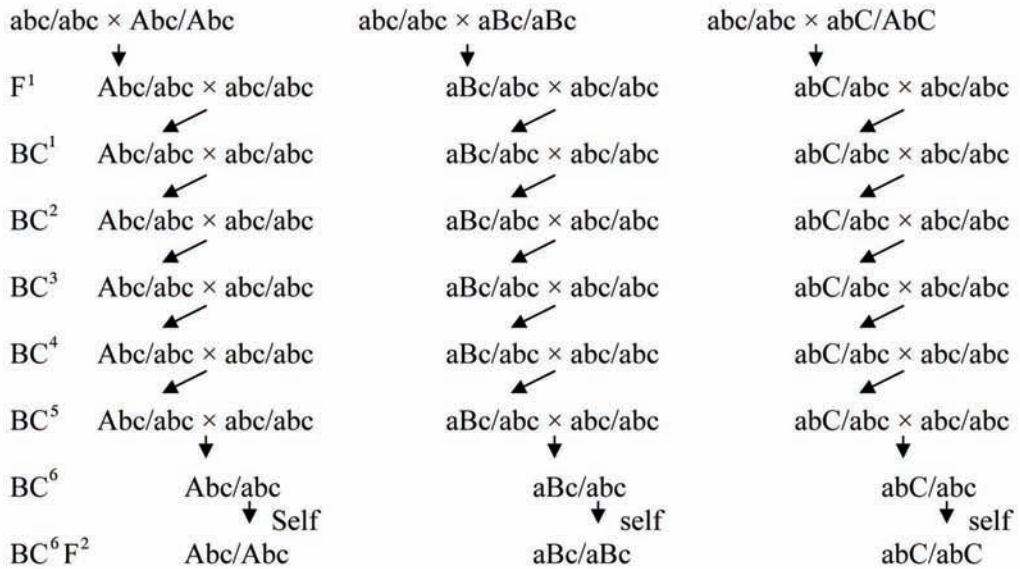
One of the most strongly argued cases for marker-assisted selection has been that background genotyping will allow the breeding program to reduce the number of generations required to recover an introgressed gene from the donor parent in a highly elite genetic background. Simulation modeling indicates that two or three

generations can be saved, but at the cost of a lot of additional marker genotyping. In a short-lived crop like sorghum, it is often going to be faster to make minimal use of background genotyping (at least until DArT markers are readily available) and instead let rapid generation advance in a backcrossing program reduce the average percentage of the donor genome in the segregating population by 50% per backcross generation.

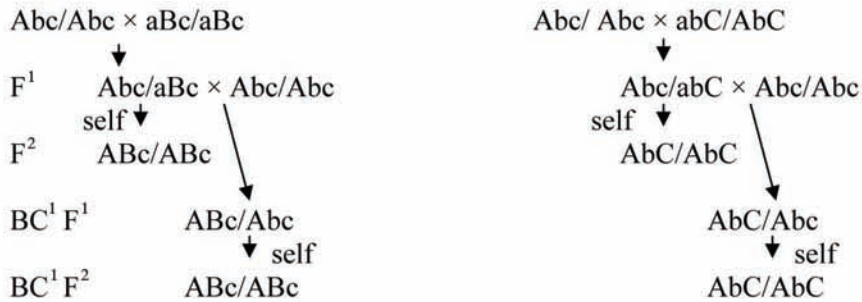
Rapid generation advance: When marker genotyping is relatively slow compared to the crop life cycle (for example, if you are forced to use autoradiography to visualize allelic differences in RFLP markers), then it may be expedient to advance two crop generations between stages with marker genotyping. For example, one could restrict marker genotyping to the BC_2F_1 , BC_4F_1 , BC_6F_1 and BC_6F_3 generations. When this is done, you may end up actually needing to generate more marker data as you need to increase population sizes (with appropriate population structure) in order to ensure that you carry forward the target QTL. For example, in order to ensure the same probability of carrying forward a target QTL to BC_2F_1 that could be obtained by foreground genotyping 7 BC_1F_1 individuals and then foreground genotyping a single family of 7 BC_2F_1 individuals from the selected BC_1F_1 (genotyping a total of 14 individuals), you would need to foreground genotype 7 BC_2F_1 families each containing 7 individuals (genotyping a total of 49 individuals). However, regardless of whether marker genotyping is fast or slow compared to the crop life cycle, the breeding program should push forward the generations as quickly as possible so as to generate a useful product in the shortest possible time and maximize the return on resources invested in the marker-assisted breeding program.

Thus molecular marker-assisted breeding is an expensive process involving selection and crossing of appropriate parents, characterizing marker polymorphism of those parents, production of a segregating population (or segregating populations) of appropriate size (one that ensures a high probability of obtaining desirable segregants in the number required), DNA sampling all individuals in the segregating population, marker genotyping all individuals in the segregating population at “foreground” loci flanking the target QTL, and selection among the individuals in the segregating population on the basis of this foreground marker data. The individual segregants selected on the basis of this foreground marker data are selfed and/or backcrossed. If the number of such desirable segregants is larger than can be advanced to the next generation, then there is an opportunity for optional background genotyping to increase the degree of agronomic eliteness of individuals selected for advance. Rapid generation advance—with or without marker genotyping every generation—with some inbuilt redundancy will help ensure that the marker-assisted breeding program takes as little time as possible to generate useful products.

Introgression of individual QTLs:



First pyramiding cycle:



Second pyramiding cycle:

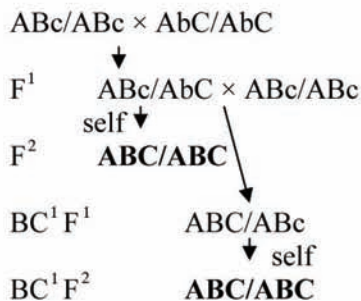


Fig. 30. Scheme for pyramiding three QTLs in a common background (assuming capitalized alleles are favorable).

Current status of QTL mapping and gene tagging in sorghum

QTL mapping and other gene tagging procedures have identified flanking markers for several sorghum target traits, including

- tolerance to pre-flowering drought stress (Tuinstra et al. 1996)
- the stay-green component of terminal drought tolerance (Tuinstra et al. 1997, 1998; Crasta et al. 1999; Subudhi et al. 2000a, 2000b; Tao et al. 2000; Xu et al. 2000; Kebede et al. 2001; Sanchez et al. 2002; Haussmann et al. 2002b; Harris et al. 2007)
- tolerance to aluminum (Magalhaes et al. 2004)
- resistance to *Striga hermonthica* (Haussmann et al. 2004)
- resistance to several insect pests including
 - green bug (Agrama et al. 2002; Katsar et al. 2002; Nagaraj et al. 2005)
 - head bug (Deu et al. 2001)
 - yellow sugarcane aphid
 - sorghum shoot fly (Folkertsma et al., submitted; Sajjanar 2002; Deshpande 2005; Mehtre 2006)
 - sorghum midge (Tao et al. 2003)
- resistance to foliar diseases
 - downy mildew (Gowda et al. 1995, Oh et al. 1996)
 - anthracnose (Boora et al. 1998, Mehta 2002)
 - leaf blight (Boora et al. 1999)
 - rust (Tao et al. 1998)
- resistance to grain and panicle diseases
 - head smut (Oh et al. 1994)
 - components of grain mold resistance (Klein et al. 2001a)
 - plant height
 - grain hardness
- resistance to sprouting (Lijavetsky et al. 2000)
- domestication-related traits, weediness and perenniality (Paterson et al. 1995a, 1995b; Hu et al. 2003)
- plant height (Lin et al. 1995; Pereira and Lee 1995; Ming et al. 2002)
- flowering time (Lin et al. 1995; Ming et al. 2002)
- grain quality, productivity and other morphological traits (Rami et al. 1998; Sanchez-Gomez 2002)
- tillering and other morphological traits (Hart et al. 2002) and
- photoperiod-sensitivity of flowering time (Trouche et al. 1998; Chantereau et al. 2001).

In the case of rust resistance, the gene responsible for the QTL detected has been identified and several sequence variants characterized (McIntyre et al. 2004). Similarly, it appears that the gene responsible for Al⁺⁺⁺ tolerance has also been identified (J. Magalhaes, pers. comm.).

Conclusions

It is now practical, in at least some cases, to use marker-assisted methods for step-wise maintenance and enhancement of elite cultivars in sorghum hybrid programs. This can be done to extend the economically useful lifespan of important sorghum hybrids, and their parental lines, and to more efficiently target step-wise improvement of the sorghum hybrid combinations that best meet farmers' needs. Considerable additional work is required to make the set of molecular genetic tools currently available for marker-assisted breeding in sorghum both more broadly applicable and more breeder friendly, but the work required to achieve this is well underway and application of these tools in public- and private-sector sorghum programs has begun.

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Development and testing of transgenics in sorghum

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Introduction

Advances in transgenic technology have enabled the transfer of agronomically desirable traits into crop species from diverse sources across reproductive barriers. Entire process of crop improvement through transgenic technology can be divided into (i) production of transgenic plants (ii) transgenic breeding program (iii) release of products.

Production of transgenic plants

The gene of interest is introduced to the crop gene pool through the process of genetic transformation. The chief methods employed for transfer of genes are (i) particle bombardment (ii) *Agrobacterium*-mediated process (iii) *in planta* methods. The former two methods of transformation are frequently reported and are based on the efficiency of protocols in tissue culture. Tissue culture protocols are, in general, genotype dependent and hence, allow the transformation of a few selected genotypes. Therefore, the gene of interest is initially introduced into a transformation amenable model genotype(s) and is backcrossed to desirable genotype backgrounds. In rice, genotypes belonging to *japonica* groups such as T 309, and a few genotypes of *indica* group such as IR 72, IR 64, Radon and aromatic rice varieties such as Pusa Basmati 1, Basmati 370, are identified as model genotypes. A set of transgenes responsible for production of vitamin A were initially introduced into the model genotype, T 309 and were further backcrossed into elite genotypes cultivated in India. Similarly in maize, A 188 and its derivatives are used for acquiring new genetic traits. Bobwhite lines in wheat, Golden Promise and Igri in barley were identified as the most responsive to genetic transformation. Sorghum is recalcitrant to tissue culture and thereby to genetic transformation compared to other cereals. Model genotypes that can be readily transformed with far greater efficiency and reproducibility are not available in sorghum, and thus the genotypes of interest are directly used.

In order to overcome the difficulties encountered in *in vitro* protocols, *in planta* methods and direct transformation of developing tissues with gene guns are

employed, though the transformation efficiency is far lower than the methods described above. After production, the transgenic plants are evaluated for the levels of expression of transgene trait and the stable inheritance of the transgene in subsequent generations. Development of transgenic sorghum plants for agronomically important traits at research level is presented in Table 18.

Transgenic breeding program

Transgenic plants that are regenerated immediately after transformation are usually designated as T_0 generation and carry the transgene in hemizygous condition. These plants are selfed to obtain T_1 generation that segregate in monogenic pattern of inheritance and segregate in 1:2:1 ratio (1 homozygous for transgene, 2 heterozygous for transgenes and with no transgene).

Most of the transgenic plants show carryover effects due to the prolonged culture during genetic transformation. To overcome these undesirable genetic changes due to somaclonal variation, one or two backcrosses to the parent and selection of desirable phenotypes from the backcross progeny are practiced. After obtaining the isogenic lines for the transgene, the plant population can be subjected to the evaluation for transgene expression. This holds true especially for transgenes related to tolerance to biotic and abiotic stresses. Therefore, it is ideal to test for the stress tolerance after achieving refined genetic background. In case of value added traits, like nutritional quality, dough quality and quality protein, testing can be initiated in early generations. Thus, preliminary trials for stress tolerance are conducted in elite genetic backgrounds and the promising transgene events can be transferred to other genotypes of interest. If the transgenes are developed in hybrid parental lines, agronomic advantage of transgenic trait is tested initially in heterotic combinations and the promising events in hybrid combinations are used for development of products.

Release of products

Finished breeding products developed using transgenic technology is passed through bio-safety regulations. India is ready with its policy for bio-safety measures. The introduced molecule/trait should be tested and declared safe for consumption and the environment. Field trials for agronomic advantage are conducted at two stages.

Stage 1: Limited field trials are carried out at research level to identify promising events from the transgenic material.

Stage 2: Large scale trials are conducted for release of the transgenic variety in the targeted geographic location to declare their agronomic superiority over the existing

cultivars. These trials are conducted by regulatory bodies of the government. In India, committees as RCGM (for limited trials) and GEAC (for approval for cultivation) are regulatory bodies.

Public acceptance of the transgenic product is the key for measuring the success of transgenic technology. Before the release of the transgenic variety it is ensured that the variety is acceptable for cultivation in general, eg, all transgenic cotton varieties in India can be released only if they express higher levels at least by 15% of resistance to CLSV (Curly Leaf Spot Virus) disease. The genetically modified (GM) potato varieties are tested for their cooking and other characters before they are released for commercial cultivation.

In the era of globalization and enactment of plant variety protection acts in most countries, it is expected that transgenic crop varieties will fetch huge revenue and thus trigger increased investment for transgenic breeding by private sector. The public sector research and development organizations are investing more on basic research on developing transgenic technology in economically important crops. A few examples of transformation of sorghum for important traits are provided in Table 18.

Table 18. Transformation of sorghum with agronomically important traits at research level.

Trait	Transgenes	Method	Organization	References
Resistance to stem borer	<i>Bt cry1Ac</i>	Bombardment	ICRISAT, India	Girijashankar et al. (2005)
Resistance to stem borer	<i>Bt cry1Aa & cry1B</i>	Bombardment	NRCS (ICAR), India	Visarada et al. (2006)
Resistance to stalk rot	<i>Rice chitinase</i>	<i>Agrobacterium</i> and bombardment	Kansas Univ., USA	Zhu et al. (1988), Krishnaveni et al. (2001)
Enrichment of lysozyme (nutritional improvement)	<i>dhcps-r1</i>	Bombardment	Vrije Univ., Belgium	Tadesse et al. (2003)
Drought resistance	<i>HVA1</i>	Bombardment	Michigan Univ., USA	Devi et al. (2004)
Drought resistance	<i>mtID</i> , <i>p5CSf129A</i> and <i>codA</i>	<i>Agrobacterium</i> and Bombardment	CRIDA (ICAR), India	Maheswari et al. (2006)

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Bio-safety in transgenic crops – sorghum

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The United Nations Conference on the Human Environment held at Stockholm in June 1972, in which India participated, took a decision to take appropriate steps for the protection and improvement of human environment, other living creatures, plants and property. Accordingly, to exercise the powers conferred by sections 6, 8 and 25 of the Environment (Protection) Act, 1986 (29 of 1986) and with a view to protecting the environment, nature and health, in connection with the application of gene technology and micro-organisms, the Government of India (GOI) under the Ministry of Science and Technology and Department of Biotechnology formulated the Bio-safety Rules. These Rules are applicable to:

1. Import and storage of micro-organisms and gene-technological products.
2. Genetically engineered organisms, micro-organisms and cells and any substances and products and food stuffs, etc, of which such cells, organisms or tissues hereof form part.
3. New gene technologies, organisms/micro-organisms and cells generated by the utilization of such other gene-technologies and to substances and products of which such organisms and cells form part.

At the Institute level, the Institutional Bio-safety Committee (IBSC) will oversee the work where recombinant (r) - DNA techniques are used. IBSC shall be constituted by an occupier or any person including research institutions handling microorganisms/genetically engineered organisms. The committee shall comprise the Head of the Institution, scientists engaged in r-DNA work, a medical expert and a nominee of the Department of Biotechnology (DBT). The occupier or any person including research institutions having microorganisms/genetically engineered organisms shall prepare with the assistance of the Institutional Bio-safety Committee (IBSC) an up-to-date on-site emergency plan according to the manuals/guidelines of the Review Committee on Genetic Manipulation (RCGM) and make available copies to the District Level Committee/State Biotechnology Co-coordinating Committee and the Genetic Engineering Approval Committee (GEAC).

Generally, the IBSC will meet twice in a year to review the progress of r-DNA work and report the same to the DBT. It will also review and recommend applications for large scale/field testing of the transgenic materials to the RCGM. When such trial(s)

are under progress, the IBSC will meet more frequently as deemed necessary along with other committees.

The National Research Centre for Sorghum (NRCS) is actively engaged in the development of transgenic sorghums for resistance to stem borer, salinity, low stalk HCN content in forage sorghums and improved grain dough quality.

Principles of seed production and seed quality maintenance in sorghum

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Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is grown world wide for food, feed, fodder, and fuel. In India sorghum is the most important cereal for poor people in the semi-arid tropics. Sorghum production, as is true for any crop production, is dependent on several inputs and production environment. Of all the inputs, seed is the key input in sorghum production as the quality of seed determines the quantum of economic yield. The high quality seed in terms of high genetic and physical purity, healthiness, high germination, vigor and viability ensure maximum production under suitable and favorable agro-climatic conditions. The impact of improved cultivars can only be realized if and only if seed of the cultivars in purest form reaches the target farmers. Several steps and procedures are involved in multiplication of seed of improved cultivars for timely supply of genetically pure seed to target farmers in the required quantity. The principles of quality seed production in sorghum are detailed in the following sections.

Seed multiplication chain

In India, the seed is multiplied in four stages viz., a) Nucleus seed, b) Breeder seed, c) Foundation seed, d) Certified seed. Nucleus seed is the initial handful of seed that has originated through selection/breeding by the breeder. It is the only class of seed that is regenerated from itself and is produced in very small quantity under the supervision of the originating breeder. The purity of the subsequently multiplied breeder seed, foundation seed and certified seed depends on the purity of nucleus seed and therefore, it must be produced with utmost care. The breeder seed is produced from nucleus seed in small quantities in research stations by the sponsoring breeder under his direct supervision. The organization sponsoring cultivar release has the responsibility for production, safe storage and supply of breeder seed. The foundation seed is produced from breeder seed in farmers' fields/ research stations of recognized institutions/organizations under the supervision of the concerned breeder and needs certification. The certified seed is produced from foundation seed in farmers' fields under the supervision of the concerned breeder and needs certification. The seed certification under Indian law is voluntary. Apart

from certified seed, the seed is also sold as truthfully labeled seed (TLC). However, certified seed has advantages like high genetic and physical purity, freedom from diseases and pests, high germination and seedling vigor. The seed of different classes can be produced by forecasting the demand of annual certified seed requirement based on multiplication ratio, replacement rate and additional seed requirement. The area to be planted and target seed of each class is calculated by multiplying area and quantity with a factor $\times 200$ (Table 19).

Table 19. Estimation of seed targets (t) and area requirement (ha) for different classes of seed multiplication.				
	Breeder seed	Foundation seed	Certified seed	Grain
Area (ha)	0.5 ha =	100 ha =	20,000 ha =	4 m ha
Quantity (t)	1 t	200 t	40,000 t	

Genetic purity maintenance

The genetically pure seed of a variety is expected to have all the unique economic and diagnostic characters. In cross pollinated species like sorghum inbred lines and varieties, the deterioration will be much faster due to contamination with undesirable pollen of other genotypes. The major factors causing deterioration of varieties or inbreds leading to the production of seeds with low genetic purity are (a) residual genetic variation, (b) developmental variations, (c) cross-pollination due to undesirable pollen (d) mechanical mixtures, (e) damage due to pest and diseases. The other factors that may have influence on genetic purity are (i) plant breeder's techniques during selection programs and (ii) mutations. The following important measures should be followed during sorghum seed production in order to maintain genetic purity.

- 1) Adequate attention must be given to land requirements, isolation, rouging, harvesting, drying, sorting of ears, threshing of ears, etc, so as to maintain maximum possible genetic purity.
- 2) Proper class of seed should be the source for further multiplication.
- 3) The best cultural practices should be followed.
- 4) Inspection should be done of seed plots at all critical stages.
- 5) Mechanical mixtures should be avoided at sowing, harvesting, threshing, processing and storage.

Essential requirements of genetic purity maintenance

Seed source: The seed for sowing should be collected from authentic source depending on the stage of seed multiplication.

Isolation of seed production plots: Sorghum is generally a self pollinated crop but cross pollination up to 5–6% may also occur. It varies from 2–10% depending on the genotype and panicle type. Isolation distance for seed production depends on the class of seed, ie, foundation or certified seed and the presence of other types in the same species and related species. The pollen of forage sorghum types can spread to more distance and hence require more isolation distance (Table 20).

Rouging: Rouging of the seed field is very important for quality sorghum seed production. The rouging is done at three stages of crop growth (i) before flowering ii) at flowering time and iii) pre-harvest stage. The procedure for the same is described below (Kannababu and Seetharama 2002).

Table 20. Isolation standards for the production of different classes of sorghum seed.

Contaminants 1.1	Minimum distance (m)	
	Foundation seed	Certified seed
Fields of other varieties	400	200
Fields of same variety but not confirming to purity	400	200
Johnson grass (<i>Sorghum halepense</i>)	400	400
Forage sorghum with high tillering and grassy panicle	400	400

Note: Different flowering dates for modifying isolation distances are not permitted.

Rouging before flowering

- Start the rouging operation before off types, volunteers or shedders in the female rows start shedding pollen.
- All rouges and volunteer plants must be cut from ground level or pulled out to prevent re-growth and subsequent contamination of seed crop.
- Out crosses can be identified because of their greater height and should be removed as soon as these are noticeable.

Rouging at flowering

- Rouging should be done every day to remove pollen shedders in the seed rows. The sterile types have only the stigma, or a few abortive anthers exerted. These should not be mistaken for normal fertile plants. Normal fertile plants will have rich yellow anthers, which are full of pollen out to the tips of both lobes. On shedding, these lobes rupture on distal and discharge pollen. All plants out of place, ie, plants in between the rows, male plants in female rows and vice-versa have also to be removed. Special attention should be given at the ends where the border rows and seed rows meet, as male seed may fall in female rows (or female in male rows).

- In addition, to remove off types and volunteers within the field, eliminate other sorghum types and other related plants such as Johnsongrass, Sudangrass and forage plants from within the isolation distance. These sources of undesirable pollen must also be eliminated before pollen is produced.

Pre-harvest rouging

- The field should also be rouged thoroughly before harvest and after seed maturity to the stage when the true plant and seed characters are 'apparent'.

The permissible off-types in different classes of sorghum seed production are given in Table 21.

Table 21: Permissible frequency off-types in different classes of sorghum seed production.

Factor	Maximum permitted (%)	
	Foundation seed	Certified seed
Off-types (earheads) at any one inspection at and after flowering	0.05	0.10
Earheads infected by kernal smut and grain smut and head smut at final inspection	0.05	0.10

Note: Seed fields should be thoroughly rouged to remove plants infected by sugary disease (*Sphacelia sorghi*)/Ergot (*Claviceps* spp.), so that the prescribed standards are met at seed stage. However the seed fields shall not be rejected on account of presence of sugary/ergot infected earheads.

- Seed fields can however be certified if diseased panicles are removed and burnt and the fields show on re-inspection, infection not more than maximum permissible level. Only one such re-inspection is permitted (Kannababu et al. 2004).

Seed crop management

Production environment: The area where the temperature during flowering ranges from 27–32°C is best suited for good seed production of sorghum. Night temperature should not fall below 11°C for longer period since it affects the seed development. Flowering and seed development stages should not coincide with the rains as the pollen loss and grain mold deteriorate seed quality. The fields where sorghum was not grown in the previous season should be selected. In addition, there should be no Johnsongrass in the seed field or within isolation distance. The field should be well leveled and drained. Saline, alkaline or very light soils are not suitable. Uniform and level piece of land with good drainage should be selected. The pH should be around 5.5 to 8.5. Good irrigation facilities are essential for sorghum seed crop.

Season: Sorghum seed production is mostly undertaken during *kharif* in Maharashtra, Madhya Pradesh, Rajasthan and Gujarat. In the other sorghum growing areas, it is taken up in *rabi* or summer season. Seed produced in seasons other than *kharif* has good germination and vigor. During *kharif*, problems due to grain mold arise frequently. The sowings should be carried out before the end of June and September in *kharif* and *rabi* seasons, respectively. Early sowing eliminates shoot fly attack and seed crop passes through its life cycle at the most optimum environmental condition for crop growth and seed development. The seed of commercial hybrids is produced in *rabi* and summer seasons both by public and private seed agencies and marketed in summer season itself. However, seed for *rabi* has to be stored till the next *rabi*. Consequently, there is some difficulty in the availability of seed for *rabi* leading to stagnant production of *rabi* sorghum. The seed production in *rabi* is predominantly concentrated in Andhra Pradesh and adjacent areas of Karnataka due to favorable ecological conditions.

Seed treatment: Shoot fly can be effectively controlled by treating the seed with carbofuran before sowing. Seeds are treated with 50% soluble powder at 100 g for every kg of sorghum seed.

Sowing: Seed should be placed at 3-4 cm depth. Maintenance of male sterile line (A-line) involves sowing of two parents ie, A-line (male sterile) and B-line (male fertile, non-pollen restorers). Similarly, certified seed production of hybrids includes male sterile A-line and fertility restorer R-line. The border rows (4–6) should be sown with male line all round the seed production plot. To facilitate frequent rouging operation, a spacing of 60 cm (row to row) and 15–20 cm (plant to plant) is advisable. Precautions should be taken to avoid admixing two parental lines at the time of sowing. For A-line seed production the seed rate is 7.5 kg ha⁻¹ of A-line and 5 kg ha⁻¹ of B-line. The usual planting row ratio of A- and B-lines is 4:2 for breeder seed production. For certified hybrid seed production, the female and male lines in 4:2 can be sown. However, the proportion can be widened to 6:2. The general seed rate varies from 7–8 kg/hectare depending on spacing.

Fertilizer application: Recommended dose of fertilizers (80 kg N: 40 kg P₂O₅; 40 kg K₂O ha⁻¹) should be applied for obtaining optimum yield and good quality seed. Higher fertilizer dose (100 kg N:50 kg P₂O₅:50 kg K₂O ha⁻¹) enables better expression of plant characters.

Irrigation: Risk should not be taken for growing seed crop in unirrigated area. Crops for all seed categories of seeds should be grown under assured source of irrigation. In sorghum flower primordial initiation, boot leaf, flowering and grain development are the most critical stages. Moisture stress at any of the stages will result in significant reduction in seed yield. Hence, irrigation is essential if there is drought at these stages during *kharif* season. In *rabi* season, irrigation should be given at all the critical stages.

Plant protection: Successful disease and insect pest management is one of the most important factors in raising a healthy seed crop. Seed plots of all categories of seeds should be raised from seeds treated with proper fungicide/insecticide. Rouging of diseased plants should be done promptly. Spraying with insecticides and fungicides in proper doses is recommended throughout the crop growing season.

Pest control

Shoot fly: Seed treatment with Carbofuran before sowing controls shoot fly infestation. Seeds are treated with 50% soluble powder at 100 g kg⁻¹ of sorghum seed. It can also be controlled by applying Furadan 3G or Phorate 10G in the seed furrows @20 kg ha⁻¹ at the time of sowing.

Stem borer: Stem borer can be effectively controlled by application of Endosulfan 4G/4D, or Carbaryl 3G or Malathion 10D or Furadan 3G @8-12 kg ha⁻¹ at 20 and 35 days after emergence.

Midge: High levels of midge infestation can be controlled by spraying any of these insecticides: Endosulfan 35 EC 1 litre, or lindane 20 EC 1.2 litres, or malathion 50 EC 1 litre per hectare in 500–600 litres of water followed by second application 4–6 days later.

Head bug: The population density (50 nymphs/panicle) at pre bloom and 50% flowering stage requires dusting of Malathion 10D@20kg ha⁻¹.

Disease control

Grain molds in *kharif* and charcoal rot in *rabi* are the key diseases. The seed should be treated with Thiram or Captan @3g kg⁻¹ of seed. Grain mold can be checked with Aureofungin solution @30g/10 lt of water + Captan (30g/10 lt of water) or Dithane M 45 + Captan @ 3% concentration). Charcoal rot can be reduced by proper soil management practices to conserve moisture, besides growing tolerant cultivars. Leaf spot such as rust can become serious in favorable climate during the *kharif* and *rabi* seasons, can be controlled by spraying Dithane M 45 @ 3% concentration. Sugary disease in hybrid sorghum seed production plots where female parent become infested, can be managed to a certain extent by spraying Dithane M 45 or Dithane Z-78 @2g/lt of water or Bavistin @ 5g/10 lt of water at flowering stage. For chemical control of honeydew stage of ergot disease, spray Benlate (0.1%) at the stage of 50% flowering. For downy mildew control, spraying of Dithane M 45 (0.4%) four times at an interval of one week starting from seventh day after planting has proved to be the best.

Intercultivation: Hand weeding after 20 days of sowing is preferable. Intercultivation with guntaka (blade harrow or Danti cultivator) will help to control weeds and conserve moisture. Pre-emergence spraying of Atrazine (atrataf) at 0.5kg active ingredient per hectare or propazine 50% wettable powder @1 kg in 1000lt of water can control weeds.

Harvesting and threshing: The seed crop must be fully ripe before harvesting. Harvesting should be done at physiological maturity stage when the black layer formation appears at the point of attachment of seed with the caryopsis. In general, the seeds harvested 35–45 days after flowering have superior seed quality. Artificial drying is followed for seed production in *kharif* to avoid grain mold incidence. The harvested heads should be sorted out to remove diseased or otherwise undesirable heads, and dried on the threshing floor for a week or so in a thin layer before threshing. Doubtful ear heads are rejected. The border rows of seed plots should be avoided to prevent the chances of natural contamination. The male rows should be harvested first and kept separately to avoid mechanical mixtures. After this, the female rows should be harvested. Threshing can be done by clean machine threshers at proper seed moisture content (13–14%). Seed should be dried to 10–12% moisture content before storage. Care should be taken to avoid mechanical mixtures while threshing.

Hybrid seed production

Sorghum hybrid seed production is a highly commercial venture. It is essential to maintain efficient level of crop management in order to maximize production at minimum cost. The basics for hybrid seed production are as follows.

Determining the hybrid seed quantity targets: The quantities of hybrid seed required should be roughly estimated on an annual basis in advance, depending upon the projected demand for the commercial hybrid under cultivation. It is desirable to maintain significant quantities of carry-over seed as an insurance against unforeseen seed crop losses.

Planting ratio: Male sterile (A) and restorer (R) lines are sown in alternate strips of rows, normally in a ratio of 4A:2R, depending on the local experience of success and the ability of the R-line to disperse the pollen. The borders on all four sides of the hybrid seed production field are sown with the restorer (R) lines to ensure an adequate supply of pollen and guard against incoming stray pollen. The ideal planting ratio between male and female lines is two male rows alternated by 4 to 6 female rows. Where the male lines have the smaller earheads and shorter span of flowering compared to the female ones, (as in case of CSH 14 and CSH 15R) it is desirable to allow only four female rows for each pair of male rows. The female

rows for each pair of male rows can be increased to six if the male lines have larger panicle and longer span of flowering. A five row thick border all around the seed production plots must always be provided. Economizing on male lines both within the plots and borders, which many seed growers tend to do, may affect the seed set and is not a wise step.

Isolation requirement for hybrid seed plot: Getting required isolations (300 to 400 meters for foundation and 200 to 400 meters for certified) is increasingly becoming difficult for sorghum seed production. Hence, it is necessary that the hybrid wise seed production be planned in a few clusters of villages. Each cluster can have 2–3 contiguous villages with about 200 or more hectares. The number of clusters may depend on the total seed required based on demand and supply. Compact blocks are easy for supervision, maintenance of quality, minimizing the nicking and isolation problems to a major extent and will also serve as demonstration blocks.

Plant height: Most of the parental lines of sorghum hybrids have matching heights in the *rabi* season facilitating easy pollination process. The problem of disparity of heights can be avoided to some extent by planting the short parent on the raised ridges and the taller parent in the furrows below. Selective urea sprays also enable to increase the height to some extent by elongating the peduncle.

Synchronization: It is essential that the parental lines chosen for hybrid seed production flower at the same time ie, the viable pollen is available when stigmas are receptive. Therefore, a prior knowledge on the flowering patterns of both the parents in hybrid seed production is necessary. The male and female parents of the various hybrids, with different degrees of photo and thermo-sensitivities may react variably under different day length and temperatures at various locations or seasons. Several methods are employed to ensure synchrony. These are:

- The growth stages of male and female parents should be critically examined at 4 weeks stage or even later depending upon the length of their vegetative growth period.
- The flower primordia and the apex of male and female plants should be sampled randomly and observed critically by stripping the leaves of stem. The difference in the time of initiation and size of the panicle bud would indicate the difference in their time to 50% flowering.
- The parent lagging behind can be hastened by selective measures like supplementation of nitrogen in the soil (additional dose of 50 kg N/ha) followed by foliar spray of urea spray (2%), soaking of seeds in water, GA spray at primordial initiation stage (Kannababu et al. 2002).
- Alternatively, selective irrigation of one parent (late flowering) and delayed irrigation of the other will also help in synchronizing the flowering date of the parents.

- Careful manipulation of nitrogenous fertilizers, foliar spray of GA and irrigation can synchronize the flowering of parents that differ by up to one week.
- If the male is advanced in the early stage due to adverse seasonal conditions, cut alternate plants to allow the tillers to come up and boost such tillers with additional dose of nitrogen.
- In case of partial seed setting, sugary disease (ergot) may occur. Spray Thiram/Captan to control the disease and avoid prolonged sowings in the same areas, since the disease may invade the late sown crop in epiphytotic proportion. However, making available pollen to achieve good seed set ensures better control of ergot disease.

Pollen production and dispersal: The pollen production is influenced by temperatures. During the winter months, especially in areas where the night temperatures are rather low, pollen production and dispersal is appreciably reduced. 2% borax spray (on both male and female lines), two times from ear-head emergence till the completion of anthesis would greatly solve the problem. In fact, the staggered planting of the two male rows ensures adequate and prolonged availability of pollen. It is not safe to rely entirely on natural winds to aid in pollen dispersal. It is desirable to use artificial aids of pollen dispersal like tapping the male plant or blowing air through empty duster over the male heads. It is also advisable to spray 2% Borax to improve the pollen production and dispersal. If the pollen is not available in the same plot, collect the pollen in the morning from neighboring plots and instantly spray with water or dust on the earheads of the female parent. If there is dew fall hampering spread of pollen, blow empty power duster on the male rows to disperse pollen towards female heads or tap the male heads.

Stigma receptivity: Generally, the stigma retains good receptivity up to 4–5 days (MS 2219A, MS 296A and AKMS 14A) after flower opening, although in some lines it is extended beyond that period as in MS 2077A. However, during the hot summer months, the receptivity is lost faster owing to desiccation of stigmas.

Rouging: Regular rouging should follow the commencement of flowering. Apart from off-types, pollen shedders can also be a problem in A-lines. Shedders are plants that look similar to the A-line but exhibit fertile anthers and shed pollen. Such plants can only be identified at anthesis and should be uprooted immediately. Shedders can also arise from partial breakdown of sterility in the A-lines due to high temperatures (>38°C). Delay in identifying shedders will result in out crossing to male-steriles and subsequently contaminate the hybrid causing genetic deterioration. Therefore, it is recommended that rouging be carried out in the early morning hours before pollen shedding takes place. The R-line should also be rouged periodically.

Harvest of hybrid seed: All possible precautions against seed contamination should be taken during harvesting of hybrid seed production plots and threshing of

panicles from the A-line rows. Usually, the R-line is harvested first and the harvest removed from the field. Later the A-line rows are carefully inspected for off-types and other chance admixtures and then harvested. Hybrid seed yield (on the A-line) depends upon the yield potential of the A-line, percent seed set, and environmental conditions (Kannababu and Rana 2003).

Seed processing

Seed processing is an integral part of sorghum seed technology, which encompasses steps such as drying, cleaning, grading, treating and bagging. Sorghum seed properly threshed can often be cleaned to the desired purity on the air screen cleaner alone. However, the gravity separator is commonly used, to remove light materials and improve germination.

Physical purity: The threshed seeds should be physically pure and should not contain weed seeds, disease and pest infested seed, other crop seed, other cultivar seed, undesirable seed and damaged seeds. Specific seed standards for different classes of sorghum seed are furnished in Table 22. It is not desirable to sow the seed along with these contaminants as the yields and quality of resultant produce will be low. Seed processing includes several distinct steps that must be followed in a specific sequence. Sorghum seed of different classes should possess the following minimum seed purity standards.

Seed drying: Seeds contain varying amounts of moisture at harvest, and if they are to be stored for subsequent planting it is essential that their moisture content is reduced to a safe level. High moisture in seeds reduces seed viability and causes mechanical damage during processing. In addition to this, high moisture in seeds provides favorable atmosphere for pest and disease attack in storage. Sorghum seeds should be dried to the moisture content of 9% to avoid the breeding and multiplication of insect population in storage. The moisture content fluctuates during storage in cloth or hessian bags, but if the seed store is reasonably moisture vapor proof, the fluctuation in seed moisture content would be small. In sorghum, proper sun drying of earheads is essential to bring down the seed moisture to desirable level to avoid seed deterioration. In sun drying, seeds are normally dried by spreading them on the floor under diffused sunlight or under shade. Frequent stirring of the seeds is required to facilitate rapid drying. If higher quantities are produced, artificial drying can be considered. Maximum recommended air temperature for seed drying is 40°C, however in order to reduce the risk of damage, drying temperatures should be lower than the maximum. If seed moisture is more than 18%, maximum recommended drying temperature is 32°C and if lower than 18%, 40°C is the temperature for drying.

Seed cleaning and grading: In order to maintain the physical purity to the required standards, seed cleaning is the essential step in the processing unit to separate the inert matter, weed seed, other crop seed, other variety seed, disease and pest infested seed and any other undesirable contaminants. Sorghum seed cleaning and upgrading is mainly based on physical differences in seed volume, test weight and density. The sieve aperture sizes of top and bottom screens of air screen cleaner differ with genotypes. Generally, the top screen may be around 12/64” or 4.75 mm with round holes and the bottom screen at 9/64” or 3.5 mm with round holes. The specific gravity separator helps in upgrading the quality of seeds by rejecting the seed that is inferior in specific gravity.

Seed Treatment: Seed treatment refers to the application of fungicides, insecticides, or a combination of both to disinfect the seed from seed borne pathogens and to protect from soil borne organisms. Some chemicals offer a systemic protection against diseases. The use of insecticides reduces the damage to seed by insects. It must be remembered that seed treatment with chemical disinfectants does not improve the germination percentage. Sorghum seed after seed treatment can be protected from systemic pathogens like loose and head smut and non systemic ones like Helminthosporium blight, Fusarium and bacterial blights. Seed treatment also provides protection against storage pests (rice weevil) and shoot fly. The fungicides like Thiram or captan @3g kg⁻¹ and insecticides like Malathion dust (5%) (Premium grade) @0.6g kg⁻¹ seed are recommended for sorghum seed treatment. Thiram (75 WDP)g in 0.5lt water can be used to slurry treat the sorghum seeds in the processing chain.

Seed packing: Processed seed can be packed in cloth bags or poly lined bags @3–4 kg bag⁻¹, sewed with proper label of particular seed class and can be sealed with lead seal.

Table 22. Specific seed standards for different classes of sorghum seed.

Factor	Standards for each class	
	Foundation seed	Certified seed
Pure seed (minimum)	98.0%	98.0%
Inert matter (maximum)	2.0%	2.0%
Other crop seeds (maximum)	5/kg	10/kg
Weed seeds (maximum)	5/kg	10/kg
Other distinguishable varieties (maximum)	10/kg	20/kg
Ergot, sclerotia, seed entirely or partially modified as sclerotia, broken sclerotia, or ergotted seed (Spheccelia sorghi-Mc Rae & Claviceps spp) (maximum)	0.020% (by no.)	0.040% (by no.)
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapor proof containers (maximum)	8.0%	8.0%

Seed storage management

Storage under controlled environment: The genetic damage will not show up in the crop grown from that seed in the first generation, but will begin to segregate in the subsequent generations. Therefore, it is very important to store breeder seed and germplasm material carefully so that the loss of viability and genetic damage is minimized. As soon as seed germination falls by 20–30% from the initial seed germination of 90–95%, the seed should be regenerated. Such seeds should be stored under controlled conditions at a particular temperature and relative humidity regime (Table 23).

Table 23. Suggested conditions for storing sorghum breeder seed and germplasm.

S.No.	Duration of storage (years)	Temperature (°C)	Relative humidity (%)
1	5–7	15–20	45–50
2	20–25	2–4	40–45
3	50 or more	-10	40–45

Short term storage under ambient condition: Seeds of most of the sorghum species can be stored under ambient conditions for seed certification for at least 12–15 months, if seed moisture does not exceed 9–10%. Relative humidity of the atmosphere and storage temperature are the two important factors that influence seed viability during storage. The relative humidity of 70% and temperature of 20°C can be accepted as maximum permissible for safe storage. But in tropical countries like India, places registering mean temperature of 20°C throughout the year are few. The seed godowns must be rain proof, relatively moisture vapor proof and insect proof. There should be no cracks in the wall or on the floors. The bags should not be kept directly on the floor, but on wooden pallets and should be at least 50 cm away from the walls. An exhaust fan may be fixed for ventilation when outside temperature is lower than the seeds stores, the relative humidity of the outside air should also be considered while planning to ventilate the seed stores. Careful ventilation can reduce both storage temperature and seed moisture. Seeds are invariably attacked by different insect pests during storage. To avoid storage losses and to keep seeds free from insect pests during storage, one must adopt the following preventive and remedial measures.

Preventive measures before storing the seed

1. The seed moisture content should be preferably below 9%. The moisture content fluctuates during storage in cloth and hessian bags, but if seed store is reasonably moisture vapor proof, the fluctuation in seed moisture content would be low.

2. New bags should be used to avoid both insect infestation and mechanical mixture.
3. The storage structure should be thoroughly cleaned and white washed.
4. The storage structure should be disinfected with residual sprays of insecticides such as Malathion 50EC (one part in 100 parts of water) @ 5litres per 100 sq.m.
5. Proper stacking should be followed for arranging seed bags in storage structures.
6. It should be ascertained that the seed is properly treated with disinfectants before keeping the seeds in storage.
7. Seeds of different types such as cereals, pulses and vegetables should be stored separately to avoid the spread of insect infestation.

Maintenance of seed storage

1. The processing units and storage structures should be clean.
2. All sweeps should be kept far away from the premises of seed godowns so that insects will not breed and reinfest seeds.
3. The inspection of seed lots in storage structures should be carried out every fortnight. Seeds must be thoroughly fumigated at regular intervals.
4. Fumigation can be done with 1) Aluminum phosphide, 2-3 tablets (3g each) per ton of material with an exposure period of 5-7 days or 1 tablet per cu. m. space. 2) Ethylene dibromide (EDB) @ 32g per cu.m. space with an exposure period of 5-7 days. 3) Ethylene dichloride carbon tetrachloride (3:1) (EDCT) mixture @ 320-480 g per cu. m. space with an exposure period of 24-48 hrs.
5. Of all these fumigants, Aluminum phosphide is safest. Its repeated application does not impair seed quality. A maximum of 3 fumigations may be given at an interval of 40-50 days.
6. During fumigation and surface sprays, handle the chemicals carefully as they are highly toxic to human beings.
7. Seed structures should be aerated and thoroughly cleaned with brush or hard broomsticks to remove all dead and moribund insects.
8. To prevent reinfestation, surface treatment with Malathion 50EC or Finitrothion 50EC @4-5 litres per sq.m. area or Malathion dust 5% @3-4kg per 100 sq.m. should be given.
9. Surface treatment of seed godowns and processing units should be carried out at an interval of 2-4 weeks depending upon the severity of pest. Check on re-infestation prevents insects developing resistance to insecticides.

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Seed processing, testing, storage and marketing in sorghum

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Seed processing

Seed processing is an integral part of sorghum seed production, which encompasses steps such as drying, cleaning, grading, treating and bagging. Properly threshed sorghum seed can often be cleaned to the desired purity on the air screen cleaner alone. However, the gravity separator is commonly used to remove light materials and improve germination.

Physical purity

The threshed seeds should be physically pure and should not contain weed seeds, disease and pest infested seed, other crop seed, other cultivar seed, undesirable seed and damaged seeds. Sorghum seed of different classes should possess certain minimum seed purity standards (Table 24).

Table 24. Specific seed standards for foundation and certified seed of sorghum.

Factor	Standards for each class	
	Foundation seed	Certified seed
Pure seed (minimum)	98.0%	98.0%
Inert matter (maximum)	2.0%	2.0%
Other crop seeds (maximum)	5/kg	10/kg
Weed seeds (maximum)	5/kg	10/kg
Other distinguishable varieties (maximum)	10/kg	20/kg
Ergot, sclerotia, seed entirely or partially modified as sclerotia, broken sclerotia, or ergotted seed (<i>Sphacelia sorghi</i> -Mc Rae, & <i>Claviceps</i> spp) (maximum)	0.020% (by number)	0.040% (by number)
Germination (minimum)	75%	75%
Moisture (maximum)	12.0%	12.0%
For vapor proof containers (maximum)	8.0%	8.0%

Seed drying

Seeds contain varying amounts of moisture at harvest, and if they are to be stored for subsequent planting it is essential that their moisture content is reduced to a safe level. High moisture in seeds deteriorates seed viability and causes mechanical damage during processing. In addition to this, high moisture in seeds provides favorable atmosphere for pest infestation and disease infection storage. Sorghum seeds should be dried to 10-11% moisture content to avoid the breeding and multiplication of insect population in storage. The seed moisture content fluctuates during storage in cloth or hessian bags, but if the seed store is reasonably moisture vapor proof, the fluctuation in seed moisture content would be minimal. In sorghum, proper sun drying of panicle is essential to bring down the seed moisture to desirable level to avoid seed deterioration. In sun drying, seeds are normally dried by spreading them on the floor under diffused sunlight or under shade. Frequent stirring of the seeds is required to facilitate rapid and uniform drying. For bulk seed drying, seeds can be dried in forced air driers. Maximum recommended air temperature for seed drying is 40°C. However, in order to reduce the risk of heat damage to the seeds, drying temperatures should be lower than the maximum recommended. If seed moisture is more than 18%, maximum recommended drying temperature is 32°C and if lower than 18%, 40°C is the temperature for drying.

Seed cleaning and grading

In order to maintain the physical purity to the required standards, seed cleaning is the essential step in the processing unit to separate the inert matter, weed seed, other crop seed, other variety seed, disease and pest infested seed and any other undesirable contaminants. Sorghum seed cleaning and grading is mainly based on physical differences in seed volume, test weight and density. The sieve aperture sizes of top and bottom screens of air screen cleaner differ with genotypes. Generally, the top screen has holes measuring 12/64" or 4.75mm with round holes and the bottom screen has holes measuring 9/64" or 3.5mm with round holes. The specific gravity separator helps in upgrading the quality of seeds by rejecting the seed that is inferior in specific gravity (Fig. 31).

Seed treatment

Seed treatment refers to the application of fungicides, insecticides, or a combination of both to disinfect the seed from seed borne pathogens and to protect from soil borne organisms. Some chemicals offer a systemic protection against diseases. The use of insecticides reduces the damage to seed by insects. Sorghum seed after seed treatment can be protected from systemic pathogens like loose and head smut and non-systemic pathogens like *Helminthosporium* blight, *Fusarium*



Fig. 31. A view of seed pre-cleaner cum grader and specific gravity and indented cylinder separators.

and bacterial blights. Seed treatment also provides protection against storage pests (rice weevil) and shoot fly. The fungicides like Thiram or captan @3g/kg and insecticides like Malathion dust (5%) (Premium grade) @0.6g kg⁻¹ seed are recommended for sorghum seed treatment.

Packing

Processed seed can be packed in cloth bags or poly lined bags @3-4 kg bag⁻¹. The bags are then sewed with proper label of particular seed class and sealed with lead seal (Fig. 32).

Crop -----	Label no. -----
Variety -----	Pure seed* % -----
Class of seed -----	Inert matter* %-----
Production institutes -----	Germination* %-----
(Name, address and seal)	Genetic purity*-----
	Date of test -----
	Net contents-----
	(when packed -----% moisture)



Fig. 32. Bulk stacking and storage of processed seeds.

Seeds are packed in containers of specified weight after processing and treatment. Cloth bags of 3.0 or 1.5 kg capacity are used for sorghum. Cloth bags of 5.0 or 8.0 kg capacity are also used. A label must be placed in, or on the bags to maintain positive identity of the seed. The outer label or tag can be sewn to the bag when bags are sewed with a sewing machine.

Seed testing principles and procedures

The international rules and regulations for seed testing (1976, 1999), as finalized at the seventeenth International Congress of Seed Testing, Warsaw (1974), became effective on 1 January 1977 in the Southern Hemisphere. For the purpose of better understanding, the principles and procedures for testing samples of different crop groups have been discussed under the following sub-heads:

1. Sampling
2. Moisture testing (oven method, by moisture meters)
3. Purity analysis
4. Evaluation of seedlings for germination
5. Vigor and viability tests
6. Seed health testing
7. Verification of genetic purity (grow-out test)

Sampling

A sample is obtained from a seed lot by taking small quantities at random from different stocks or positions at the time of processing or packaging or from sealed bags in the lot and pooling them. The classification of samples drawn at various stages for laboratory tests is given below:

Primary sample: A primary sample is a small portion taken from a lot, at a particular stage, with the purpose of forming a composite sample.

Composite sample: A composite sample is formed by combining and mixing all the primary samples taken from a lot and a part of this sample is sent to the laboratory for testing.

Submitted sample: A composite sample is usually large in quantity than what is required for testing and so needs to be reduced in quantity as per requirement. A composite sample, when so reduced in quantity and sent to the testing station, is called a submitted sample.

Working sample: The working sample is a part taken from the submitted sample in the laboratory, to be subjected to quality tests as described in the rules.

Analysis of purity components

In accomplishing the objectives of the International Seed Testing Agency (ISTA) Rules, the sample is separated into three components: (1) pure seed; (2) inert matter; and (iii) other seeds.

Pure seed: Pure seed refers to seed of that species which is stated by the sender to be the dominant one in the seed lot. Seeds such as immature seeds, undersized, shriveled, diseased, or germinated seeds, unless transformed into fungal sclerotia, smut balls or nematode galls, may be regarded as pure seed, provided they can be identified as of that species.

Inert matter: Inert matter includes such seed like structures as pieces of broken or damaged seed, empty glumes or any other extraneous matter such as soil, sand, stone, chaff, stems, leaves, pieces of bark, flower, nematode galls, fungal bodies, insect larvae, etc.

Other seeds: This refers to any kind of seed or seed like structures of any plant species other than that of pure seed. The distinguishable characteristics set out for the pure seed shall also be applicable to other seeds except certain weed seeds that are classified separately.

Evaluation of seedlings for germination

Germination: Germination in a laboratory test refers to the emergence from the seed embryo of those essential structures, which, for the kind of seed being tested, indicate its ability to develop into a normal plant under favorable conditions.

Percentage of germination: The percentage of germination reported in a Seed Analysis Report indicate the proportion by number of seeds which have produced

seedlings classified as normal under the conditions and within the specified period.

Normal seedlings: It is necessary to distinguish the normal seedlings that are counted for percentage of germination from abnormal seedlings. According to the ISTA Rules, 1976, normal seedlings are those that come under one of the following categories:

1. Seedlings which show the capacity for continued development into normal plants when grown in good quality soil and under favorable conditions of water supply, temperature and light
2. Seedlings which are found to possess all the essential structures when tested on artificial substrate
3. Seedlings which show a vigorous and balanced development of its structures except having some slight defects, such as having a damaged primary root or superficially damaged other essential structure, or having only one cotyledon when they are dicotyledonous
4. Seedlings with all the essential structures intact but in a state of decay caused by fungi or bacteria. It should, however, be clear that the parent seed is not the source of infection.

Abnormal seedlings: Abnormal seedlings are those that do not show the capacity for continued development into normal plants when grown in good quality soil and under favorable conditions of water supply, temperature and light. Seedlings with the defects (damaged seedlings, decayed seedlings, deformed seedlings, hard seeds, fresh un-germinated seeds, dead seeds) when tested on artificial media shall be classified as abnormal.

Kinds of vigor tests

Direct tests: These tests simulate pertinent favorable field conditions on a laboratory scale. The principle advantage of such tests is that it simultaneously evaluates all the factors affecting seed vigor.

Indirect tests: These tests measure certain physiological attributes of seeds. These tests have the advantage that the variables can be precisely controlled, allowing reproducibility of results.

Viability tests: Only two methods, the topographical tetrazolium test (TZ) and the embryo excision test have been accepted as official methods.

TZ test is a biochemical test. In this test, living cells are made visible by reduction of an indicator dye. The indicator used in the TZ test is a colorless tetrazolium salt. Although a number of tetrazolium compounds can be used, 2, 3, 4, triphenyl-tetrazolium chloride is preferred by most seed technologists. The colorless tetrazolium is reduced in living cells by action of dehydrogenase enzymes to form a red water insoluble formazan product. The red color makes it possible to distinguish the living parts of the seeds from the colorless dead parts. Treated seeds fall into three groups: (a) completely stained, viable seeds, (b) completely unstained, non-viable seeds and (c) partially stained seeds. Varying proportions of necrotic tissues occur in different parts of partially stained seeds. Localization and spread of necrosis in the embryo and/or endosperm determines whether seeds are viable or non-viable.

In the embryo excision method, embryos are excised from the seeds, in accordance with the methods of Flemion (1938) and Heit (1955) and then placed on moist filter paper or blotter discs in petri dishes. The tests are placed at ordinary room temperature and light intensity. However, the temperature should not exceed 24°C. The embryos should germinate within a few days to two weeks. Viable non-germinated seeds can be easily distinguished from dead seeds after two weeks.

Seed storage management

Storage under controlled environment

It is very important to store breeder seed and germplasm material carefully so that the loss of viability is minimized. As soon as seed germination falls by 20-30% from the initial seed germination of 90-95%, the seed should be regenerated (Fig. 33).



Fig. 33. Breeder and Foundation seed cold storage.

Such seeds should be stored under controlled conditions at a particular temperature and relative humidity regime (refer table 23 on page 219).

Tips for better seed storage

1. The seed moisture content should be preferably below 9%. The moisture content fluctuates during storage in cloth and hessian bags, but if seed store is reasonably moisture vapor proof, the fluctuation in seed moisture content would be minimal.
2. New bags should be used to avoid both insect infestation and mechanical mixture.
3. The storage structure should be thoroughly cleaned and white-washed.
4. The storage structure should be disinfected with residual sprays of insecticides such as Malathion 50EC (one part in 100 parts of water) @ 5 litres per 100 sq. m.
5. Proper stacking should be followed for arranging seed bags in storage structures.
6. It should be ascertained that the seed is properly treated with disinfectants before keeping the seeds in storage.
7. Different types of seeds such as cereals, pulses and vegetables should be stored separately to avoid the spread of insect infestation.

Maintenance of seed storage

1. The processing units and storage structures should be clean.
2. All sweeps should be kept far away from the premises of seed godowns so that insects will not breed and reinfest seeds.
3. The inspection of seed lots in storage structures should be carried out every fortnight. Seeds must be thoroughly fumigated at regular intervals.
4. Fumigation can be done with a) Aluminum phosphide, 2–3 tablets (3g each) per ton of material with an exposure period of 5–7 days or 1 tablet/m³ space. b) Ethylene dibromide (EDB) @32g/m³ space with an exposure period of 5–7 days. c) Ethylene dichloride carbon tetrachloride (3:1) (EDCT) mixture @320–480g/m³ space with an exposure period of 24–48 hours.
5. Of all these fumigants, Aluminum phosphide is safest. It's repeated application does not impair seed quality. A maximum of 3 fumigations may be given at an interval of 40–50 days.
6. During fumigation and surface sprays, handle the chemicals carefully as they are highly toxic.

7. Seed structures should be aerated and thoroughly cleaned with brush or hard broomsticks to remove all dead and moribund insects.
8. To prevent reinfestation, surface treatment with Malathion 50EC or Finitrothion 50EC @4–5 litres/m² area or Malathion dust 5% @3–4 kg/100 m² should be given.
9. Surface treatment of seed godowns and processing units should be carried out at an interval of 2–4 weeks depending upon the severity of pest. Check on reinfestation prevents insect developing resistance to insecticides.

Buffer stock

It is necessary to keep a buffer stock of seed to meet contingencies such as natural calamities and to safeguard against shortfalls in seed production. The quantity of seed to be kept in buffer should not be less than 10% of the projected requirement in the subsequent year in the case of certified seed, 20% in the case of foundation seed, and 50–100% (depending upon the hybrids/varieties) in the case of breeder seed. The quantity of carry-over stocks must be kept at a minimum.

Seed marketing

The National Seed Corporation (NSC) and State Seed Corporations (SSCs) are the public sector organizations chiefly involved in the distribution and marketing of seeds. NSC seeds reach the farmers at the state, district, tehsil and village level through distributors or through their sales counters at the tehsil level. The SSC seeds are supplied through appointed distributor, state agricultural department seed stores or through SSC sales counters. The NSC and SSCs also distribute seed via agro industries corporation and government godowns (The Seed Act 1966).

Private seed companies follow their own marketing and distribution channels. Their seeds reach farmers via one or more distributors at district or town level. Each distributor has a number of dealers at the village level, who sell seeds directly to farmers. In general, dealers are responsible to the company's distributor, and sell seed on prices fixed by the company. Normally, the cost of certified seed is two to three times more than that of procurement price of the grain. The price of the breeder seed is normally 50% higher than the foundation seed.

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PPV and FR Act and National Seed Policy

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Introduction

After India became a signatory to the Trade Related Aspects of Intellectual Property Rights Agreement (TRIPs) in 1994, a legislation was required to be formulated. Article 27.3 (b) of this agreement requires the member countries to provide for protection of plant varieties either by a patent or by an effective *sui generis* system or by any combination thereof. Thus, the member countries had the choice to frame legislations that suit their own system, and India exercised this option. The existing Indian Patent Act, 1970 excluded agriculture and horticultural methods of production from patentability. The *sui generis* system for protection of plant varieties was developed integrating the rights of breeders, farmers and village communities, and taking care of the concerns for equitable sharing of benefits. India has opted for a *sui generis* system of protection of plant varieties and has provided for farmers' rights, breeders' rights, researchers' rights and equity concerns in the same legislation. All these provisions in one Indian legislation on protection of plant varieties is an outcome of intense public debate involving all interested groups and an elaborate exercise by a Joint Convention on Biological Diversity and the FAO Declaration on Farmer's Rights to which India is a party. The Protection of Plant Varieties and Farmers' Rights Act was passed by the Indian Government in 2001; it seeks to establish an independent National Plant Variety and Farmer's Rights Protection Authority with a Chairman appointed by the Government of India and 15 ex-officio and nominated members, as well as a subordinate office of Registrar General of Plant Varieties. The Registry will have its headquarters at New Delhi, with regional offices at Bangalore, Pune, Bhopal, Patna and Guwahati. The act also provides to establish a Plant Variety Tribunal with the status of District Court for settlement of disputes connected with this Act. The main functions of the Authority are: 1) registration of plant varieties, 2) characterization and documentation of registered varieties, 3) documentation indexing and cataloguing of farmers' varieties, 4) providing compulsory cataloguing facility for all plant varieties from India and abroad, 5) ensuring seeds of all registered varieties are made available to the farmers, 6) collection of comprehensive statistics on plant varieties, and 7) maintenance of national register of plant varieties legislation, making it a unique Act when compared to similar legislations in other countries. Only a few countries have provided for farmers' rights. However, unlike in India, rights for farmers, viz., as breeder, conserver, seed producer and consumer have not been considered

elsewhere in the world. It is this uniqueness of the Act that poses many challenges for its effective implementation. The balance between breeders' rights and farmers' rights could be tough to strike and difficult to implement. In this paper the *pros and cons* of legislations on seed industry and their representative views are analyzed.

Salient features of PPV & FR Act

Protection of Plant Varieties (PPV) and Farmers' Rights (FR) Authority

This Authority (Protection of Plant Varieties and Farmers' Rights Authority) is being established by the Central Government to carry-out the purpose of the Act. The Authority is composed of organizations and State Governments – The Registrar General will be the Member Secretary of the authority. The Authority will be assisted by committees and officers such as Standing Committee, other committees and the Registrar, for efficient discharge of its functions under the Act.

Functions of the Authority

1. It shall be the duty of the Authority to promote, by such measures as it thinks fit, the encouragement for the development of new varieties of plants and to protect the rights of the farmers and breeders;
2. In particular, and without prejudice to the generality of the foregoing provisions, the measures referred to in Sub-section (1) may provide for:
 - a. The registration of extant, new plant varieties and essentially derived varieties subject to such terms and conditions and in the manner as may be prescribed;
 - b. Developing characterization and documentation of varieties registered under this Act;
 - c. Documentation, indexing and cataloguing of farmer's varieties;
 - d. Compulsory cataloguing facilities for all varieties of plants;
 - e. Ensuring that seeds of the various varieties registered under this Act are available to the farmers and providing for compulsory licensing of such varieties if the breeder of such varieties or any other person entitled to produce such variety under this Act does not arrange for production and sale of seeds in the manner as may be prescribed;
 - f. Collecting statistics with regard to plant varieties, including the contribution of any person at any time in the evolution or development of any plant variety, in India or in any other country, for compilation and publication;
 - g. Ensuring the maintenance of the Registrar.

Power of authority of the Registrar

1. The Registrar shall have all the powers of a Civil Court for the purpose of receiving evidence administering oaths, enforcing the attendance of witnesses, compelling the discovery and production of documents and issuing commissions for the examination of witnesses;
2. May order as to cost as it considers responsible, and any such order shall be executable as a decree of a Civil Court.

Registry

1. Establishment of a Registry known as "Plant Varieties Registry";
2. A National Registry of Plant Varieties shall be kept at the head-office of the Registry and also at branch offices duly entering in it all the particulars of all varieties registered.

This Register will be maintained under the superintendence and directions of the Central Government, and it shall be kept under the control and management of the Authority.

Criteria for registration of varieties

Only varieties that fulfill the criteria of novelty, distinctiveness uniformity and stability will be registered.

Persons who may apply

An application for registration under Section 14 shall be made by:

- a) Any person claiming to be the breeder of the variety; or
- b) Any successor of the breeder of the variety; or
- c) Any person being the assignee of the breeder of the variety in respect of the right to make such application; or
- d) Any farmer or group of farmers or community of farmers claiming to be the breeder of the variety; or
- e) Any person authorized in the prescribed manner by the person specified under clauses (a) to (d) to make application on his behalf; or
- f) Any university or publicly-funded agricultural institution claiming to be the breeder of the variety.

Duration and effect and benefit sharing

On registration of a variety it will be initially for a period of

- a) Nine years for trees and vines;
- b) Six years for other crops.

This could be renewed further for the remaining period of

- a) 18 years in respect of trees and vines;
- b) In the case of extant varieties, 15 years from the date of notification of variety under Section (5) of the Seeds Act, 1966;
- c) In other cases, 15 years from the date of registration. Depositing the seeds or propagating material by the breeder in the National Seed Bank is mandatory.

Rights of breeder or his successor

A certificate of registration for a variety shall confer an exclusive right on the breeder or his successor, his agent or licensee to:

- a) Produce;
- b) Sell, market, distribute, import or export the variety;
- c) A breeder may authorize any person to produce, sell/market, or otherwise deal with the variety registered under the act subject to such limitations and conditions as may be specified in the regulations.

Exclusion of certain varieties

No registration will be granted for a variety in a case where prevention of commercial exploitation of such variety is necessary to protect public order or public morality or human, animal and plant life and health, or to avoid serious prejudice to the environment.

Researcher's rights

For the purpose of research, any person can use such variety to conduct an experiment or research as an initial source of variety for the purpose of creating other varieties. For further repeated use of the variety as a parental line, permission of the breeder is required.

Farmer's rights

- a) A farmer who has bred or developed a new variety shall be entitled for registration and other protection in like manner as a breeder of a variety under this Act;
- b) The farmers' variety shall be entitled for registration if the application contains a declaration as specified in Clause (h) or Sub-section (1) of Section 18;

- c) A farmer who is engaged in the conservation of genetic resources of landraces and wild relatives of economic plants and their improvement through selection and preservation shall be entitled in the prescribed manner for recognition and reward from the Gene Fund, provided that material so selected and preserved has been used as donors of genes in varieties registerable under this Act;
- d) A farmer shall be deemed to be entitled to save, use, sow, re-sow, exchange and share or sell his farm produce including seed of a variety protected under this Act in the same manner as he was entitled before the coming into force of this Act, and provided that the farmer shall not be entitled to sell branded seed of a variety protected under this Act.

Right to claim compensation and exception from payment of fees

In case the expected performance under given conditions is lacking and if the propagating material fails to provide such performance under such given conditions, the farmers or the group of farmers or the organization of the farmers, as the case may be, can claim compensation in the prescribed manner before the authority. However, farmers are protected from innocent infringement of the provisions of the Act. The farmers are also exempted from paying any fee in any proceedings before the Authority or Registrar or Tribunal or the High Court under the provisions of the Act and Rules.

Constitution of a Gene Fund

The central government shall constitute a fund to be called “The National Gene Fund”, which will receive funds from:

- a) The benefit sharing received in the prescribed manner from the breeder of a variety or any essentially derived variety registered under this Act or propagating material of such variety, as the case may be;
- b) The annual fee payable to the authority by way of royalty under Sub-section. (1) of Section 35;
- c) The compensation deposited in the Gene Fund under Sub-section (4) of Section 41;
- d) The contribution from any National and International Organization and other sources.

The Gene Fund shall be used in the prescribed manner, to meet the following expenses:

- a) Any amount to be paid by way of benefit sharing under Sub-section (5) of Section 26;

- b) The compensation payable under Sub-section (3) of Section 41;
- c) The expenditure for supporting the conservation and sustainable use of genetic resources including *in-situ* and *ex-situ* collections and for strengthening the capability of the Panchayat in carrying out such conservation and sustainable use;
- d) The expenditure of the schemes relating to benefit sharing framed under Section 46.

Compulsory license

The Breeder having got the Compulsory License from the authority may license to an individual or a company the production, distribution and sharing activity of the seed, other propagated and essentially derived variety.

Plant Variety Protection – Appellate Tribunal

The Central Government will constitute an Appellate Tribunal to hear the grievances, arising out of the decisions of the Authority or Registrar regarding registration of a variety, orders relating to claim, benefits, regarding revocation or modification of compulsory license or others regarding payment of compensation.

The Protection of Plant Variety and Farmers Rights Act (PPVFR), 2001 is yet to be enforced since rules have not yet been framed and notified. Administrative problems could be visualized only after issue of rules and regulations under various provisions of the Act and the authority.

Implications of PPVFR on agricultural research

At the outset, the implementation of PPVFR will give monetary returns to the institutions and individuals for their investment in research. Recognizing the efforts of plant breeders by granting Plant Breeders Rights (PBRs) will definitely encourage further plant breeding activity and stimulate enhanced research in developing new varieties of plants. The competition henceforth, would not only be with the peers in public and private sector but also with the farmers in development of location specific varietal and other sustainable crop management technologies and innovations. There would now be increased interest towards commercialization and commercial potential of research. There is already a concern that some of the research projects with public influence or those of basic research with little income potential will be left behind as the need for income generation would have a direct impact on the research agenda. There would be increased pressure to seek new sources of funding, and more and more collaborative programs on mutually agreed conditions in any public-private or private-private partnership may become the order of the day.

With the enactment of PPVFR, the country is gearing up to implement the grant of PBR to recognize and protect the rights of farmers and breeders. PBR can only be granted for a variety that is new, distinct, uniform, and stable or in other words only after DUS testing. Farmers' varieties need not comply with the novelty provisions but otherwise all varieties claiming PBR need to prove their distinctness. For this, the Department of Agriculture and Cooperation (DAC), Ministry of Agriculture and the Indian Council of Agricultural Research (ICAR) have initiated the process. DAC has been providing funds since 2000 to various ICAR institutes to strengthen the DUS test facilities. ICAR has identified 35 crops and 43 centers for finalizing the DUS test guidelines and undertaking the test activities. Many of the centers are project coordinating units already engaged in All-India Coordinated multilocation testing of elite lines. One of the formidable tasks for DUS testing units would be to characterize the extent (national releases) of varieties to develop a database for reference collection of varieties (RCV). Though strictly only the latest varieties would be tested for DUS, the requirement of manpower and finances for precise total characterization, which would go for legal censure as well in case of dispute, would be a limiting factor. As DUS today would be done on a cost recovery basis, the onus is on the ICAR institutes to maintain the DUS test plots in excellent condition to prove the distinctness of the varieties, maintain the database and relevant records in order.

Further, to claim PBR, the variety must be clearly distinguishable for at least one essential character from the varieties of common knowledge in India and abroad. Such distinctness has to be bred into the varieties while incorporating other economically important traits. Establishing such distinct features will be more and more difficult in future. Initially there may be a rush of varieties for DUS testing, but this is expected to slow down eventually for want of distinctness. Further distinctness does not guarantee commercial viability of the variety and breeders have to combine both; unless this is achieved through planned breeding activity, the genotypes developed cannot be commercially exploited fully through PBR.

The most important ingredients for crop improvement program is the availability of genetic diversity. Until recently, the germplasm was considered worldwide as the heritage of mankind to be shared and used by all. With the new world order that is emerging in the post WTO era, the availability of germplasm may be restricted. Any breeder trying to develop new varieties should obtain explicit permission to use germplasm or landraces from the region from where this germplasm was originally collected. Prior Informed Consent (PIC) and Material Transfer Agreement (MTA) although do assure the benefit sharing arrangements; the willingness to share the germplasm rests with the breeder/farmer and/or with the community. Unless available in the national genebank, from where the material can be accessed by signing a MTA, there is no way of getting the material if the party is not willing to share the germplasm.

Further, the second step for the development of new varieties is to test the elite lines in the All India Crop Improvement Programs. The breeders nominate the materials in good faith and further selection would depend on the performance of cultures in the program. Simultaneously, other breeders also nominate the material and are free to use available material in the trial if they wish to do so. Now, if a particular line is doing extraordinarily well in station or state level testing, the breeder may not be as readily agreeable as he was in the earlier days to part with the line for multilocation trials, as his peers may want to identify it for their use or select and modify as an Essentially Derived Variety (EDV). Unless divulged, the benefit sharing becomes a bone of contention between the breeders and the organizations. Thus, the free exchange of germplasm and breeding lines may become the casualty of the PPVFR act unless some new innovative practices are devised.

Increasing use of biotechnology in producing transgenic crop varieties and genetically modified organisms (GMOs) also requires development of biosafety norms to regulate trade in such crops, animals and products. The trade in GMOs will need to be strictly regulated, and for that, capacities need to be created urgently. This will ensure that we attract investment in this sector with a responsible regulatory system. Prior informed consent of farmers must also be ensured while pursuing on-farm trials on transgenics. Public notice must be given for all such trials and informed debate should take place on these issues rather than exposing people to only populist propaganda, as has often been the case. It must however be remembered that much greater environmental damage takes place due to existing chemical pesticides compared to the possible damage that may be caused by a transgenic pest tolerant crop. To prevent biotechnologically produced varieties to take away the benefits of conventionally bred varieties by transferring one or a few genes into or from the same, the concept of EDV was developed. However, EDV does not deal with incorporation of gene from a protected variety into an unprotected variety. The fact that conventional breeding by farmers or plant breeders made the expression of a particular critical gene possible, has to be recognised. Therefore, the claimant for plant variety protection for a biotechnologically produced variety should disclose the source parents and must agree to contribute part of the gain with the breeders of the source variety.

Provisions of the PPVFR act will encourage conserver farmer and farmer breeders. The former are custodians of local germplasm and the latter through pure line selections developed very important landraces that have unique biotic/abiotic stress tolerance or quality attributes or medicinal value. Such farmers as individuals or as a community or through any NGO will be able to claim PBR or get benefited through the benefit sharing provision under clause 26(2) of the PPVFR in respect of any breeder's claim of new variety wherein the local germplasm or landrace has been used in the development of such variety.

The explicit provisions of farmers' rights would safeguard the farmers' interest in growing the seed and preserving the produce to grow the next crop and also to share, exchange and sell the produce without the risk of infringement of PPVFR. As what has been happening in the country for a long time as a traditional practice has been regularized as a full fledged farmers' right, the public and private seed companies should now intensify their efforts in producing good quality seed and make it available to the farming community. The seed village programs may get a shot in the arm and may pose a problem for the seed producing agencies in future. The public sector seed companies have focussed largely on high volume – low value crops, while the private sector operates the other way round. At present, of the total seed business of Rs 4000 crores, the private sector garners more than 75%. Although the public sector seed corporations played a historic role in the spread of HYVs in the post-green revolution era, now the private sector is playing a dominant role in supplying quality seeds at highly competitive prices. Once the farmers take up seed production on a community basis, this may pose a further threat to the public seed agencies although both public and private seed sectors have a critical role to play in Indian agriculture. Perhaps, it is time that they become more competitive and the National Seed Board has a vital role to play in seed program planning, production, supply and quality assurance. Managerial and “freedom to operate” need greater attention, and not mere technical or regulatory matters for the success of public sector seed industry.

To sum up, WTO and other provisions in the Agreement on agriculture need not always be viewed as deterring factors. The TRIPS and the PPVFR acts in the long run would act as catalysts for enhancing the agricultural production and productivity in the country. We are moving from green revolution to gene revolution with a strategic integration of biotechnology tools into Indian agricultural systems. It is time to strengthen and support the agricultural systems through better funding, and the scientists too should learn to adapt to the new IPR regime. As it is said, **“the best way to learn to compete is to compete”**, and the right way to do this is through right type of deliverables and innovative policies, especially on human resource development.

Salient features of Draft Seeds Bill 2004

Based on the recommendations of the Seed Policy Group headed by Padmasri Dr MV Rao, Ex-Vice Chancellor of ANGRAU, Rajendranagar, Hyderabad on the New Seed Policy, the Government of India has proposed a New Seeds Bill, 2004, regulating the seeds business and in the matter of Registration of a kind or varieties of seeds, their production, processing, quality control and law enforcement. Draft Seeds Bill, 2002, as a single enactment is proposed by amalgamating the provisions of Seeds Act, 1966 and Seeds Control Order, 1983.

This is an Act to provide for regulating the sale, import and export of seeds, to facilitate supply of quality seeds to farmers throughout the country, and to establish a National Seeds Board to advise the government in all matters connected therewith.

Views of the Seedsmen Association on Draft Seeds Bill

In the short title after Production, “Processing” may also be included – this would regulate seed processing also and would help the quality regulators in regulating the quality of all the seeds even before they are marketed.

Definition

1. The word ‘hybrid’ may also be defined in the definitions;
2. Under misbrand, the sale of a duplicate of a popular registered variety or hybrid may also be included and defined so that sale of duplicates also becomes a major offence.

National Seeds Board

Seed is a highly specialized subject and to formulate all the important issues such as minimum standards, required procedure, etc, the presence of at least five experienced and qualified representatives (representing the geographical regions of the country) from the seed industry is essential so as to strengthen the National Seed Programme. There are already twenty-four members in the Board, and adding three more members should not be a problem.

Constitution of Committees

In all the committees constituted under the proposed New Seeds Act as per the decisions of the National Seed Board (NSB), 33% (one third) of the membership may be reserved for qualified and experienced representatives of the seed industry. In any of the developed countries, when the constitution of important committees is examined, we can see that the majority are from the industry. This gives a practical approach in the working of the committee.

Central Seed Testing Laboratories

There should be at least four central seed testing laboratories to monitor the working of notified seed testing laboratories in the country. At present, there is no audit on the working of the notified seed testing laboratories, which needs to be monitored closely in future.

On several occasions, the seed lots that record excellent performance at the farmers fields are declared as substandard by the notified state seed testing laboratories – the adherence of the standard practices and maintenance of the required conditions for germination in SSTL needs to be audited closely for which more number of central seed testing laboratories are required.

Labeling

Along with other particulars to be mentioned on the label as provided in the earlier Seeds Act, 1966, it is now proposed in the new Act to state the expected performance of the seeds (varieties/hybrids) under given conditions.

Working out a format for giving these particulars will be very difficult and the performance depends on not only the genetic purity but also on the environmental factors such as soil fertility, soil reaction, managerial practices including pest and disease management, as well as the climatic factors such as rainfall, temperature, etc, which vary from year to year even within a specific geographical region. This would result in confusion among the seed producers as well as the seed consuming farmers and might lead to meaningless litigation. As such, expected agronomic performance of the seed in given conditions should be deleted as required in Section 19.

Compensation to the Farmers (Section 15)

The cost of the seeds supplied by the seed company in most of the cases varies from 3% to 10% of the cost of the cultivation of a crop. The contribution of seed alone could be to the extent of 20–30% towards the yield. Therefore, the returns on investment made by the farmer for seed are very high and attractive. In spite of this, due to healthy competition in the Indian Seed Industry, the prices of the seeds for most of the crops are much lower as compared to the seed prices even in the neighboring Asian countries. Putting the burden of compensating the farmers on the industry would result either in:

- a. The companies closing shops to escape the burden;
- b. The surviving companies jacking up the prices to hedge for the probable compensations.

This would result in reduced competition, lesser availability of options to the farmers and higher costs of seeds, which is not desirable. If at all the compensation is to be awarded, it should be limited to the value of the seeds or two times the value of the seeds at most, so that the companies are careful enough in maintaining the minimum standards in the seed supplied by them.

- c. The very proposal mentioned in the proposed Act would lead to endless litigation, which might not really serve the purpose of compensating the farmers.

2. General

A peculiar situation is arising with regard to award of compensation, the remedy is provided independently under the following legislation:

- i) Consumer Protection Act, 1986;
- ii) Protection of Plant Varieties and Farmers Rights Act, 2001 (Section 39 (2));
- iii) Proposed New Seeds Act, 2002: This needs review, vesting powers in a single enactment concerned with seeds has made the section an over riding one in the proposed Act.

Transgenic varieties

The environmental safety for a particular gene or for a particular transgenic event involving the gene in a specific crop is thoroughly examined by the regulatory system before it is released (de-regulated) into the environment. Once the specific gene with a specific transformation event is de-regulated, there is no need for the concurrence of EPA for releasing each and every variety or hybrid carrying the same gene. Therefore, this clause may be modified to state: "No seed of any transgenic kind or variety shall be registered unless the (alien) gene that it is carrying has been approved by the Environmental Protection Act, 1986".

The Environmental Protection Agency of the US, Australia and other countries where transgenics are grown commercially has similar provisions. For example, in the US, the Bt gene *cry 1ac* is de-regulated and any seed company can introgress the gene into their variety by the simple breeding technique of back crossing and release for commercial cultivation without going for EPA again.

Provisional Registration

Provisional registration may be granted to the seed companies till the decision is finalized instead of for three years as proposed. This is being suggested as the decision for final registration may take longer than three years.

Power of the Seed Inspector - Section 31 (1) c

Since seed sales of a particular crop happens for a very short period, sometimes ranging from 7 to 15 days in a year, the powers for issuing the stop sale order for a period of 30 days is too long and can ruin a seed producer. Therefore, this power should be conditional. The following paragraph should be added to Section 31 (1) c:

“When the producer has furnished information clarifying the objections raised by the Seed Inspector, the detention orders should be revoked within 24 hours”.

or

“If the reasons are inadequate, notices calling for further information should be served within 24 hours”.

Offences and Punishments - Section 35 (1)

For minor infringements, the proposed fine is high. Seed is a biological product and germination may at times deteriorate very fast due to harsh climatic conditions that prevail in India particularly in the *kharif* season. Therefore, the penalty should be limited to between Rs 1000 and Rs 5000.

The minor offences should be compoundable by the Seed Inspector himself to avoid wastage of time in filing cases and prosecution through the courts, which leads to loss of precious time for the Seed Inspector as well as the seed companies.

Views of the seed industry on new seed bill

1. No fee exemption for the farmer to register his varieties unlike in the PPVFR Act. This may be looked into to strike harmony between this bill and the PPVFR Act.
2. Multilocation testing (MLT) and value for cultivation and use (VCU): For registering a variety, MLT data is to be submitted. Data generated by private companies may not have any validity for assessment of ‘agronomic performance’ required for registration. This may make ICAR testing mandatory.
3. Too much power being vested in the Seed Inspectors. This may sometimes result in unfair practices.
4. Declaration of expected performance through a label/tag on the container/bag is tricky. The farmer is entitled to claim compensation if the variety fails to provide the expected performance under such given conditions. This may result in more oral cases and huge compensations.
5. There may not be compulsory registration for transgenic varieties as they will be getting the clearance or de-regulation by EPA (Environment Protection Act, 1986). This may delay the process of release of transgenic varieties. For example, in the US, the Bt gene-*cry 1 Ac* is de-regulated and any seed company can introgress the gene into their variety by the simple breeding technique of back crossing and release for commercial cultivation without going for EPA again.

Integrated and effective implementation of PVPFR Act and new seed bill

There is a need for the effective and integrated implementation of various new acts/bills concerning biodiversity, environment and seed, which have shared interfaces in implementation of certain provisions of each law, since the common entity that is addressed in all these laws is "SEED". Although some overlapping issues have been sorted out to bring harmony between the Seed Bill and the PPVFR Act, a lot still needs to be looked into. For example, the seed bill requires mandatory registration of the varieties/seeds, but registration is under the purview of the PPVFR Act. Key differences exist between the seed bill and the PPVFR relating to declaring the origin/pedigree (parentage) of the variety, the conditions for MLT and who will conduct these tests, level of transparency maintained on grant of registration, price control and the treatment of farmers' varieties. While the PPVFR requires the declaration of the origin of the variety with pedigree details, the seed bill does not. With respect to testing of the new variety, the PPVFR lays down that the national authority will conduct the tests for DUS of the variety. The seed bill does not specify who will conduct the tests for establishing the usefulness of the new variety. This lacuna can be misused unless it is clarified. People have an opportunity to raise objections if they have reason to think that the variety is not what is claimed. In the case of the seed bill, the registered varieties will be made known only through periodic publications. The public has no opportunity to object to a new variety for any reason. This lack of transparency could mean that the varieties of poor performance could get registered without giving people a chance to oppose such grants.

As there are certain loopholes in the PPVFR and the seed bill, there is every need to look into each and every aspect of these laws to strike harmony between these two. Hence, there is every need to harmonize this seed bill with the PPVFR and Biodiversity Act, 2002. In PPVFR, the breeder applies for registration for a PBR. This right is valid for a period of 15 years for crop varieties and 18 years for tree species. The seed bill allows the period of protection to be doubled so that the seed variety can be protected by the seed producer for 30 years and 36 years, respectively. This extension of seed owner's right is a positive sign for the seed industry.

One of the greatest impacts of compulsory VCU based registration may cause a significant delay in release of new hybrids and varieties with superior pedigree. Any new hybrid or variety is identified after several years of breeding and testing efforts. To impose additional testing requirements, which would take an additional 2 or 3 years, would mean that the farmers would be deprived of the benefits of new value added products for another 2 or 3 years. Therefore, it will have serious economic consequences not only for the seed industry, but also for the farmers

and the nation as a whole. It is not clear how the proposed National Seed Board will manage the testing of several thousand new varieties and hybrids through loosely networked institutes and research centers. The existing manpower and infrastructure resources may not be sufficient to handle such a mammoth task.

It is evident that the industry needs to be encouraged by way of further liberalizing seed related regulations to ensure that the growth momentum attained over the last sixteen years is maintained. The proposed VCU registration, if it is mandatory, can be reduced to one year testing to save time and it will reduce lock in period affecting the economy of the seed company that has invested a huge amount of money in developing that hybrid. Accreditation of organizations for certification and private organizations to conduct agronomic trials is a positive point for the industry. It is also indirectly stated in the bill that the dealers cannot sell the seed of local unregistered varieties. It is good for the seed industry because of the proprietary nature of their products. Stock display system by dealers and distributors is a new development to ensure good quality seed to the farmers. Involving representatives from the seed industry in the National Seed Board (NSB) is an encouraging process for the industry to make representations.

Challenges ahead

It is generally perceived that IPR promotes innovation and increases return from investment and the investment in R&D. Under the Indian scenario, varietal improvement hitherto was largely undertaken by the public research system with very little involvement by the private sector. The impact of this Act on public research and private research could possibly be different. The liberal Farmers' Rights on the use of seed of protected varieties and Researchers' Rights provided in the Act may restrict the entry of increased research investment from private sector in self- pollinated and vegetatively propagated crop plants, but the public sector is expected to continue its predominant role, and the interest of our farmers in the best national interest is expected to be protected as per policy of the Government of India. Also, private interest in pollinated crops with high volumes of annual seed trade is likely to continue to supplement public sector efforts. Thus, private investment in varietal improvement under this legislative regime is expected to increase in selective sectors, such as hybrid varieties of commercially attractive crop species and self/vegetatively propagated crop species offering high volume annual seed sale. Consequently, the public sector research is expected to face stiff competition in these sectors from the private sector.

Technological superiority supported with genetic diversity, increased investment, advancement in frontier areas and efficiency may critically determine the competitive edge of these sectors. With several of the crop species not offering a commercially

attractive scale of operation for the private sector, public research may be required to continue its attention on the varietal improvement of these crop species with least competition from the private sector.

Varietal protection may influence the public research priorities. While it may be possible that all varieties of crop plants bred by public research may not be protected or that all protected varieties may not be licensed out, all competitive varieties of major crop plants are expected to be protected and licensed for a consideration. Such flow of return may provide incentive to the concerned breeders and help in encouraging their competitiveness and a general improvement in the competitiveness across the public system. The public sector can also be equally successful as the private sector in acquiring new genetic diversity from elsewhere through material transfers agreements. Public research can put up a match to the private sector by streamlining its research management to enhance efficiency, speed and competitiveness. Such a change may also encourage private-public collaboration to take advantage of the impressive infrastructure and HRD capability available with the public sector in the diverse areas of crop improvement.

It is estimated that currently more than 400 private firms are involved in the Indian seed sector. Out of these, only very few have their own R&D capability on varietal improvement. In other words, these firms have been existing in the seed business with the varieties and hybrids freely available from the public sector. Under the emerging regime, viability of those firms with little R&D backup may also become increasingly difficult. Accordingly, Indian private seed industry has also been changing with its lead players acquiring foreign tie-ups. Such tie-ups are likely to lead to acquisitions and mergers to eventually create a few private majors with increased compatibility and monopolistic control on varietal improvement and seed trade. Public sector research has a lesson to be learnt from the European experience with the introduction of varietal protection. Within a decade of introduction of such protection in Europe, much of its public research ceased to operate with reputed public research institutions sold out to the private sector. While such changes are not likely to happen with such speed in India, the possibility of such change in the long run cannot be ruled out. Considering the necessity of the public research and development system, including the general realization for its further strengthening, it is expected that the Protection of Plant Varieties and Farmers' Rights Act is likely to bring an advantageous change in crop improvement and variety development efforts to fulfill the all round interests of Indian agriculture.

National seeds policy and its implications

Agriculture development in its comprehensive definition is central to all strategies for the planned socio-economic development in India. Agriculture being a State

subject will continue to receive the fullest attention of the State Governments and the role of the Central Government will be to complement the efforts of the State Governments to ensure progress in agriculture and minimize regional imbalances. Agrarian reforms implemented through the first three Five Year Plans provided the first momentum for the growth of agriculture in the Indian economy. Investments in irrigation including agricultural education were stepped up side by side. This was followed by a period of introduction of high yielding varieties of seeds. A well-designated extension network for spreading knowledge and skills was also developed side by side. This ushered in a new beginning in Indian Agriculture called “Green Revolution”. The spread of high yielding variety technology particularly in wheat and rice crops in the mid sixties in conjunction with associated inputs and efficient delivery services culminated in dramatic change in India’s agriculture. Production of food grains has almost quadrupled in the last five decades. Impressive growth has also been made in the case of commercial crops like oilseeds, sugarcane and cotton. Fruits and vegetables, particularly potatoes have also shown spectacular growth.

The increase in agricultural production, however, has been brought in its wake, uneven development across regions, crops and also across different sections of the farming community. In the decade of the ‘nineties’ a marked slackening in the pace of growth has occurred, pointing to the need of infusing a new vitality in the agricultural sector. Of various agricultural inputs like fertilizer nutrient, seeds, agrochemicals, etc, seed is perhaps the most important determinant of agricultural production on which the efficacy of other agricultural inputs is dependent. Seeds of appropriate characteristics are required to meet the demand of diverse agro climatic conditions and cropping systems. Sustained increase in agricultural production and productivity enhancement is dependent, to a large extent, on development of new and improved varieties of crops and an efficient system through which timely supply in desired quantity of quality seeds to farmers is made. The seed sector has made impressive progress over the last three decades. The area under certified seed production has been increased from less than 500 hectares in 1962-64 to over 5 lakh hectares in 1999–2000. The quantum of quality seeds has crossed 100 lakh quintals (10 million tons). The Seeds Act, 1966 and Seed Control Order promulgated thereunder and the New Policy on Seeds Development, 1988 form the basis of promotion and regulation of Seed Industry. Far-reaching changes, however, have taken place in the national economic and agricultural scenario and in the international environment since the enactment of the existing seed legislation and the announcement of the 1998 policy. The framework for seed sector reforms covers seed production and distribution, quality control and seed legislation, import and export of seeds, plant quarantine and plant breeders and farmers’ rights.

Promotion of seed industry

It has become evident that in order to achieve the food production targets of the future, major efforts will be required to enhance the seed replacement rates of various crops. This would require substantial increase in the production of quality seeds, in which the private sector is expected to play a major role. The creation of a facilitative climate for growth of the seed industry, encouragement of import of useful germplasm and boosting of exports are core elements of the agricultural strategy of the new millennium.

Biotechnology and seed development

Biotechnology is likely to be a key factor in agricultural development in the coming decades. Genetic engineering/modification techniques hold enormous promise in developing crop varieties with a higher level of tolerance to biotic and abiotic stresses. A conducive atmosphere for application of frontier sciences in varietal development and for enhanced investments in research and development is a pressing requirement. At the same time, concerns relating to possible harm to human and animal health and bio-safety as well as interests of the farmers must be borne in mind.

Economic liberalization and seed trade

Globalization and economic liberalization have opened up several new opportunities as well as challenges. While providing the appropriate climate for the seed industry to utilize available and prospective opportunities, safeguarding the interests of Indian farmers, protecting and conserving agro biodiversity and traditional knowledge are also of central concern. While unnecessary regulation needs to be avoided, it must be ensured that gullible farmers are not exploited by unscrupulous elements. A regulatory system of new type is, therefore, needed which will encompass quality assurance mechanisms coupled with facilitation of a vibrant and responsible seed industry.

Varietal development and plant variety protection

The development of new and improved varieties of plants and availability of such varieties to Indian farmers is of crucial importance for a sustained increase in agricultural productivity. Appropriate policy framework and programmatic interventions are to be adopted to stimulate varietal development in tune with market trends, scientific-technological advances, suitability for various biotic and abiotic stresses and farmers' needs. An effective *sui-generis* system for intellectual property protection will be implemented to stimulate investment in research and

development of new plant varieties and to facilitate the growth of the Seed Industry in the country. A Plant Varieties & Farmers' Rights Protection (PVP) Authority will be established which will undertake registration of extant and new plant varieties through the Plant Varieties Registry to accord protection. The registration of new plant varieties by the PVP Authority will be based on the criteria of novelty, distinctiveness, uniformity and stability. The criteria of distinctiveness, uniformity and stability (DUS) will be relaxed for registration of extant varieties, which will be done within a specified period to be decided by the Authority. Registration of all plant genera or species as notified by the Authority will be done in a phased manner.

The PVP Authority will develop characterization and documentation of plant varieties registered under the PVP Act and compulsory cataloguing facilities for all varieties of plants. The rights of farmers to save, use, exchange, share or sell farm produce or protected varieties will be protected with the proviso that farmers shall not be entitled to sell branded seed of a protected variety. The rights of researchers to use the seed/planting material of protected varieties for bonafide research and breeding new plant varieties will be ensured. Equitable sharing of benefit arising out of the use of plant genetic resources that may accrue to a breeder from commercialization of seed planting materials of a new variety will be ensured. Farmers/group of farmers/village community will be rewarded suitably for their significant contribution in evolution of a plant variety. A National Gene Fund will be established for implementation of the benefit sharing arrangement, and payment of compensation to village communities for their contribution to the development and conservation of plant genetic resources and also to promote conservation and sustainable use of genetic resources. Plant Genetic Resources for Food and Agriculture Crops will be permitted to be accessed by Research Organizations and Seed Companies from public collections as per the provisions of the 'Material Transfer Agreement' of the International Undertaking on Plant Genetic Resources and the Biological Diversity Bill. Regular interaction amongst the private and public researchers, seed companies/organizations and development agencies will be fostered to develop and promote growth of a healthy seed industry in the country. For keeping abreast with the global developments in the field of Plant Variety Protection and for technical collaboration, India may consider joining various regional and international organizations. The PVP Authority will resort to compulsory licensing of a protected variety in public interest on the ground that requirements of the farming community for seeds and propagating material of a variety are not being met or that the production of the seeds or planting material of the protected variety is not being facilitated to the fullest possible extent.

Seed production

To meet the needs of the nation's food and nutritional security, it is important to make available to Indian farmers seeds of superior quality, in adequate quantity on a timely basis. Currently, the Indian seed program adheres to the limited three generations system of seed multiplication, namely breeder, foundation and certified seed. Breeder seed is the progeny of nucleus seed. Nucleus seed is the seed produced by the breeder to develop the particular variety and is directly used for multiplication as breeder seed. Breeder seed is the seed material directly controlled by the originating or the sponsoring breeder or institution for the initial and recurring increase of foundation seed. Public Sector Seed Production Agencies will continue to have free access to breeder seed under the National Agricultural Research System. Private Seed Production Agencies will also have access to breeder seed subject to terms and conditions to be decided by the Government of India. State Agriculture Universities will have the primary responsibility for production of breeder seed as per the requirement of the respective state.

Foundation seed is the progeny of breeder seed. Foundation seed may also be produced from foundation seed. Thus, production of foundation seed stage-1 and stage-2 may be permitted, if supervised and approved by the Certification Agency and if the production process is so handled as to maintain specific genetic purity and identity. Certified seed is the progeny of foundation seed or the progeny of certified seed. If the certified seed is the progeny of certified seed, then this reproduction will not exceed three generations beyond foundation stage – 1 and it will be ascertained by the Certification Agency that genetic identity and genetic purity are not significantly altered. Seed replacement rates (SRR) will be raised progressively with the objective of enhancing the use of quality seeds. Attention will also be given to upgrading the quality of farmers' 'saved seeds'. DAC, in consultation with ICAR and states, will prepare a National Seed Map to identify potential areas for seed production of specific crops. To put in place an effective seed production program, each state will do careful advance planning and prepare a perspective plan for seed production and distribution over a rolling ten-year period. The 'Seed Village Scheme' will be promoted to facilitate production and timely availability of seed of desired crop/varieties at the local level. Special emphasis will be given to seed multiplication for building adequate stocks of certified quality seed by providing foundation seed to farmers. For popularizing newly developed varieties and promoting seed production of these varieties, seed minikits will be supplied to farmers. For making available seeds of minimum time gap, the seed producing agencies will be encouraged to tie up with research institutions for popularization and commercialization of the varieties. As the hybrid varieties have potential to improve plant vigor and increase yield, support for production of hybrid seed will

be provided. Seed production will also be extended to areas that are outside the traditional seed growing areas.

Seed Banks will be established for stocking specified quantities of seed of required crop/variety for ensuring timely and adequate supply of seeds to farmers during adverse conditions such as a natural calamity, shortfall in production, etc. For seed storage at the village level farmers are encouraged to take Seed Crop Insurance, which covers risk factors involved in production of seeds in the event of any unforeseen situation.

Quality assurance

The Seeds Act, 2001 will regulate the sale, import and export of seeds and planting materials of agriculture crops including fodder and green manure, horticulture, forestry, medicinal and aromatic plants and supply of quality seeds and planting materials to farmers throughout the country. National Seed Board (NSB) will be established in place of existing Central Seed Committee (CSC) and Central Seed Certification Board (CSCB). The NSB will have permanent existence with the responsibility of executing and implementing the provisions of the Seeds Act and advising the government on all matters relating to seed planning and development. The Board will function as the apex body in the seed sector.

All seeds that are placed on the market for sale and distribution will be registered. The Board will undertake registration of kind or variety of seeds that are to be offered for sale in the market, on the basis of identified parameters for establishing value for cultivation and usage (VCU) through testing and trials. Registration of varieties will be granted for a fixed period as prescribed for multilocational trials that determine VCU over a minimum period of three seasons or, as otherwise prescribed based on varieties that are in the market at the time this Act comes into force. These will have to be registered within a fixed time period, to be notified by the government. The Board will accredit ICAR, SAU and private organizations to conduct VCU trials for the purpose of registration.

The Board will maintain the National Seed Register containing details of varieties that are registered and coordinate and assist activities of the states in their effort to provide quality seeds to farmers. The Board will prescribe minimum standards ie, germination, genetic purity, physical purity and seed health, etc, as well as suitable guidelines for registration of seed and planting materials. Provisional registration would be granted on the basis of information provided by the applicant relating to trials over one season to tide over the stipulation of testing over three seasons before the grant of registration.

The government will have the right to exclude certain kinds of varieties from registration to protect public order or public morality or human, animal and plant life and health or to avoid serious prejudice to the environment. The Board will have the power to cancel the registration granted to a variety if the registration has been obtained by misrepresentation or concealment of essential data, the variety is obsolete and has outlived its utility and that the prevention of commercial exploitation of such variety is necessary in public interest.

The Seed Processing Unit will be registered. Registered Seed Processing Units should meet minimum standards of processing seed. Seed certification will continue to be voluntary. The certification tag/label will provide an assurance of quality to the farmer. To meet quality assurance requirements for export of seeds, seed testing facilities will be established in conformity with guidelines and standards specified by the Board, will establish one or more State Seed Testing Laboratories or declare any Seed Testing Laboratory where analysis of seeds is carried out in the prescribed manner. The Board will accredit individuals or organizations to carry out seed certification, including self certification, on fulfillment of criteria as prescribed.

Farmers will retain their right to save, use, exchange, share or sell their farm seeds and planting materials without any restriction. They will be free to sell their seed on their own premises or in the local market without any hindrance provided that the seed is not branded. The sale of spurious or misbranded seed will carry a major penalty, minor infringements committed by dealers and producers will attract minor penalty to obviate an opportunity for harassment by enforcement staff.

Seed distribution and marketing

The availability of high quality seeds to farmers through an improved distribution system and efficient marketing set up will be ensured to facilitate greater security of seed supply. For promoting efficient and timely distribution and marketing of seed throughout the country, a supportive environment will be provided to encourage expansion of the role of the private seed sector. The private seed sector will be encouraged and motivated to restructure and reorient their activities for catering to nontraditional areas.

A system will be established for collection and dissemination of market intelligence regarding preference of consumers and farmers. A National Seeds Grid will be established for collection and monitoring of information on requirement of seed, its production, distribution and preference of farmers on a district-wise basis. For developing efficient seed distribution and marketing facilities, access to term finance from commercial banks will be facilitated for growth of seed sector. Distribution and

marketing of seed of any variety for the purpose of sowing and planting will be allowed only if the said variety has been registered by the National Seed Board. The National Seed Board can direct a dealer to sell or distribute seeds in a specified manner if it is considered necessary to the public interest.

Infrastructure facilities

To meet the enhanced requirement of quality/certified seeds, focus will be on creation of new infrastructure facilities along with strengthening of existing facilities. The National Seed Training Centre being set up at Varanasi (UP) will impart training and build capacity in various disciplines of the seed sector. The Central Seed Testing Laboratory will be established at the National Seed Training Centre to perform the referral and other functions as required under the Seeds Act, 2001. Seed processing capacity will be augmented to meet the enhanced seed processing requirement in view of increased seed production. Modernization of seed processing facilities will be supported in terms of modern equipment and latest techniques, such as seed treatment for enhancement of performance of seed, etc. Conditioned storage for breeder and foundation seed and aerated storage for certified seed would be created in different states. A Computerized National Seed Grid will be established to provide information on availability of different varieties of seeds with production agencies, their location and quality to facilitate optimum utilization of available seeds during any given season. Initially, seed production agencies in the public sector would be connected with the National Seed Grid, but progressively the private sector will be encouraged to join the Grid for providing a clear assessment of demand and supply of seeds.

State governments or the National Seed Board in consultation with the concerned state government may establish one or more seed Certification Agency in the states. State governments will establish appropriate systems for effective execution and implementation of the objectives and provisions of the Seeds Act, 2001 for promoting seed growers, seed associations and cooperatives.

Transgenic plant varieties

Biotechnology has an important role in the development of the agriculture sector. This technology could be used not only to develop new crops/varieties which are tolerant to disease, pest and abiotic stresses but also to improve productivity and nutritional quality of food products. All genetically engineered crops/varieties will be tested for environment and biosafety aspects before their commercial release as per the regulation and guidelines of the Environment Protection Act (EPA), 1986. Seeds of the transgenic plant varieties for research purposes will be imported through National Bureau of Plant Genetic Resources (NBPGR) as per the EPA, 1986. The transgenic

crops/varieties will be tested to determine their agronomic value for at least two seasons under the All India Coordinated Project Trials of ICAR in coordination with the tests for environment and biosafety clearance as per the Environment Protection Act, before any variety is commercially released in the market.

Once the transgenic plant variety is commercially released, its seed will be marketed in the country as per the provisions of the seeds Legislation. Transgenic Seeds and planting materials, if and when placed on sale, would be required to bear a label indicating their transgenic character. After commercial release of a transgenic plant variety, its performance in the field will be monitored for at least 3 to 5 years by the Ministry of Agriculture and the State Department of Agriculture.

Transgenic varieties can be protected under the PVP legislation in the same manner as non-transgenic varieties after their release for commercial cultivation. All seeds imported into the country will be required to carry a declaration and a certificate from the Competent Authority of the exporting country certifying transgenic character of the seed. If the seed or planting material is a product of transgenic manipulation, it will be allowed to be imported only with the approval of the Genetic Engineering Approval Committee (GEAC), set up under the EPA, 1986.

Imports of seeds and planting material

The object of the import policy is to provide the best planting material available anywhere in the world to the Indian farmers, to increase productivity, farm income and export earnings, while ensuring that there is no deleterious effect on environment, health and biosafety. While importing seeds and planting material, care will be taken to ensure that there is absolutely no compromise on the requirements under prevailing plant quarantine procedures to prevent entry into the country of exotic pest diseases and weeds detrimental to Indian agriculture. All import of seeds and planting materials, etc, will be allowed freely subject to requirements of the Plants, Fruits and Seeds (Regulation of import into India) Order, 1989 as amended from time to time. Seeds and planting materials imported for sale into the country will have to meet minimum seed standards of seed health, germination, genetic and physical purity as prescribed. All the imports of seeds will require a permit granted by the Plant Protection Advisor to the Government of India. All importers will make available a small, specified quantity of imported seeds for accession to the Genebank maintained by NBPGR. The existing policy which permits free import of seeds of vegetables, flowers and ornamental plants, cuttings, saplings of flowers, tubers and bulbs of flowers by certain specified categories of importers will continue. Tubers and bulbs of flowers will be subjected to post-entry quarantine. After the arrival of consignments at the port of entry, quarantine checks would be undertaken which may include visual inspection, laboratory inspection, fumigation

and grow out tests. For the purpose of these checks, samples will be drawn and the tests will be conducted concurrently.

Export of seeds

Given the diversity of agro-climatic conditions, a strong seed production infrastructure and market opportunity, India has significant prospects for export of seeds. The Government will evolve a long term policy for export of seeds with a view to raise India's share of global seed export from the present level of less than 1 to 10% by the year 2015. The export policy will specifically encourage custom production of seeds for export and will be based on a long term perspective policy, dispensing with case to case consideration of proposals. Establishment and strengthening of seeds export promotion zones with special incentives from the government will be a focus area. A data bank will be created to provide information on International Market/export potential of Indian varieties in different parts of the world. A database on availability of seeds of different crops to assess impact of exports on domestic availability of seeds will be created. Various promotional programs to improve the quality of Indian seeds to enhance its acceptability in the international market will be taken up.

Promotion of domestic seed industry

Incentives will be provided to domestic seed industry to encourage and promote its growth to enable it to produce seeds of high yielding varieties and hybrid seeds at a faster pace to meet domestic requirement. The seed industry will be provided congenial and liberalized climate for increasing seed production and marketing, both domestic and international. Membership to International Organizations and Seed Associations like ISTA, OECD, UPOV, ASSINSEL, WIPO at the national level or at the level of individual seed producing agencies, including both private and public sector, will be encouraged.

Emphasis will be given on improving the quality of seed produced and special efforts will be directed towards increasing the quality of farmers' saved seeds. Financial support for capital investment, working capital and infrastructure strengthening will be facilitated through NABARD/Commercial Banks/Cooperative Banks. Tax rebate/concessions will be considered on the expenditure incurred on in-house research and development for development of new varieties and other seed related research aspects. For encouraging seed production in non-traditional areas including backward areas, special incentives such as transport subsidy will be provided to seed producing agencies operating in these marginalized areas. Reduction of import duty will be considered on machines and equipment used for seed production and processing which are otherwise not manufactured in the country.

Strengthening of monitoring system

The Seeds Division of the Department of Agriculture and Cooperation will supervise the overall implementation of the National Seed Policy. Adequate infrastructure support could be required for undertaking, monitoring and servicing the proposed Board and the activities there under. Human resource development in overall improvement in the seed sector is another important area that requires adequate attention.

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Guidelines for the conduct of test for Distinctness, Uniformity and Stability (DUS) on sorghum [*Sorghum bicolor* (L.) Moench]

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With the signing of the TRIPS agreement of the World Trade Organization (WTO), India has agreed to provide protection to plant varieties. The Government of India has enacted legislation on Protection of Plant Varieties and Farmers' Rights (PPV & FR) in 2001 to provide legal framework for Plant Breeders' and Farmers' Rights. The rules of this Act were notified in September 2003. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) came into existence under this Act, which will be responsible for implementation of the Act.

Plant varieties seeking protection need to be registered with PPV authority. For registration, the varieties have to pass through Distinctness, Uniformity and Stability (DUS) test. DUS test ensures distinctness of a character or characters, uniformity of those characters in a population and stability of characters over generation. The DUS test will be carried out for a minimum of two years to ascertain the distinctness of a candidate variety.

The preparation of DUS test guidelines for sorghum was initiated by National Research Centre for Sorghum during 2000–01 and was based on the International Union for the Protection of New Varieties of Plants (UPOV)'s guidelines. The UPOV guidelines were modified to suit Indian materials and conditions. The guidelines for sorghum were presented during various meetings of sorghum group (AICSIP), and other experts, and all important suggestions were incorporated.

As per the earlier national test guidelines (with 41 characteristics), the experiments were carried out for the past four years at four centers of sorghum DUS test, viz: NRCS, Hyderabad & Mahatma Phule Krishi Vidyapeeth (MPKV), Rahuri for grain sorghum; and GB Pant University of Agriculture and Technology (GBPUA&T), Pantnagar & CCS HAU, Hisar for forage sorghum. Based on the data collected during the past four years, the earlier guidelines were suitably modified and utmost care was taken in giving sample varieties for most of the attributes. These guidelines (with 33 characteristics) submitted to PPV&FRA were further corrected by the 'Task Force (1/2005)' and published by the Authority during February 2007. These guidelines contain the following sections.

- I) Subject
- II) Seed material required
- III) Conduct of tests
- IV) Methods and observations
- V) Grouping of varieties
- VI) Characteristics and symbols
- VII) Table of characteristics
- VIII) Explanation of the table of characteristics
- IX) Literature
- X) Working Group details

Subject

These test guidelines shall apply to all varieties, hybrids and parental lines of Sorghum [*Sorghum bicolor* (L.) Moench].

Seed material required

1. The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide when, where and in what quantity and quality the seed material are required for testing a variety denomination applied for registration under the Protection of Plant Variety and Farmers' Rights (PPV & FR) Act, 2001. Applicants submitting such seed material from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislations and regulations are complied with. The minimum quantity of the seed to be provided by the applicant shall be 3000 gram in the case of the candidate variety or hybrid and 1500 gram for each of the parental lines of the hybrid. Each of these seed lots shall be packed and sealed in ten equal weighing packets and submitted in one lot. If requested by the competent authority, an additional 100 panicles shall be submitted.
2. The seeds submitted shall have the following standards for germination capacity, moisture content and physical purity.
 - a. Germination capacity
 - i. Inbred lines and single cross hybrids : 80% (minimum)
 - ii. Varieties and double cross hybrids : 90% (minimum)
 - b. Moisture content : 10-12% (maximum)
 - c. Physical purity : 98% (minimum)

3. The applicant shall also submit along with the seed certified data on the germination test made not more than one month prior to the date of submission. It also shall possess the highest genetic purity, uniformity, sanitary and phyto-sanitary standards.
4. The seed material shall not have been subjected to any chemical or bio-physical treatment.

Conduct of tests

1. The minimum duration of the DUS tests shall normally be at least two independent similar growing seasons.
2. The test shall normally be conducted at least at two test locations. If any essential characteristics of the candidate variety are not expressed for visual observation at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on expressed request of the applicant.
3. The field tests shall be carried out under conditions favouring normal growth and expression of all test characteristics. The size of the plots shall be such that plants or parts of plants could be removed for measurement and observation without prejudicing the other observations on the standing plants until the end of the growing period. Each test shall include about 900 plants in the plot size and planting space specified below across four replications. Separate plots for observation and measurement can only be used if they have been subjected to similar environmental conditions. All the replications shall be sharing similar environmental conditions of the test location.
4. Test plot design:

Number of rows	: 6
Row length	: 6 m
Row to row distance	: 60 cm
Plant to plant distance	: 15 cm
Number of replications	: 4
5. Observations shall not be recorded on plants in border rows.
6. Additional tests for special purpose shall be established by the PPV & FR Authority.

Methods and observations

1. The characteristics described in the table of characteristics shall be used for the testing of varieties, inbred lines and hybrids for their DUS.
2. For the assessment of Distinctness and Stability, observations shall be made on 40 plants or parts of 40 plants, which shall be divided among 4 replications (10 plants in each replication).
3. For the assessment of Uniformity of characteristics on the plot as a whole (visual assessment by a single observation of a group of plants or parts of plants), the number of aberrant plants or parts of plants shall not exceed 6 in 100.
4. For the assessment of Uniformity of characteristics on single ear-rows, plants or parts of plants (visual assessment by observations of a number of individual ear-rows, plants or parts of plants) the number of aberrant ear-rows, plants or parts of plants shall not exceed 6 in 100.
5. For the assessment of color characteristics, the latest Royal Horticultural Society (RHS) color chart shall be used.

Grouping of varieties

1. The candidate varieties for DUS testing shall be divided into groups to facilitate the assessment of distinctness. Characteristics, which are known from experience not to vary, or to vary only slightly within a variety and which in their various states are fairly evenly distributed across all varieties in the collection are suitable for grouping purposes.
2. The following characteristics are proposed to be used for grouping sorghum varieties:
 - a. Adaptation of season: *Kharif/Rabi/Others*
 - b. Plant: Time of panicle emergence (50% of the plants with 50% anthesis) (Characteristic 4)
 - c. Plant: Total height (at physiological maturity) (Characteristic 15)
 - d. Panicle: Shape (at physiological maturity) (Characteristic 22)
 - e. Caryopsis: Color (after threshing) (Characteristic 26)

Characteristics and symbols

1. To assess Distinctness, Uniformity and Stability, the characteristics and their states as given in the table of characteristics (Section VII) shall be used.
2. Note (1 to 9) shall be used to describe the state of each character for the purpose of digital data processing.

3. Legend:

(*) Characteristics that shall be observed during every growing period on all varieties and shall always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall be provided.

(+) See explanation on the table of characteristics in Section VIII. It is to be noted that for certain characteristics the plant parts on which observations to be taken are given in the explanation or figure(s) for clarity and not the color variation.

4. The optimum stage of plant growth for assessment of each characteristic is given in the sixth column in the section VII on table of characteristics.

5. Type of assessment of characteristics indicated in column seven of table of characteristics is as follows:

MG: Measurement by a single observation of a group of plants or parts of plants

MS: Measurement of a number of individual plants or parts of plants

VG: Visual assessment by a single observation of a group of plants or parts of plants

VS: Visual assessment by observation of individual plants or parts of plants

Table of characteristics.							
S. No.	Characteristics	States	Note	Example varieties/ lines	Stage of observation	Type of assessment	
1	2	3	4	5	6	7	
1	Seedling: Anthocyanin coloration of coleoptile	Yellow-green (RHS 144-N144) Greyed-purple (RHS 183-187)	1 2	AKMS 14B M 35-1, AKR 150	Seedling 7-8 days after sowing	VS	
2	Leaf sheath: Anthocyanin coloration	Yellow-green (RHS 144-N144) Greyed-purple (RHS 183-187)	1 2	AKMS 14B, Pant Chari 4	5 leaf	VS	
3	Leaf: Mid rib color (5 th fully developed leaf)	White (RHS 155-N155) Yellow-green (RHS 144-N144) Greyed-yellow (RHS 162) Greyed-purple (RHS 183-187)	1 2 3 4	SPV462, JJ 1041 CS 3541 - -	5 th leaf	VS	
4	Plant: Time of panicle emergence (50% of the plants with 50% anthesis)	Very early (<56 days) Early (56-65 days) Medium (66-75 days) Late (76-85 days) Very late (>85 days)	1 3 5 7 9	GFS 4 CSH 14 CSH 16 Pant Chari 5 SSV 84	Panicle emergence	VG	
5	Plant: Natural height of plant up to base of flag leaf	Very short (<76 cm) Short (76-150 cm) Medium (151-225 cm) Tall (226-300 cm) Very tall (>300 cm)	1 3 5 7 9	- 296B RS 29 Pant Chari 3 -	Panicle emergence	MS	

Contd.

6 (*)	Flag leaf: Yellow coloration of midrib	Absent Present	1 5	CS 3541 27B	Panicle emergence	VS
7 (*)	Lemma: Arista formation	Absent Present	1 5	CS 3541 296B	Flowering	VS
8 (*)	Stigma: Anthocyanin coloration	Absent Present	1 5	CS 3541 SSG 59-3	Upper portion of the panicle at the end of flowering	VS
9 (*)	Stigma: Yellow coloration	Absent Present	1 5	CS 3541 27B	Flowering	VS
10	Stigma: Length	Short (<1mm) Medium (1–2mm) Long (>2mm)	3 5 9	AKMS 14B IMS 9B MAN T1	Flowering	MS
11 (+)	Flower with pedicel: Length of flower	Very short Short Medium Long Very long	1 3 5 7 9	- - CS 3541 27B SSG 59-3	Flowering	VG
12	Anther: Length	Short (<3mm) Medium (3–4mm) Long (>4mm)	3 5 7	C 43 27B -	Flowering	MS
13	Anther: Color of dry anther	Yellow-orange (RHS 14-23) Orange (RHS 24-29) Orange-red (RHS 30-35) Greyed-orange (RHS 163-177)	1 2 3 4	2219B CS 3541 - CSH 16	End of flowering	VG

Contd.

14 (*) (+)	Glume: Color	Green-white (RHS 157) Yellow-white (RHS 158) Greyed-yellow (RHS 160-162) Greyed-orange (RHS 163-177) Greyed-red (RHS 178-182) Greyed-purple (RHS 183- N187)	1 2 3 4 5 6	- 2077B Pant Chart 5 UPMC 503 - Pant Chart 4	Physiological maturity of grain	VG
15 (*)	Plant: Total height	Very short (<76 cm) Short (76–150 cm) Medium (151–225 cm) Long (226–300 cm) Very long (>300 cm)	1 3 5 7 9	- 2219 B RS 673 GJ 39 -	Physiological maturity	MS
16	Stem: Diameter (at lower one-third height of plant)	Small (<2 cm) Medium (2–4 cm) Large (>4 cm)	3 5 7	CS 3541 2077B -	Physiological maturity	MS
17	Leaf: Length of blade (the third leaf from top including flag leaf)	Short (<41 cm) Medium (41–60 cm) Long (61–80 cm) Very long (>80 cm)	3 5 7 9	- 2219B CS 3541 CSH 18	Physiological maturity	MS
18	Leaf: Width of blade (the third leaf from top including flag leaf)	Narrow (<4.1 cm) Medium (4.1–6.0 cm) Broad (6.1–8.0 cm) Very broad (>8.0 cm)	3 5 7 9	GFS 4 - CSV 17 CSH 16	Physiological maturity	MS
19 (*)	Panicle: Length without peduncle	Very short (<11 cm) Short (11–20 cm) Medium (21–30 cm) Long (31–40 cm) Very long (>40 cm)	1 3 5 7 9	- SSV 84 CS 3541 IMS 9B SSG 59-3	Physiological maturity	MS

Contd.

20	Panicle: Length of branches (middle third of panicle)	Short (<5.1 cm) Medium (5.1–10 cm) Long (10.1–15 cm) Very long (>15 cm)	3 5 7 9	- CS 3541 2077B SSG 59-3	Physiological maturity	MS
21 (*)	Panicle: Density at maturity (ear head compactness)	Very loose Loose Semi loose Semi compact Compact	1 3 5 7 9	SSG 59-3 Pant Char 4 CSH 16 C 43 Surat 1	Physiological maturity	VG
22 (*) (+)	Panicle: Shape	Reversed pyramid Panicle broader in upper part Symmetric Panicle broader in lower part Pyramidal	1 2 3 4 5	- JJ 741 CSH 9 MAN T1 SSG 59-3	Physiological maturity	VG
23 (*)	Neck of panicle: Visible length above sheath	Absent or very short (<5.1 cm) Short (5.1–10 cm) Medium (10.1–15 cm) Long (15.1–20 cm) Very long (>20cm)	1 3 5 7 9	296B JJ 1041 Pant Char 4 GJ 37 CSH 16	Physiological maturity	MS
24 (+)	Glume: Length	Very short (25% of grain covered) Short (50% of grain covered) Medium (75% of grain covered) Long (100% of grain covered) Very long (longer than the grain)	1 3 5 7 9	CSH 9 CSV 15 2219B SSG 59-3 -	Physiological maturity	MS

Contd.

	Grain: Thresholdability				C 43	Maturity	VG
25 (+)		Freely threshable (<11% unthreshed grain) Partly threshable (11–50% unthreshed grain) Difficult to thresh (>50% unthreshed grain)	1 5 7		C 43 MR 750 SSG 59-3		VG
26 (*)	Caryopsis: Color after threshing	White (RHS 155) Greyed-white (RHS 156) Yellow-white (RHS 158) Yellow-orange (RHS 14-20) Greyed-orange (RHS 165)	1 2 3 4 5		MAN T1 Pant Chari 4 Pant Chari 5 27B UPMS 503	After threshing	VG
27	Grain: Weight of 1000 grains	Very low (<16 g) Low (16–25 g) Medium (26–35 g) High (36–45 g) Very high (>45 g)	1 3 5 7 9		SSG 59-3 2219B C 43 - -	After threshing	MG
28 (+)	Grain: Shape (in dorsal view)	Narrow elliptic Elliptic Circular	1 2 3		SSG 59-3 2219B 27B	After threshing	VG
29 (+)	Grain: Shape in profile view	Narrow elliptic Elliptic Circular	1 2 3		SSG 59-3 2219B 27B	After threshing	VG

Contd.

30 (+)	Grain: Size of mark of germ	Very small Small Medium Large Very large	1 3 5 7 9	- RS 29 296B C 43 DSV 4	After threshing	VG
31 (*)	Grain: Texture of endosperm (in longitudinal section)	Fully vitreous (100% corneous) ¾ vitreous (75% corneous) Half vitreous (50% corneous) ¾ farinaceous (25% corneous) Fully farinaceous (0% corneous)	1 3 5 7 9	- - AKMS 14B 296B -	After threshing	VG
32	Grain: Color of vitreous albumen	Greyed-yellow (RHS 160-162) Greyed-orange (RHS 166) Greyed-purple (RHS N187)	1 2 3	AKMS 14B SSG 59-3, UPMC 503 Pant Chari 4	After threshing	VG
33 (*)	Grain: Lustre	Non-lustrous Lustrous	1 5	296B CS 3541, M 35-1	After threshing	VG

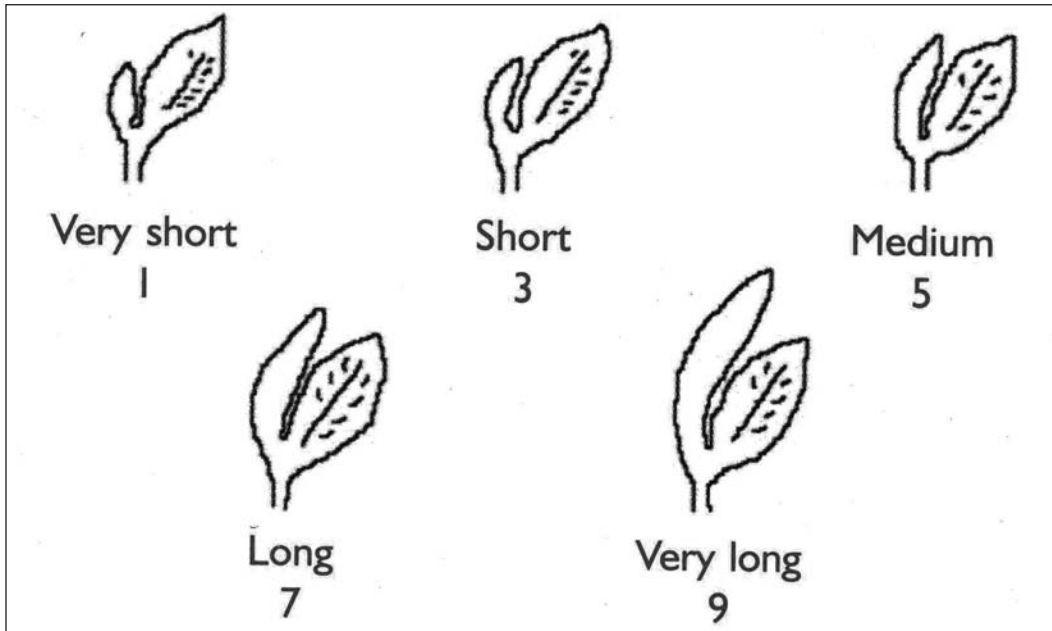
Explanation of the Table of characteristics

Characteristic 1. Seedlings: Anthocyanin coloration of coleoptile

Cultivation for production of seedlings of sorghum under controlled conditions as per Payne et al. 1980.

Soil	:	1/3 compost + 2/3 sand
Temperature	:	24°C
Lighting	:	Continuous light at 24000 lux
Duration of test	:	About 14 days with the day of sowing included
Actual observation	:	2 times on 25 seedlings

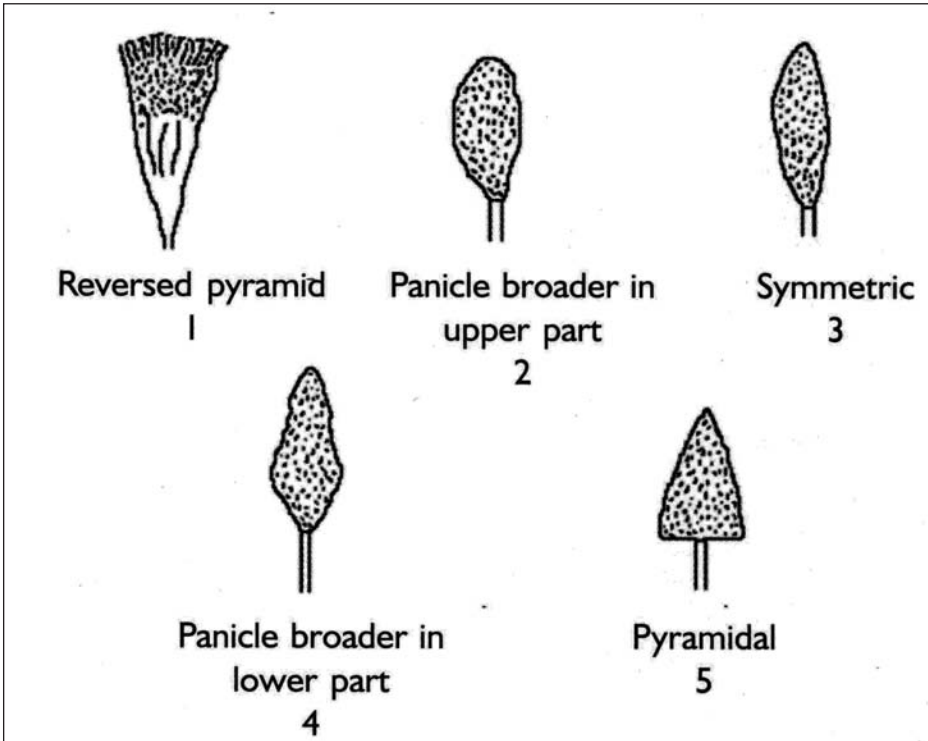
Characteristic 11. Flower with pedicel: Length of flower



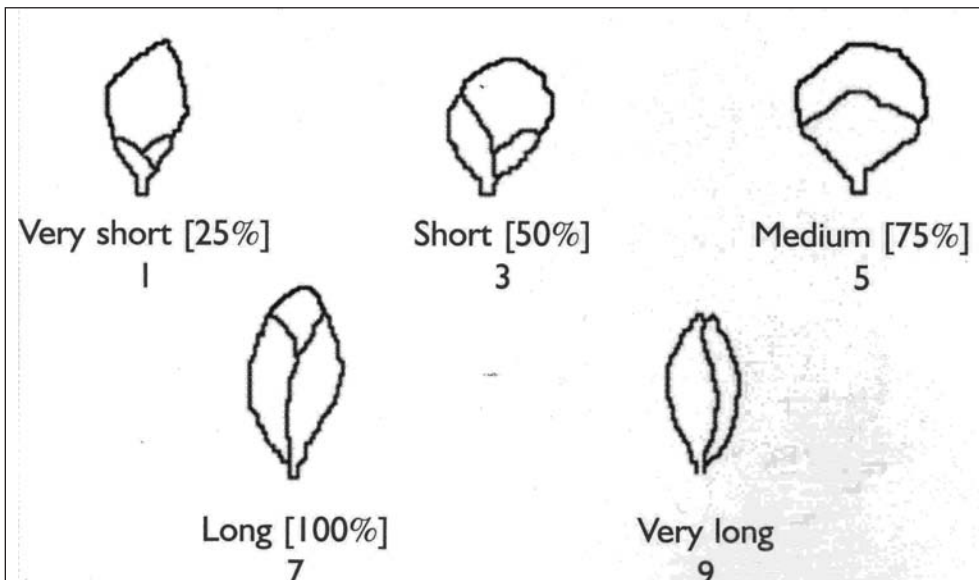
Characteristics 14. Glume: Color

Glume color is to be recorded at the time of physiological maturity, ie, when the black layer is formed on the base of the grain.

Characteristic 22. Panicle: Shape



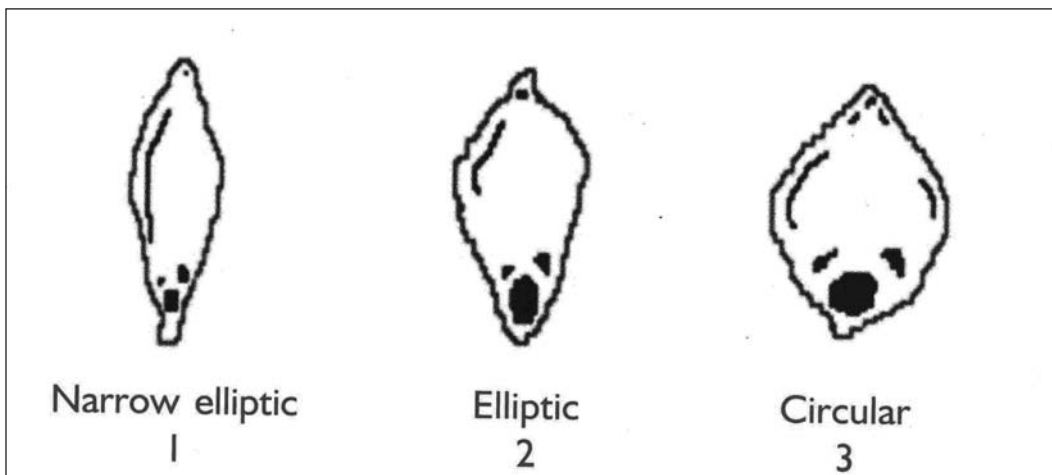
Characteristic 24. Glume: Length



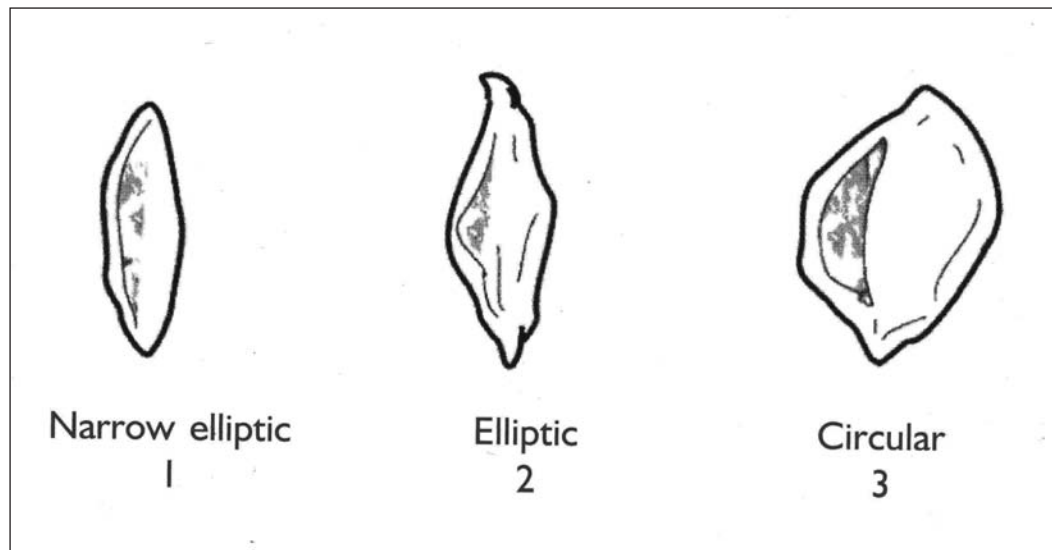
Characteristic 25. Grain: Threshability

Three primary branches each from top, middle and bottom portions (total 9 primary branches) of the panicle shall be selected after one week of physiological maturity and hand threshed. Ten panicles per replication shall be randomly selected for this purpose.

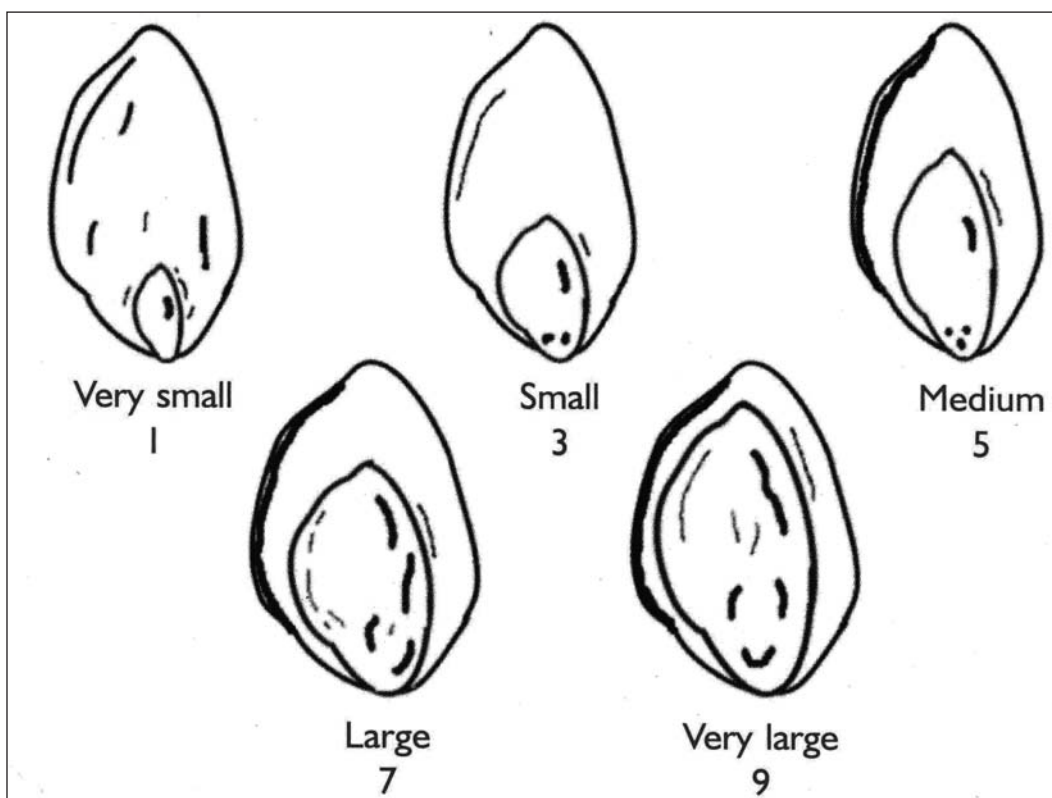
Characteristic 28. Grain: Shape in dorsal view



Characteristic 29. Grain: Shape in profile view



Characteristic 30. Grain: Size of mark of germ



Literature

1. **UPOV**. 1989. TG/122/3 Guidelines for the conduct of tests for distinctness, homogeneity and stability in Sorghum.
2. **IBPGR** and **ICRISAT**. 1984. Revised Sorghum Descriptors.
3. **IBPGR** and **ICRISAT**. 1993. Descriptors for Sorghum (*Sorghum bicolor* (L.) Moench) 99.
4. **Payne RC**, **Koszykolowski TU** and **Morris LP**. 1980. Differentiation of Sorghum, Sudan grass and Sorghum x Sudan Grass Cultivars by seedling pigmentation patterns. Journal of Seed Technology Nr 1.

Working Group details

The Test Guideline developed by the National Core Committee in consultation with the Director, National Research Centre for Sorghum (NRCS), Hyderabad, the Nodal Officer, DUS Testing, NRCS, Hyderabad and the Task Force (1/2005) constituted by the PPV & FR Authority.

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Alternative uses of sorghum grain and other products

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Introduction

Sorghum is an important crop for food and fodder in the semi-arid tropic regions in India. It is mainly grown in *kharif* (rainy), *rabi* (postrainy) and summer seasons. The area planted to sorghum in postrainy season has remained stable over the years. However, area planted to sorghum in rainy season has declined over the last two decades because of poor demand for the grains due to frequent infection by grain mold. Of late, sorghum grains and its products are finding several alternative uses. In this article, potential alternative uses of sorghum apart from its food uses are described.

Food uses

In India, sorghum is traditionally consumed in the form of unleavened pan cake (*bhakri*). In southern India it is consumed in the form of *sankati*, *annam* and *kanji* (thin porridge). Sorghum *sankati* is a type of thick porridge consumed in South India. Sorghum *sankati* is consumed in the Rayalaseema tracts of Andhra Pradesh, the southern tracts of Karnataka, and all over Tamil Nadu. Boiled sorghum (rice-like) called *annam* or *soru* is one of the common products prepared in India, particularly in southern regions and it accounts for about 10% of the total sorghum grain produced.

The consumption of sorghum *roti* is a traditional practice in southern states of India, especially in Maharashtra, Karnataka and Andhra Pradesh. Sorghum *roti* preparation requires a special skill due to lack of stickiness in flour and hence designing suitable machinery to prepare large number of *rotis* in a short span is required for popularizing sorghum *roti* in urban areas. A machine suited to medium to large scale *roti* preparation is available at present. However, the National Research Centre for Sorghum (NRCS) is making efforts to test a machine with higher capacity (already in use for wheat *roti*) for making *roti* from sorghum grain on a large scale, and market it.

To reap the nutritional benefits of sorghum, the grain can be processed into different grades of milled products (eg, *suji*) and used for the preparation of different traditional and non-traditional foods as an alternative to rice and wheat. Sorghum can be flaked or popped to be used as breakfast or snack. Sorghum flour is suitable for the preparation of noodles, an important snack food in today's urban life. The other possible promising alternative food products that can be produced from sorghum grain are bakery products, malto-dextrins as fat replacers in cookies, liquid/powder glucose, high fructose syrup and sorbitol. The technologies for production of glucose, malto-dextrins and high fructose syrup are yet to be scaled up.

Bakery products

Substitution of wheat with local cereals like maize and sorghum in biscuit production was studied to improve the nutritional quality of biscuits. At NRCS, efforts were made to prepare common bakery products like bread, cakes and biscuits (Fig. 34). Fine sorghum flour made out of *kharif* grain (pearled) equal to the consistency of maida was used in combination with maida for the preparation of various bakery products like bread (whole sorghum), mixed bread (bread made from sorghum, ragi and bajra in 2:1:1 ratio), plum cake and biscuits. The methodology for the preparation of these products from sorghum flour is similar to that used for wheat flour.



Fig. 34. Different food products developed from sorghum.

Products from sweet sorghum

The juicy stalks of sweet sorghum, which is similar to sugarcane, can be utilized for preparation of syrup, jaggery and fuel-grade ethanol. Sweet sorghum juice-based syrup and jaggery received good attention, when the same was demonstrated on a large scale (Fig. 35). Shelf life and nutritive value of syrup and jaggery produced from sweet sorghum juice are the same as those produced from sugarcane juice.

Natural syrup: Sweet sorghum juice concentrated and sterilized to make natural syrup can be used in the dairy and confectionery industry as a sweetener. This syrup can be used in place of honey and can be served along with breakfast foods. This syrup called “sorghum honey” can be marketed immediately. The chemical composition of sweet sorghum syrup is similar to honey.



Fig. 35. Sweet sorghum - promising crop for ethanol and syrup.

Jaggery: Sweet sorghum juice can be concentrated and jaggery can be prepared using the same methodology used in sugarcane. Sugars in sweet sorghum stalk include sucrose, glucose and fructose. Depending on the sugar profile in juice, sweet sorghum stalk was processed to produce jaggery (low reducing sugars) with an average jaggery yield of about 30 to 35 q ha⁻¹. The cost will also be economical compared to sugarcane jaggery and may be about Rs 5 per kg. Jaggery preparation is mainly dependent on the invertase activity. Some genotypes that have very low invertase and the sucrose in the stalks do not get inverted are ideal for jaggery preparation.

Fuel-grade ethanol: Sweet sorghum with a potential of 30–50 t ha⁻¹ of juicy biomass and 40-60% of juice recovery can serve as a potential alternative feedstock

for fuel-grade ethanol. Because of seed propagation, short duration and lower cost of production, lower water requirement, and lower per liter cost of ethanol production, compared to sugarcane (molasses, the byproduct of sugarcane is the sole feedstock for ethanol production), policy makers and researchers are favoring the use of sweet sorghum as an alternative to molasses for ethanol production. A pilot study conducted with Renuka Sugar Factory, Belgaum, has recorded an ethanol recovery of 9% from the juice. The bagasse thus obtained was utilized for co-generation of electricity (2086 net calories per ton). Cultivation of sweet sorghum is economical in rain-fed areas where growing sugarcane is not economical. The seed rate and planting pattern recommended for grain sorghum can be adopted. Sweet sorghum has the ability to yield 40–45 t ha⁻¹ millable cane and 1–1.5 t grain ha⁻¹ and an average Brix of 18.4%. The juice has a minimum of 12% sucrose and at least 15% total fermentable sugars.

Industrial uses

Grain sorghum finds a potential place in the animal feed sector, alcohol, starch, textile, pharmaceutical, brewery and energy sectors. Commercialization of potable alcohol produced from sorghum grain is already in practice. Sorghum grain requirement in alcohol and starch industry would be around 2–5 lakh tons and 30–80 thousand tons by 2010.

Sorghum as a feed: A comparative analysis of various feed grains as economical sources of nutrients indicated that sorghum is the cheapest source of energy at the prevailing price. Grain sorghum is very similar to shelled corn in chemical composition except that grain sorghum is slightly higher in protein and contains little, if any, of carotene. Grain sorghum can be used to replace as much as 50% of the corn in the animal feed rations without affecting animal performance. Recent studies from the industrial survey conducted at NRCS indicate that sorghum can replace maize to the extent of 100 %.

Potable alcohol: Severely damaged sorghum grain not preferred even for cattle feed but a rich source of carbohydrates can serve as an economically viable raw material for potable alcohol production. For instance, hybrids CSH 16 and CSH 18 with a high starch content yield high recovery of ethanol and can be used in commercial production. Molded sorghum grain is grinded, gelatinized and digested with amylase and amyloglucosidase and is fermented for 72 h for the production of potable alcohol. The alcohol obtained from sorghum is significantly cleaner (low sulphur) than that obtained from sugarcane molasses. The biproducts left after fermentation like proteins, fats and fibers in the stillage can serve as an excellent protein supplement in cattle feed.

Lager beer: Lager beer can be produced from barley malt and any cereal adjunct including sorghum. Properties of sorghum malt were in general poor compared to barley malt, but total soluble sugars and cold water (%) extract of the sorghum malt were relatively higher than those of barley malt. Production of lager beer from 100% sorghum grain is entirely a new concept. The cultivars that have low gelatinization temperature, low polypeptides, low lipid, high diastatic power and readily soluble protein are good for lager beer preparation. Beer was prepared using 100% sorghum malt, sorghum adjuncts and hops on the ratio 96:3:1. Pilot experiments are being carried out at NRCS for use of sorghum in lager beer preparation.

Malt: Barley is used normally for the preparation of malt. Sorghum can also replace barley for malt preparation, as the diastatic activity of sorghum is 80% that of barley. The standardization of conditions for malt preparation was carried out at NRCS. The diastatic activity (SDU) varied from 144 SDU in IS 14387 to 200 SDU in WS 1297. The malting loss of 27–39% reported in these genotypes can be minimized with air rest and minimized water supply. Malt prepared from sorghum grain can be used in the preparation of baby food beverages. Fine sorghum malt flour is used in the preparation of baby foods. Sorghum grain is processed into flour of 200 μ to 300 μ particle grade and grit of 300 μ to 1100 μ particle grade through dry milling system. 300 μ particle grade flour is used in traditional consumption.

Starch and starch by-products: Sorghum grain contains 63.4–72.5% starch, 17.8–21.9% amylose, 7.9–11.5% protein, 1.86–3.08% fat and 1.57–2.41% fiber. Sorghum is a good, cheap source for the production of starch and starch by-products. The recovery of starch from sorghum grain is 5–8% less than maize but equally lustrous. The recovery can be increased by increasing grain size, reducing protein and fiber content. Liquid glucose and high fructose syrup (HFS) can be prepared from starch. Maltodextrins prepared from sorghum starch are used in the preparation of low calorie-low fat cookies in the baking industry.

High fructose syrup (HFS): High fructose syrup can be prepared from sorghum grain. This is a highly valued product in the pharmaceutical industry. HFS is 1.6 times sweeter than sucrose (common sugar). HFS can be utilized as a sweetener in pharmaceuticals, soft drinks and food processing industries.

Glucose: Liquid and powder glucose can be prepared economically from sorghum grain. Sorghum can be a competitive raw material for the production of glucose. The technology is available with Agro Product Development Centre, PDKV, Akola.

Food safety in sorghum

Due to frequent infection by grain mold, *kharif* grain gets severely infested with mycotoxins making it unsafe for consumption or processing into a food product.

Systematic research work at NRCS reveals that mycotoxin contamination is less in sorghum compared to maize. The contamination level is safe for human consumption.

Conclusion

Sorghum grain is nutritionally superior and should be used extensively in all food preparations to exploit its nutritional value. Milled products of sorghum should be popularized in urban areas to increase its consumption. Ready to eat products like flakes, noodles, etc, are expected to attract greater demand in urban areas. Sorghum can completely replace maize in animal feed rations. Heavily molded sorghum is a suitable raw material for potable ethanol production. Sorghum, either malted or unmalted, is suitable for lager beer preparation. Natural syrup can be made with sweet sorghum stalk juice.

Sweet sorghum - a potential energy crop for biofuel production in India

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Sorghum [*Sorghum bicolor* (L.) Moench] is the principal dryland coarse cereal grown in India (9.50 m ha) and around the world (45.8 m ha) for food, animal feed, fodder and energy.

Why sweet sorghum for ethanol production?

The Government of India's policy to blend petrol with ethanol (to reduce fuel-import bill and environmental pollution) initially up to 5% has triggered production of bioethanol from different feedstocks. Requirement of ethanol in the country is estimated to be around 0.64 billion liters annum⁻¹ at the 5% level of doping in petrol. Currently, molasses is the sole feedstock to produce alcohol. The price of molasses is highly variable between Rs 2000 and Rs 5000 ton⁻¹ and the cost of ethanol production from molasses also varies accordingly. The process of ethanol production from molasses is highly polluting as molasses itself is a byproduct from the sugar industry. Also, production of ethanol from molasses alone is unlikely to meet the requirement of ethanol for doping with petrol @ 5%, as the scope for increasing sugarcane area beyond the current 4.0 million ha in the country is bleak due to depleting water reserves and shrinking land area available for cultivation. Therefore, researchers and policy makers are exploring alternative feedstock for ethanol production. Sweet sorghum, similar to grain sorghum, with a potential to accumulate sugars (10–20%) in its stalks as in sugarcane (Hunter and Anderson 1997), offers a potential alternative feedstock as cost of feedstock production, water requirement and crop duration is far less than those of sugarcane from which molasses is obtained. The stillage after extraction of juice from sweet sorghum has higher calorific value and hence can be used to cogenerate power of about 2.5 MW ha⁻¹ crop (Hunsigi 2005). Stillage can also be used as animal fodder after suitable processing and also as a substrate for production of second generation ethanol. Further, economics of ethanol production is in favor of sweet sorghum. Per liter cost of production of ethanol from sweet sorghum (Rs 13.11) is lower than that from sugarcane molasses (Rs 14.98). Besides these, use of sweet sorghum will not compromise food security as only stalk juice is used for ethanol production. Considering these advantages, sweet sorghum has emerged as the best alternative

feedstock for ethanol production in India. (Ratnavathi et al. 2003; Dayakar Rao et al. 2004; Planning Commission, Government of India, New Delhi 2006; Shukla et al. 2006; Rao 2005; Reddy et al. 2005; Rao et al. 2006a and 2006b)

Distinct characteristics of sweet sorghum

- Ethanol produced from sweet sorghum stalk juice by fermentation and distillation is similar to that produced from sugarcane molasses, but without any pollution hazards.
- Sweet sorghum (4000 m³) propagated through seeds matures in 115–120 days compared to sugarcane which is propagated through cuttings and mature in 12–18 months.
- Water use or seasonal ET: Sorghum: 508 mm, Sugarcane: 1257 mm.
- Per crop water requirement of sweet sorghum is about one fourth of sugarcane (36,000 m³) per crop.
- Sweet sorghum-based ethanol production process leaves no/least hazardous pollutants such as aldehydes and sulphur compared to that based on molasses.

Adaptation and growing conditions

Sweet sorghum can be grown under dryland conditions with annual rainfall ranging from 550–750 mm. The best areas to produce this crop are central and south India, subtropical areas of Uttar Pradesh and Uttaranchal. It can be grown on well-drained soils such as silt loam or sandy silt clay loam soils with a depth ≥ 0.75 m. Atmospheric temperatures suitable for sweet sorghum growth vary between 15 and 37°C. Sorghum being a C₄ species is adapted to a wide range of environments with latitudes ranging from 40°N to 40°S of the equator. Sorghum in general has relatively deep root system (>1.5 m), and has the unique feature of becoming “dormant” under unfavorable conditions and resuming growth once environmental conditions are favorable for growth.

Sweet sorghum research and development efforts

The first major attempt in India was made at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to evaluate and identify useful high biomass producing sweet sorghum germplasm from world collections (Seetharama and Prasada Rao 1987). The sweet sorghum improvement program during the last two decades at National Research Centre for Sorghum (NRCS) and the All India Coordinated Sorghum Improvement Project (AICSIP) centers had resulted in the development of a number of breeding lines which led to national level release of

several varieties such as SSV 84 (High Brix: 18%), CSV 19SS (RSSV 9) and hybrid CSH 22 SS (NSSH 104) with productivity ranging from 40–50 t ha⁻¹. ICRISAT, Patancheru, India has developed a number of sweet sorghum breeding materials, varieties, experimental hybrids having higher stalk sugar content and superior biomass yields (Reddy et al. 2005) (Fig. 36).

NRCS was in the forefront in organizing pilot studies on sweet sorghum-based ethanol production in collaboration with many distilleries and stakeholders such as Sri Renuka Sugars, Belgaum, Karnataka and Sagar Sugars, Nelavoy, Chittoor (Dayakar Rao et al. 2004, Ratnavathi et al. 2005, Huligol et al. 2004). The alcohol yield realized in these pilot studies was from 25 to 40 liters ton⁻¹ of stalks crushed. Techno-economic feasibility studies have shown that the cost of alcohol production from sweet sorghum was Rs 1.87 less than that from molasses (Dayakar Rao et al. 2004). Recently, NRCS organized pilot studies successfully in collaboration with Sagar Sugars, Chittor, and National Sugar Institute, Kanpur (Shukla et al. 2006, Rao 2005). ICRISAT through its Agri-Business Incubator (ABI) is collaborating with Rusni Distilleries, Sangareddy, Andhra Pradesh and promoting sweet sorghum as a biofuel crop. Rusni Distilleries has already started producing ethanol from sweet sorghum (ICRISAT 2006).

Constraints

The productivity of sweet sorghum in postrainy (*rabi*) (October–November planted) season is 30–35% less than that in rainy (*kharif*) and summer seasons because of short day length and low night temperatures. In order to meet the industry demand for raw materials, especially during lean periods of sugar cane crushing, there is a need to develop sweet sorghum cultivars that are photoperiod- and thermo-insensitive with high stalk and sugar yields.

Sweet sorghum crop management practices

Land preparation and manuring: Two plowings followed by leveling for good soil tilth.

Planting time: Sweet sorghum is mainly grown during *kharif* season. The crop can also be grown during *rabi* and summer seasons depending upon the availability of soil moisture/irrigation sources. The productivity of summer season grown crop is higher than that grown during *kharif* and *rabi* seasons.

***Kharif* season crop (June–October):** Sowing should be taken up immediately after the onset of monsoon, preferably from first week of June to first week of July, (depending on the onset of monsoon). Irrigate the crop if there is no rainfall at the time of sowing to ensure uniform germination and establishment. Sow the seeds in



Fig. 36. Sweet sorghum crop at ICRISAT, Patancheru.

a furrow behind the plough or place two to three seeds at a distance of 10–15 cm in a row by hand dibbling. The locally available seed drills can be used for planting. It is preferable to place the seed at a soil depth of 5.0 cm. Make sure that the soil has fully charged with rainwater at least in the top 30 cm soil layer to ensure uniform germination.

Rabi season crop (October–February): Planting should be carried out from last week of September to first week of November. The night temperatures should be above 15°C at the time of sowing. Irrigate the crop if there is no rainfall at the time of sowing to ensure uniform germination and establishment.

Summer season crop: Planting can be done from mid January to last week of May under irrigated condition. The night temperatures should be above 15°C at the time of sowing. The crop can also be grown in rice fallows in coastal areas capitalizing residual soil moisture.

Seed rate: 8 kg ha⁻¹ (or 3 kg acre⁻¹).

Soil type & depth: The crop can be grown in areas with soil types ranging from deep black soil (*Vertisol*) to deep red loamy soil (*Alfisol*) with a soil depth of >1.0 m.

Spacing: Row to row spacing of 0.6 m and 0.15 m between plants within a row.

Plant population: Population of 1.10 to 1.20 lakh plants ha⁻¹ (40,000 to 48,000 plants acre⁻¹) is optimum for realizing maximum productivity. Raising sweet sorghum with excessive plant population than recommended will result in the production of thin stalks that will lodge due to heavy winds or rains.

Thinning: First thinning needs to be carried out at about 15 days after sowing (DAS). Retain two seedlings per hill, 15 cm apart. Second (final) thinning needs to be carried out at about 25–30 DAS to retain single plant per hill. Thinning operation is very essential for uniform stand establishment and optimum growth of plants.

De-tillering: Remove the side tillers that occur from the base of the plant manually, if they occur within 20–25 days after planting.

Intercultivation: Intercultivate the crop with blade harrow or cultivator once or twice between 20 and 35 days after sowing. This will enable the earthing up of the crop and prevent lodging.

Nutrient management

- 1) 80 kg N, 40 kg P₂O₅ and 40 kg K₂O ha⁻¹;
- 2) 50% N and full P₂O₅ at sowing as a basal
- 3) Remaining 50% N as top-dressing at two equal installments at about 30–35 days after sowing (DAS) (ie, at final thinning) and at about 45–50 DAS. K application should be based on soil test results. However, fertilizer application should be based on prior soil test results in a particular region where the soil testing facilities exist.

Weed management

1. Apply pre-emergence spray of atrazine @1.0 kg a.i ha⁻¹ one day (immediately) after sowing but before weed emergence
2. Mechanical weeding twice up to 35–40 days age of the crop will check the weed growth

Irrigation/rainwater management

Kharif: Normally the crop is raised under rainfed conditions in areas receiving rainfall of 550–750 mm. The crop may need protective irrigation if rainfall distribution is uneven during the crop growth period. In case of late onset of monsoon, plant the crop and irrigate immediately. Irrigate the crop if the dry spell continues for more than two weeks especially at critical crop growth stages such as panicle initiation (35–40 DAS) and boot stages (55–65 DAS). Maintain soil moisture always at field capacity. Always drain out the excess irrigation water or rain water from the field to avoid water logging. By and large, it may be required to arrange 2–3 irrigations during the *kharif* season based on the planting time, soil type and rainfall distribution at the particular location.

Rabi and summer: Arrange first irrigation immediately after sowing, if no rainfall occurs. Subsequently, irrigate the crop at 15 DAS, 30 DAS, 55 DAS and 80-90 DAS for realizing good cane yields. Thus a total of 4–5 irrigations are required for *rabi* and summer crops. Apply irrigation by about 50 mm of water each time. During the initial 30 days of crop growth, sprinkler irrigation is more preferable than flood

irrigation to save and conserve water as the water demand during early stages is less than during later stages.

Harvesting: Harvest the crop at about 40 days after flowering of the plants, ie, at physiological maturity of grain when black spot appears on the grain at the lower end. Alternately, the Brix of standing crop can be measured using hand refractometer. Additionally, the plants can also be sampled for small mill test (SMT) to assess the juice Brix and other quality parameters as is practiced in sugarcane. Harvest the panicles at the last internode and thresh the grains separately followed by drying. Harvest the stalks to the ground level using a sickle and remove the leaves including sheaths. The harvested canes can be stacked in small bundles of 10–15 kg and must be transported within 24 h of harvesting to the mills for crushing.

Stalk yield and quality parameters

The cane, grain, stalk juice and ethanol yields, and stalk juice content of nationally released sweet sorghum cultivars under good crop management practices during *kharif* season are furnished in the following table. However, these yields may vary according to the location, date of planting, soil type, rainfall distribution, etc.

Sl. No.	Cultivar	Stalk yield (t ha ⁻¹)	Juice yield (KL ha ⁻¹)	Juice extraction (%)	Juice Brix (%)	Ethanol yield (L ha ⁻¹)	Grain yield (t ha ⁻¹)
1	SSV 84	35–40 t ha ⁻¹	12–14	40–45	17–18	1000	1.0–1.5
2	CSV19 (RSSV 9)	35–40 t ha ⁻¹	12–14	40–45	17–18	1000	0.8–1.0
3	CSH 22 (NSSH 104)	40–45 t ha ⁻¹	14–16	40–45	17–18	1134	1.0–1.5

Crop protection: Shoot fly, stem borer, shoot bug and aphids among insect pests and downy mildew, leaf rust and leaf blight among the diseases are the major constraints for sweet sorghum stalk and grain production. These insect pests and diseases can be managed by following chemical control methods as detailed in the following table.

Constraint	Management
Insect pests	
Shoot fly	Application of carbofuran 3 G (@ 20kg ha ⁻¹) or phorate 10 G (@15kg ha ⁻¹) as basal at the time of sowing in soil furrows
Spotted stem borer	Application of carbofuran 3 G/endsulfan 35 EC (@ 2ml l ⁻¹ water) inside the whorl, based on leaf feeding damage symptoms
Shoot bug	Application of endsulfan or metasystox 35 EC (@ 2ml l ⁻¹ of water) in the whorls based on the damage symptoms.
Sugarcane aphids	Spraying of metasystox 35 EC (@ one l ha ⁻¹ in 500 l of water) at boot stage based on the damage symptoms.
Diseases	
Downy mildew	Seed treatment with apron excel (@ 3ml kg ⁻¹ seed)
Foliar diseases	Spray dithane M 45 (@ 2g l ⁻¹ of water) at panicle initiation stage (35 DAS)

Looking ahead

- Scaling up of sweet sorghum cultivation in collaboration with sugar and alcohol industry.
- Designing low-cost machines for harvesting and dressing of canes
- Developing cultivars with high yielding ability, photoperiod- and thermo-insensitivity and tolerance to shoot fly, stem borer and shoot bug.

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Linking producers and processors—sorghum for poultry feed: A case study from India

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Introduction

Global production of sorghum [*Sorghum bicolor* (L.) Moench] is currently estimated at 57.6 million tons, with Asian countries contributing 20% of this total [FAOSTAT 2002–04 (ave.)]. Within Asia, India is the largest producer of sorghum, producing 7.3 million t. Sorghum in India is grown in the rainy-season (June–October) and in the post-rainy season (September–January). Rainy-season sorghum accounts for about 60% of total sorghum production (CMIE 2004). Resource-poor, small-scale producers in the semi-arid tropics (SAT) with less than one-hectare land grow sorghum, to meet household requirements of food and fodder. Thus, sorghum is an important food security crop in a wide range of marginal areas in India. The importance of the crop is enhanced due to its stover, which is an important source of dry fodder for draft and dairy animals (Kelley et al. 1993, Kelley and Parthasarathy Rao 1994, Hall and Yoganand 2000).

However, during the last two decades, several factors are contributing to a change in the traditional role of sorghum as food security crop. On the supply side, sorghum area and production have been declining in India and in Asia as a whole. For example, in India, between 1980 and 2004 sorghum area declined by 2.8% and production by 2%. Low market price, lack of effective Minimum Support Price (MSP) and high tariffs on imports of edible oil (jacking up the domestic price of oilseeds) has led to substitution of sorghum with oilseeds. On the demand side, with rising incomes, urbanization and subsequent changes in consumer tastes and preferences, market demand for food uses of sorghum has declined. This is further compounded by the public distribution system (PDS) of rice and wheat at subsidized prices for low-income consumers.

But utilization patterns of rainy-season sorghum are in a dynamic phase. Besides its traditional use as food crop, it is finding niches in alternative uses such as livestock and poultry feed, potable alcohol, starch and ethanol production (Kleih et al. 2000). Of these, the fastest growing sector is the poultry feed sector. For example, poultry production in India is expected to grow at 15% to 20% per annum for broilers and 10% to 15% for layers, leading to a corresponding expansion in demand for feed (Dayakar Rao et al. 2003). Maize (*Zea mays* L) is the main cereal feed ingredient, which constitutes 30–35% of poultry rations. Presently, maize production is growing only at 3–4% per annum leaving a gap to be filled by alternative sources. Preliminary studies have shown that under tropical conditions, sorghum can replace up to 60% of maize in broiler diets and 50–100% of maize in layer chickens without any adverse effects on the performance (Ramasubba Reddy and Nageswara Rao 2000). But, lack of availability of rainy-season sorghum in bulk quantities and assured supplies throughout the year is one of the main reasons constraining its usage in the industry. Although about 35% of marketable surplus is available, these are often scattered and hence non-economical to procure in sufficient bulk quantities by industrial users (Marsland and Parthasarathy Rao 1999).

Existing market system for sorghum grain

Sorghum is sold through regulated and non-regulated channels. Regulated markets are those in which business is done in accordance with the rules and regulations framed by the statutory market organization, the Market Committee. There are a number of possible ways for the farmer to dispose off sorghum grain off-farm (Fig. 37).

Use and sale by farmers: After harvest, sorghum grain may be retained on-farm, to be used for home consumption or as seed or for payment in kind. The surplus grain is sold either in a regulated market through Commission Agents in the markets or through a broker (middleman) at the village. Despite several inefficiencies and exploitation by middlemen, Marshland and Parthasarathy Rao (1999) found that the marketing system per se does not constrain the utilization of the crop as food grain. At the same time, the study concluded that the existing marketing system may not be optimal for industrial users, and they would prefer to obtain sorghum through new institutional arrangements such as contract farming, bulk purchasing, etc, that compress the marketing chain and thus reduce transaction costs.

In short, the rationale for the new marketing arrangement include the following:

- Potential demand for sorghum grain in poultry feed and other emerging alternative uses
- Non-availability of grain in bulk quantities due to scattered and variable surplus production over subsistence needs
- Need for assured supply of quality grains in bulk quantities.

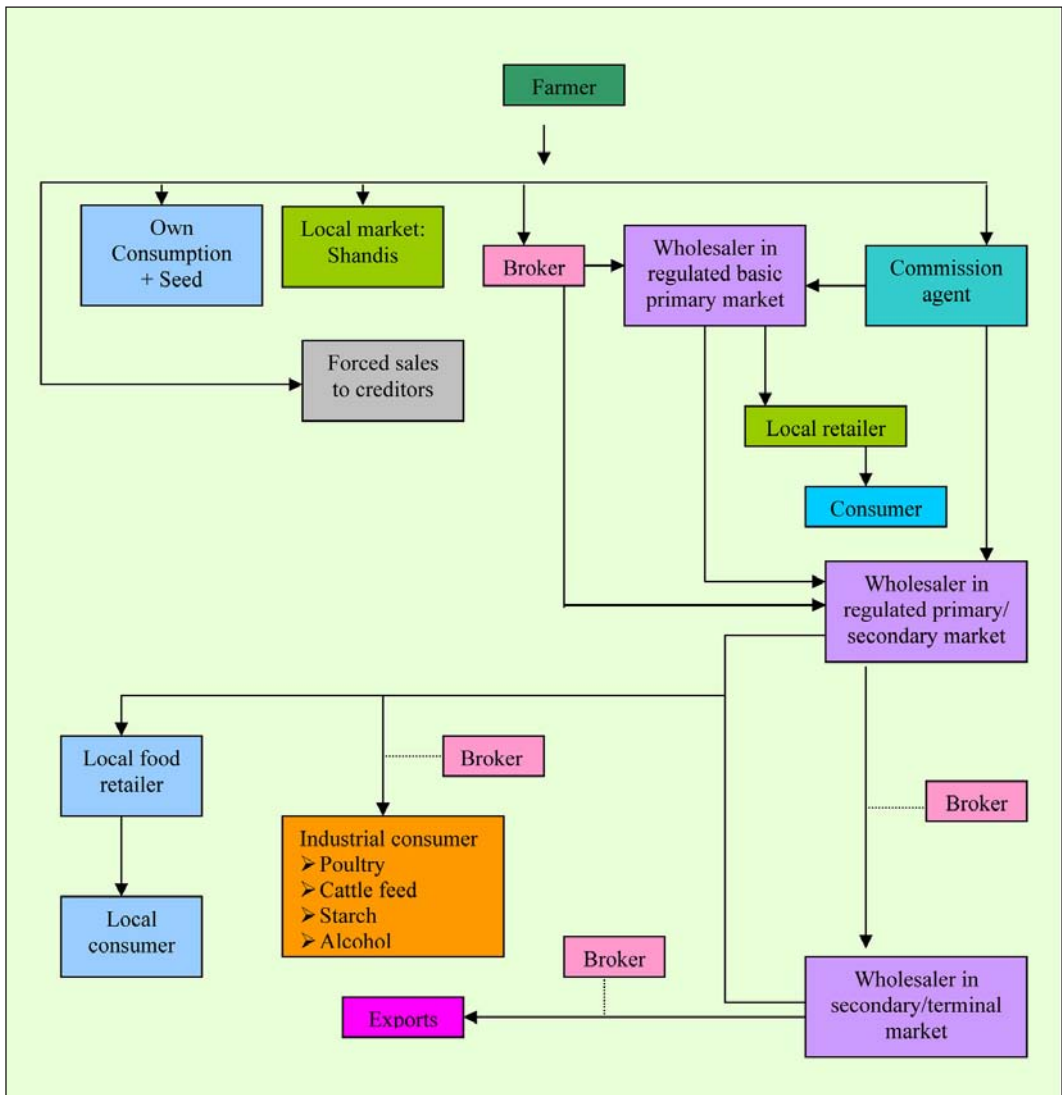


Fig. 37. Flow chart of traditional marketing chain for sorghum grain in India.

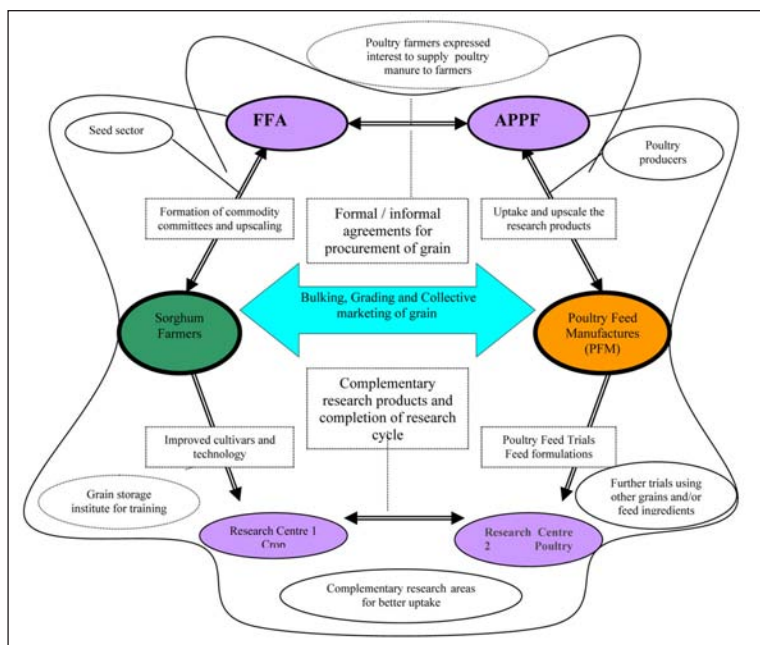
In this context, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, along with Acharya NG Ranga Agricultural University (ANGRAU), Hyderabad, India, has implemented a project funded by DFID (Department for International Development, UK) in collaboration with non-governmental organizations—Federation of Farmers Associations (FFA) and Andhra Pradesh Poultry Federation (APPF), and Janaki Feeds, a private poultry feed manufacturer, aimed at establishing market linkages between sorghum growers and poultry feed manufacturers. Under this project, farmers were supplied with seed of improved sorghum cultivars and trained in bulking and storage of

grain; poultry nutritionists conducted broiler and layer feed trials with sorghum-based feed rations; and feed manufacturers developed poultry feed rations with sorghum replacing maize in varying proportions. The common underlying goal of all the coalition partners was creation of marketing opportunities for small-scale sorghum growers by creating a sustainable economic inter-linkage between them and the poultry feed industry (Fig. 38).

Defined roles of coalition partners

Each partner/stakeholder had his or her own reasons for joining the coalition. The ICRISAT plant breeders and ANGRAU poultry scientists were interested in forming links with farmers and feed manufacturers to improve the uptake of their research outputs and findings. The sorghum farmers, represented by the FFA, saw the potential to increase the security of their livelihoods. Janaki Feeds saw the business potential of the new knowledge generated by the project and the opportunities for more reliable grain supplies. The APPF saw the potential benefits to its members by way of alternative feed ingredients and sources.

A unique feature of this approach is the 'coalition' i.e., the process in which distinct/independent entities/institutions work together as a single unit, while keeping their identity, for the common goal with synergistic effect.



FFA – Federation of Farmers Association, APPF – Andhra Pradesh Poultry Federation

Fig. 38. A coalition approach for promoting sorghum for poultry feed.

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT): Selection of suitable sorghum cultivars, multiplication and distribution of seed to participating farmers, grading and bulking of produce, networking of partners, project implementation and monitoring, and assessing impacts of the project.

Acharya NG Ranga Agricultural University (ANGRAU): Conducting poultry feed trials with sorghum as principal cereal ingredient, providing technical guidance on consumption and quality of sorghum in poultry feeds.

Federation of Farmers Associations (FFA): Represent the interest of the farmers. Identify suitable sorghum growing areas and farmers; disseminate the information on improved technology to the farmers, formation of Farmers Association and foster effective linkages with end users.

Andhra Pradesh Poultry Federation (APPF): Represent the interest of poultry producers. Take lead to interact with poultry producers; upscale use of sorghum in poultry feed rations.

Janaki Feeds: Represent the interests of feed manufacturers. Prepare feed formulations using different proportions of sorghum and up-scale project findings after completion of project.

At a later stage, a private seed company also joined the coalition for dissemination of seed of improved varieties of sorghum. The extended shoulders in Figure 38 represent further scope in enhancing partnerships between and among coalition partners. For example, poultry farmers expressed interest in supplying poultry manure to the farmers and in turn procure surplus grain. The boxes in white indicate probable areas of collaboration in the near future.

Linking farmers and poultry feed manufacturers

In the process of linking sorghum producers and poultry feed manufacturers, many simultaneous activities were carried out. For instance, Poultry Feed Trials (PFT) to further corroborate the efficiency of sorghum in poultry rations, supply of improved sorghum seed and technology to farmers, formation of farmers' groups, etc. These are briefly discussed below.

Farm trials for increased productivity

In Andhra Pradesh, rainy-season sorghum is grown on 0.30 million ha, producing 0.29 million tons of grain while the post-rainy season sorghum accounts for 0.34 million ha producing 0.35 million t of grain (GOAP 2003). The Mahabubnagar and Ranga Reddy districts of Andhra Pradesh, India, where rainy-season sorghum

cultivation is predominant, were selected for the implementation of project activities at the field level. For the 2003 and 2004 rainy-season sowings, seed of four improved high yielding sorghum cultivars, namely CSH 16, CSV 15, PSV 16 and S 35, suitable for that agro-climatic area and known to be less susceptible to grain mold attack were supplied to the chosen farmers in selected villages. The farmers fields were regularly monitored for sowing, germination, timely fertilizer application, inter cultivation, weeding and harvesting. During the meetings/field visits, ICRISAT scientists illustrated the proper harvesting stage of the crop to avoid grain mold incidence that reduces the market price of the grain. The participating farmers' realized three to four fold increase in yields by adopting improved technology (improved cultivars and practices) with proportionate increase in net farm income (Parthasarathy Rao et al. 2004).

Poultry feed trials

The use of sorghum in poultry feed is not new and its usage is quite common despite several apprehensions related to its use in place of maize (tannins, toxins, yolk color, etc). The general consensus was that sorghum can replace maize partially or fully provided its price was Rs 0.50 to 0.75/kg lower than the price of maize (US\$1 = Rs 40). To dispel some of the apprehensions on use of sorghum in poultry rations, poultry feed trials (PFTs) using sorghum were carried out by ANGRAU. The tests on both layers and broilers showed that sorghum could replace maize since all parameters like, weight gain, feed consumption and feed efficiency were comparable with those for maize (Laxmi Tulasi et al. 2004 and Rajasekher Reddy et al. 2005).

The poultry nutritionists carried out the broiler feed trials by making all the diets iso-nitrogenous, iso-caloric and homogenous in lysine, methionine and cystine levels. A second trial on part-by-part replacement of maize with sorghum was carried out at the insistence of the feed industry. The findings were not very different (except the light color of the skin of the broilers, which was recovered partially with addition of *stylosanthes* leaf meal to the diets) and it is this trial that helped in buy-in of the results by the industry. In the case of layers too, the experiments were conducted twice. Initially the trials were conducted on White Leghorn (WLH) breed but subsequently Bobcock (*commercial layer birds*) was included upon the insistence of poultry producers. It is probable that if the feed trials had been carried out in isolation, the poultry producers and feed manufacturers would have less confidence in the results.

Simultaneously, the poultry feed manufacturers prepared poultry feed rations based on varying proportions of sorghum for testing and sale. For all the above trials and their variants, information was disseminated through workshops and leaflets.

Establishing market linkages

The ultimate goal of the project was to link small-scale sorghum producers with poultry feed manufacturers through informal institutional arrangements. The process included the following steps:

- a) Formation of Farmers' Association: Farmers Association constituted in each project village consisting of farmers participating in the project.
- b) Training on specific skills: Farmers' group trained on grading the sorghum grain as per grain mold severity, bulking the surplus and storing according to scientific principles.
- c) Collective Sale: The surplus sorghum grain stored collectively by the farmers was sold to poultry feed manufacturers after careful negotiations between Farmers' Association representatives and feed manufacturers at a mutually agreeable price.

Advantages of bulk marketing

Farmers

- Better price realization
- Increase in bargaining capacity of the farmers
- Minimizes middlemen charges/transaction cost
- Increases the involvement of the farmers and makes them independent
- Improves market intelligence
- Market expansion.

Buyer

- Overcoming multiple transactions
- Assured supply of produce
- Overcoming seasonality in purchase to some extent (grain availability throughout the year)
- Quality of the produce guaranteed
- Origin ensured (from particular locality with specified qualities).

Innovation

For all stakeholders, this was their first experience of such a broad-based coalition involving different types of organizations (public, NGO, private), with different skills and expertise (breeding, poultry nutrition, farming, commerce, etc). All the coalition

partners realized that collectively they could work at a faster pace, and achieve their objectives more quickly than they would have by working in isolation. The 'coalition' allowed capitalizing on the synergies from sharing of skills from different disciplines with each member playing his/her role in the project. The conflicting interests of different stakeholders were addressed mutually in reaching the final goal.

As indicated earlier, the broiler PFTs were repeated to meet the requirements of the industry, and the layers feed trials were carried out for *commercial layer birds* also to address concerns of poultry producers. Although hypothetical, it is probable that if the scientists had been working in isolation, the poultry producers and feed manufacturers would have been less satisfied with the methods and results. The testing would not have reflected their own practices and concerns, and they would not have been in a position to make requests for adjustments after the results had been published. Innovation within the project does appear to have been propelled by linkages between people. Learning from past experience, combining different perspectives to give rise to new, synthesized ideas, and what Barnett has called 'creative imitation' (Barnett 2004), were all the product of the exchange of knowledge and experience between individuals and groups.

Lessons learnt

- The clarity and appropriateness of roles – agreed jointly at the beginning of the project – was recognised as an important ingredient of success. The monitoring plan, for example, stipulated the precise responsibilities of each partner organisation in relation to each other.
- Working in partnership required great emphatic ability and clarity on the roles each partner has to play to achieve the common overall goal of the project.
- Consensus decisions and frequent communications considered as important ingredient in the success of this attempt
- Since bulk storage is a problem in the project villages, this creates some problems related to grading and scientific storage practices. Hence storage structures are needed for success of such activities.
- The linkage between the credit and output market ie, tied transactions was one of the reasons for sales outside the Farmers Association. Need for credit from formal sources.
- Need to explore informal/formal agreements through novel market linkage models for long-run sustainability of the market linkages between the farmer and the feed industry
- More critical examination of roles and responsibilities of Farmers Association.

To address some of the above concerns and to take on board the lessons learnt, a project is now being implemented on “Enhanced utilization of sorghum and pearl millet in poultry feed industry”. This project covers a larger area, and includes sorghum and pearl millet that are grown by small-scale farmers, and have potential in poultry feed. The main emphasis under this project is linking producers with feed industry by involving all stakeholders under a coalition approach. The innovative supply chain envisaged under the project is shown in Fig. 39. Input dealers and bankers will be part of the supply chain and there is provision for storage structures at the village level for bulking and grading of produce.

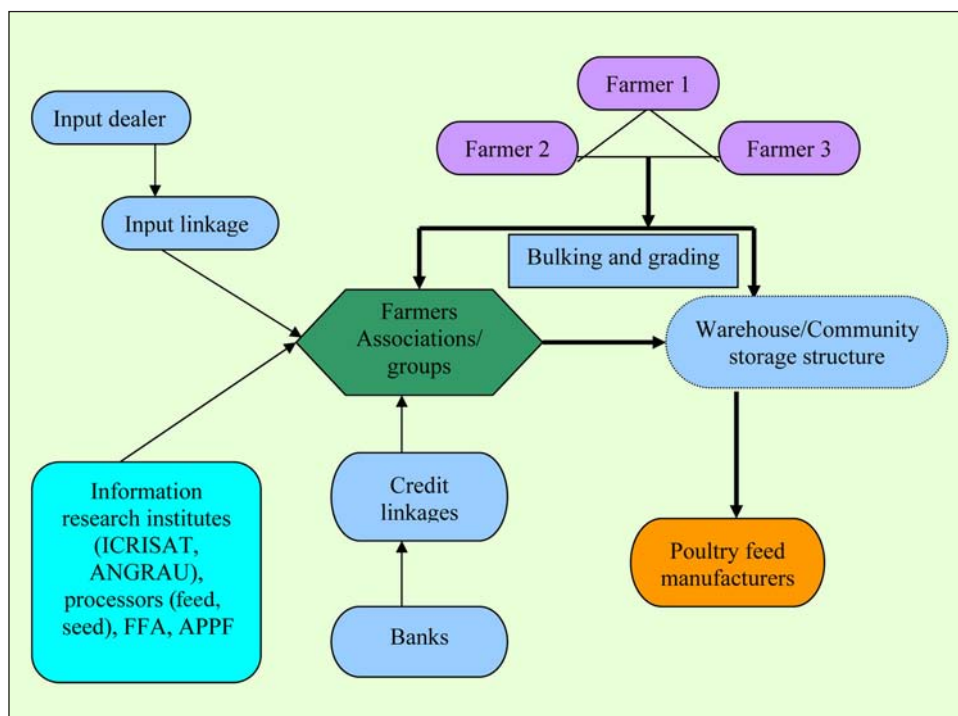


Fig. 39. Innovation in supply chain.

Concluding remarks

Creating market linkages led to four clear benefits: (1) established that sorghum could be used in poultry feed without impairing its quality and convinced poultry producers and feed manufacturers; (2) the farmers and feed manufacturers worked together to ensure the quality of the grain was suitable for poultry feed; (3) the supply chain was shortened and some middlemen made redundant, thereby decreasing the transaction cost to both farmers and feed manufacturers; (4) the direct link to industry gave the farmers the incentive to operate collectively, which increased their bargaining power and reduced marketing and transportation costs.

It is too early to assess the impact of the above project on market linkages but a good beginning has been made. The beauty of the coalition approach was that while all the partners/stakeholders worked towards a common goal, each stakeholder had their own smaller goal to achieve. For example, the crop breeder got feedback on sorghum cultivar traits preferred by the farmer, the poultry nutritionist gained knowledge on use of sorghum in poultry rations, the feed industry gained knowledge on poultry feed formulations with sorghum and the farmer, knowledge on improved cultivars, bulking and linkages to a potential market. Thus, although the stakeholders come from different institutions/organizations with different objectives, the common underlying goal is an important binding factor.

The biggest lesson from this project is perhaps the recognition of the need to involve all stakeholders who have some stake in the commodity under consideration. This ensures that stakeholders do not work at cross-purposes defeating the final goal.

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Design and analysis of experiments¹

Basic Concepts

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1. The Experiment

An experiment on research stations or on farms is a systematic inquiry undertaken to answer a specific, well-defined, research question or questions. The question(s) may relate to (a) Confirming or disproving something doubtful; (b) Discovering some unknown principle or effect; or (c) Testing, establishing or illustrating some suggested or known truth.

Experiments can be classified as *absolute* and *comparative*. A *sample survey* undertaken to assess, say, the damage caused by pod borer to chickpea in a given region is an example of an *absolute experiment*. The primary aim here is to know about the *absolute value* of the damage in that region. In *comparative experiment*, on the other hand, *two or more* treatments/interventions are compared for their effects on some chosen characteristic(s) of a certain *reference population*. Here, rather than in the absolute value of the population characteristic(s) under any one particular treatment, the primary interest lies in the differences (*comparisons*) among treatments for the chosen population characteristic(s).

The paper deals with *comparative* experiments (shortened below to *experiments*). These can be grouped into two types: *single-factor* and *multi-factor* (also called *factorial*). A factorial experiment could be either a *complete* or a *partially complete factorial*. In a single-factor experiment, only one *single factor* varies while all others are kept *constant* at some pre-specified levels. In such experiments, *the treatments are the different levels of the single factor*. For example, an insecticide trial, in which the single factor is an insecticide tested at different levels, in a unifactorial experiment. Here, only the levels of the insecticide vary from plot to plot, and all other factors, such as fertilizer and water management, are applied uniformly to all plots.

An experiment in which *the treatments are all possible combinations of the selected levels of two or more factors* is a *complete factorial experiment*. A trial having two

¹ This paper is organized in three parts. Part 1 discusses The Experiment, Part 2 The Design, and Part 3 The Analysis.

factors each at two levels, such as two levels (I1 and I2) of an insecticide and two varieties (V1 and V2), is an example of 2×2^2 factorial experiment. The four treatments in this experiment are (I1, V1), (I1, V2), (I2, V1) and (I2, V2). Adding to these four an extra *control treatment* makes this a *partially complete factorial experiment* with five treatments, four of which form a complete factorial. Here too, all other factors, as in a single factor experiment, are kept constant at some pre-specified levels.

1a. Experiments Too Need Power—To Take You There

The philosophy of *The Scientific Method* requires the research question to be transformed into a *null hypothesis*. For example, in comparing the effects of a number of different treatments, the hypothesis would be that there is no (ie, *null*) difference among treatments with respect to the effect they produce.

An experiment is then designed to *disprove* this null hypothesis if it is false. If the null hypothesis could be correctly disproved/rejected, then differences in the effects of the treatments may be inferred from the experiment to the wider (reference) population under study. The potential for an experiment to disprove a false null hypothesis depends on the *power* of the experiment. To understand the concept of power, consider, as shown below, the four possible situations that can arise when data from an experiment are analyzed using a statistical test of inference:

	Null hypothesis is	Real situation in reference population	
		False	True
Inference from experiment	Rejected	1 Correct Result	2 Type 1 Error
	Not rejected	3 Type 2 Error	4 Correct Result

Each cell represents a possible relationship between the *experimental inference* and the *real situation* in the reference population. The real situation in the population, of course, cannot be known except through statistical inference. Cells 1 and 4 represent *desirable outcomes*. Cells 2 and 3 represent *potential outcomes that are not desirable*.

In Cell 2, the experiment *rejects the null hypothesis that is true*. This is referred to as Type 1 Error. In Cell 3, the experiment *fails to reject the null hypothesis that is false*. This is referred to as Type 2 Error. The probability of committing a Type 1 Error, usually denoted by α , is known as the *level of (statistical) significance*. The probability of committing a Type 2 Error is usually denoted by β . The power is then defined as the *complement* of β , ie, Power = $1 - \beta$.

Stated in words, *power is the probability of rejecting a false null hypothesis*. A power of 0.80 implies that four times out of five a false null hypothesis will be correctly rejected. We would obviously like to maximize power in our experiment. It is possible to design an experiment in such a way that it achieves the desired power. *This comes at a cost as power (as well as statistical significance) depends on the number of replications*. This is discussed in Section 2.

Remember that none of the two error types can ever be completely eliminated since the experiment is only a representative *sample* of the reference population.

1b. Accuracy and Precision

Variation is an inherent property of biophysical systems. The challenge is to arrive at *correct* decisions in the presence of this variation. This demands dissecting the variation into a systematic (*signal*) part and a random (*noise*) part. This requires use of appropriate experimental design and a uniform spatial and temporal application of standardized experimental procedures – so that the noise is minimized allowing us to see the signal more clearly and correctly. The signal, in the context of a comparative experiment, refers to the differences among treatments. The noise refers to the variation that remains and arises from sources other than the treatments.

Noise has two aspects to it. One is the *lack of accuracy*. The other is the *lack of precision*. *Accuracy* refers to how closely the true value of treatment effect is estimated by the experiment. The closer the estimated effect is to the true value, the less it suffers from a lack of accuracy. *Precision* refers to how closely repeatable is the estimated treatment effect. The less the individual observations deviate from the estimated effect, the less the estimated suffers from a lack of precision. This is illustrated in Fig. 40. The true value is the dot in the center of the innermost circle. The two cases in column 1 represent a situation in which the average of a number of values lies

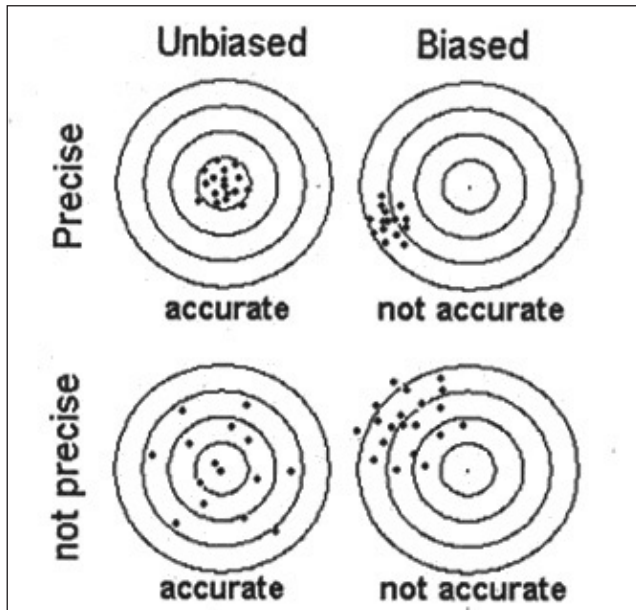


Fig. 40. Accuracy and precision.

where it should be – they are both accurate, the top one having individual values less dispersed around the average (high precision), the bottom one having individual values highly dispersed around the average (low precision). The ideal situation is of course the top one in which the estimate is both accurate and precise.

The two cases in column 2, on the other hand, exhibit a situation wherein the estimate is far away from the true value. They both lack accuracy – the top one though having high precision than the bottom one. Thus a highly precise estimate may not necessarily be accurate and vice versa. An experiment, if designed and also conducted well, can deliver both accurate and precise estimates of treatment effects.

2. The Design

A comparative experiment, in accordance with the research question, will consist of *two* basic sets of physical entities.

- a) A set of *fixed* number, say t , of *experimental treatments*; and
- b) A set of *fixed* number, say n , of *experimental units*.

The number n [(usually) a multiple of t , eg, $n = 3t$] is referred to as the *size of the experiment*. An *experimental treatment* refers to a *single set of well-defined conditions* whose effect is intended to be compared with other experimental treatments. The set may contain one, or more than one condition, depending on the objective(s) of the experiment. An *experimental unit* refers to a *well-defined unit/portion/subset* of the *experimental material*, the *entire* unit receiving *only one single* treatment.

For example, in an agricultural field trial, the experimental unit is a plot of land of certain size and shape in the field, the unit receiving one single treatment. The *experimental material* here is the *experimental field* that is divided into n smaller *nonoverlapping* units to test the t experimental treatments. *Note the distinction between experimental material and experimental treatments: experimental material is the medium on which experimental treatments are tested.* Depending on the problem, an experimental unit can be a single leaf, a whole plant, or a pot in a greenhouse.

A *statistical design* is a *set of rules* that *regulates* the *assignment* of the n experimental units to the t treatments in a manner that it

- a) Allows *valid* comparisons to be made among the treatments; and
- b) Controls the principal source(s) of *extraneous variation* in the experimental material in order to provide *accurate* and *precise* estimates of treatment comparisons.

The basic function of an experimental design is to act as an *effective filter* to clearly separate the *signal* from the *noise* and to provide accurate precise quantitative measures of these. Signal is the variability among treatments for the characteristic of interest. Noise is the non-treatment variability among experimental units – plots in a field trial, pots in a greenhouse trial – on which the treatments are tested.

It may be noted that in so far as the *design* is concerned, it does *not* take cognizance of whether the treatments constitute a randomly or purposively selected set. In *whatever manner* they have been chosen, a design treats them as *fixed* and *well defined* t distinct entities, and is concerned *solely* with how *these and only these* t should at any given occasion be assigned to the n experimental units, to ensure valid and efficient experimental conclusions. *It is at the data analysis stage that a distinction may be made as to whether the treatments are considered randomly and purposively selected.*

2a. Three Basic Design Principles

A scientifically sound experiment requires it to be designed using three basic principles: *replication of treatments*, *randomization of treatments* and *local control of extraneous variation in the experimental material*.

2a.1 Replication of treatments

This is the number of *independent* experimental units to which a treatment is assigned out of the n units available in an experiment. The t treatments are said to be *equally replicated* when each is assigned to the same number, say r, of independent experimental units; in this case $t \times r = n$. Otherwise, the treatments are said to be *unequally replicated*. When $r = 1$ for each treatment, the treatments are said to be *unreplicated* with $t \times 1 = t = n$. Correspondingly, we have an *equireplicate design*, an *unequally replicated design* and an *unreplicated design*.

Replication of treatments plays *three* roles in an experiment

- a) To estimate the experimental error variance, hence the precision of the estimates of treatment effects,
- b) To attain desired precision of the estimates of treatment effects of interest
– average from more replications is more precise than from less replications,
- c) To act as insurance against unforeseen disasters.

Experimental error variance, often shortened to *experimental error*, is the variation that arises from the *failure* of experimental units, receiving the *same* treatment, to deliver *exactly the same result*. This may happen due to one/some/all of the following reasons:

- a) Inherent variability among the experimental units;
- b) Lack of uniformity in the conduct of the experiment, ie, failure to standardize the experimental technique;
- c) Errors of observation and measurement; and
- d) Non-uniform behavior of all other extraneous factors, known, or unknown, that have not been/could not be accounted for in the experiment.

The *error* in experimental error is not synonymous with *mistake*. In a statistical context, it represents the variation that is often *unavoidable*. Experimental error is an unavoidable fact-of-life since no two experimental units receiving the same treatment, despite our best efforts and care, will hardly, if ever, match exactly in all their biophysical manifestations.

Replication provides an estimate of experimental error because experimental error arises from *variation* among *identically treated* experimental units. This variation cannot be measured when each treatment receives only one experimental unit. The *only* way to get a measure (estimate) of this variation, *except in some factorial experiments*, is to assign the treatments to a number of experimental units, even to an unequal number if the situation so warrants.

The estimate of experimental error is the *basic unit of measurement* (of random variation) on a *per unit basis*, which serves as a yardstick for

- a) Measuring the precision of the estimates of treatment; and
- b) Assessing the significance of observed treatment effects using an appropriate test of significance.

A test of significance is therefore possible *only when* the treatments have been replicated.

Replication helps attain desired precision in the following way. Let σ^2 be the experimental error variance. The *error variance of an estimated treatment mean* is then (σ^2/r) . The reciprocal of this, ie, (r/σ^2) , is defined as the *precision* of the estimated treatment mean. With $r = 2$, $(\sigma^2/r) = (\sigma^2/2)$. With $r = 3$, $(\sigma^2/r) = (\sigma^2/3)$ which is less than $(\sigma^2/2)$. The error variance of an estimated treatment mean can therefore be decreased, and hence precision increased as the number of replications, r , is increased. Beyond a certain number of replications, the improvement in precision may however be too small to be worth the additional cost and effort.

An important point that needs to be clearly understood is that *multiple observations from within experimental units do not represent genuine (independent) replications*. They are subsamples, and variation among them represents *sampling error*, not the experimental error. *It is the experimental error, not the sampling error, which is valid to test the significance of treatment differences.*

How Many Replications?

In general, more is the extraneous variability present in the experimental material, more is the number of replications needed. In any case, except in some factorial experiments, the number of replications should be *not less than two*. Some general guidelines are given below:

For estimation of precision: For the given number of treatments, and the design to be used, the number of replications should be such that the *error (ie, residual) degrees of freedom (DF)* are *at least 12* to get a reasonably good estimate of error variance, hence of precision.

For attaining desired precision and power: Given pre-specified values of (i) the expected experimental error variance (σ^2), (ii) the biologically meaningful minimum difference between any two treatments (δ) that is desired to be detected as significant, (iii) the desired level of significance (α), and (iv) the desired power ($1-\beta$), an approximation to the *minimum* number of replications per treatment can be computed as (Desu and Raghavarao 1990, Sample Size Methodology, AP Boston MA):

$$R = 2 [\text{sqrt}\{\chi^2_{(1-\alpha, t-1)} - (t-2)\} + z_{\beta}]^2 (\sigma/\delta)^2$$

where t (≥ 2) is the number of treatments in the experiment, $\chi^2_{(1-\alpha, t-1)}$ is the $100(1-\alpha)$ percentile point of the χ^2 distribution with $(t-1)$ DF and z_{β} is the β percentile point of the standard normal distribution. If the value of r obtained from the above equation is say 4.3, then the number of replications = $4+1 = 5$. Use of the above formula requires an estimate of error variance σ^2 , which could be obtained from past similar experiments conducted in conditions similar to those for the planned experiment.

For given values of t , α , β and δ , the above formula shows that, as expected, higher is the error variance σ^2 , more is the number of times each treatment will need to be replicated. Similarly, for given values of t , α , β and σ^2 , larger is the size of the difference (δ) that needs to be detected as significant between any two treatments, less is the number of times each treatment will need to be replicated.

Optimal Allocation of Limited Resources

In practice, the resources will always be limited. This sets a limit, say n , on the maximum possible number of experimental units that could be managed or are available within the available resources. One would then wish to know how to optimally allocate them to different treatments in a way that maximum precision could be obtained. Two such situations are described below:

(a) In order to maximize the precision of the comparison of $c (\geq 1)$ control treatments with $t (\geq 1)$ test treatments, the optimal number of replications for the control and the test treatments can be computed as

$$r_{\text{control}} = n / [c + \sqrt{(ct)}] \text{ for each of the } c \text{ control treatments}$$

$$r_{\text{test}} = n / [t + \sqrt{(ct)}] \text{ for each of the } t \text{ test treatments.}$$

[Reference: Theoni H (1983)].

(b) Assume, for example, there are four treatments T1, T2, T3, T4 and $n=24$. How to optimally distribute these 4 experimental units among the four treatments so that the following three treatment comparisons of interest can be made with highest possible precision?

T4 vs T1

T1 vs T2

T2 vs T3

To decide this, count the total number of times each treatment appears in the three treatment comparisons. This gives us

T1	T2	T3	T4
2	2	1	1

Take the square root of these frequencies

$$1.4 \quad 1.4 \quad 1.0 \quad 1.0 \quad \text{with their total } T=4.8 \quad (2)$$

The optimal distribution of 24 units can then be worked out as follows

T1	T2	T3	T4
$(1.4/4.8) \times 24$	$(1.4/4.8) \times 24$	$(1.0/4.8) \times 24$	$(1.0/4.8) \times 24$
= 7	= 7	= 5	= 5

A similar computational approach can be adopted in other situations. The general formula to compute the optimal number of replications for a treatment is

$$r = (\sqrt{f} / T) \times n \quad (3)$$

where f is the total number of times the treatment in question appears in all the comparisons of interest, T is the grand total [as in (2) above], and n is the number of experimental units available. **NOTE** that this is *subject to the condition* that, with treatments, the total number of treatment comparisons *must not* exceed $(t-1)$.

2a.2 Randomization of Treatments

This refers to the *random allocation* of experimental treatment to experimental units. Random allocation *does not* mean manipulation of treatments into some order that looks haphazard. Randomization needs to be an *objective impersonal procedure* that is devoid of personal bias and gives every treatment the same chance to receive any experimental unit. A design in which treatments are randomly assigned to experimental units is called a *randomized design*, otherwise a *systematic design*.

Randomization is required

- To provide validity to the estimate of experimental error, and to a statistical test of significance of treatment effects; and
- To protect against any (un)known systematic bias that may creep into treatment effects due to extraneous sources of variation that have not been/ could not be controlled.

The question is often asked whether or not to use randomization. To answer this question it is necessary first to understand the pros and cons of using randomization in relation to its above two functions. Any probability-based statistical tool, including any test of significance and any measure of precision, *to be valid*, deductively requires that the experimental observations constitute a *random sample* from some well-defined reference population. This condition will hold good when the observations are *independent* of one another. Random allocation of treatments to experimental units allows us to proceed as *though* the observations are independent. Note that this does *not* imply that randomization *guarantees* independence of observations. This distinction is based on the premise that observations from spatially/temporally adjacent experimental units will tend to be correlated. What randomization does is only to *assure* us that the effect of this correlation on treatment effects has been made as small as possible. Some degree of correlation may still persist since no amount of randomization can ever eliminate it completely. *True and complete independence of observations in any experiment is an ideal that can perhaps never be fully achieved, particularly in agricultural field trials.* In view of this, restraint and caution should be exercised in explaining experimental inferences.

That randomization protects against any (un)suspected systematic bias can be better understood through a simple example. Suppose a plant breeder, in order to compare yields of two cultivars A and B, plants each to four plots in the field systematically, in this way:

PLOT 1	PLOT 2	PLOT 3	PLOT 4	PLOT 5	PLOT 6	PLOT 7	PLOT 8
A	A	B	B	A	B	A	B

If the field has a (generally unknown) fertility gradient from left to right, variety B then consistently falls on a relatively less fertile plot. The observed yield differences between the two cultivars will therefore be subject to an unknown amount of systematic bias in favor of variety A. On the other hand, had the two cultivars been assigned randomly to the eight plots, each variety would have got an equal chance of falling on any plot that happened to possess good, or poor fertility, and the bias could have reduced.

The above arguments do *not* mean that systematic designs must never be tolerated. Provided that we possess *good* knowledge of the *form* of uncontrolled extraneous variation in our experimental material, and a systematic design is easier to work with, it may perhaps be right not to randomize. In fact, in some situations it may not be even possible to randomize. In all such circumstances, it is essential to accept the fact that any resulting inference *cannot* be supported by a meaningful probability statement, and the quoted measure of precision may be biased. So, in summary, a practical answer to the question “to randomize, or not?” is: *randomized except when there is very good reason not to; and understand that inferences from a systematic design depend heavily on the correctness of what is assumed about the underlying pattern of extraneous variation, and to report this assumption explicitly in reporting the experimental results.*

2a.3 Local Control

This refers to *blocking* the experimental units. Local control will reduce the magnitude of experimental error, which improves the efficiency of the experiment and makes the tests of significance more sensitive/powerful in detecting treatment differences.

Blocking comprises the (physical) stratification of experimental units into a number of blocks in such a manner that the units within any block are relatively homogeneous. The result is that variability within each block is minimized and variability among blocks is maximized. The experimental error thus consists of only the intrablock variation. In the absence of blocking, it would consist of both the intrablock and the interblock variation.

Two decisions must be made to ensure appropriate and effective blocking:

- a) Selection of the *source of variability* to be used as the *basis for blocking*; and
- b) Selection of *block size, shape, and orientation*.

An ideal source of variation for use as the basis for blocking is one that is large and *highly predictable*. For example, *soil heterogeneity* in a varietal or fertilizer trial where yield is of primary interest, *direction of insect migration* in an insecticide trial where insect is of primary interest, and *slope of the field* in a study of plant

reaction to drought stress. Block size, shape and orientation should be such that they *maximize* interblock variability. Some guidelines for blocking are as follows:

- For unidirectional gradient, use long and narrow blocks; orient these blocks with their length perpendicular to the direction of gradient.
- For bidirectional gradient with one gradient much greater than the other, ignore the weaker gradient and take steps as above for unidirectional gradient.
- For bidirectional gradient with both gradients the same and perpendicular to each other, choose one of the following:
 - Use blocks that are as square as possible.
 - Use long and narrow blocks with their length perpendicular to the direction of one gradient, and use the covariance technique to account for the other gradient.
 - Use a suitable row-column design with two-way blocking—one for each gradient.
 - When gradient(s) is/are suspected to be operative, but when its/their direction(s) cannot be predicted, use blocks that are as square as possible.

Local control using blocks removes only the interblock environmental variation. The variation from plot to plot within blocks still remains, which forms the experimental error. A further reduction in experimental error may thus be achieved by measuring, in some suitable way, the varying effects of the plots within blocks, and using this plot-measure as a *covariate* to obtain corrected (adjusted) estimates of treatment effects with higher precision through *covariance analysis*. A caution to keep in mind is that the covariance values, intended to measure *environmental effects only*, *must not* be affected by treatments.

A third way to reduce experimental error is to *take proper care* in physical conduct of the experiment. Any lack of care, either at the beginning, during, or towards the end of the experiment, can be a potential source for increased experimental error. When this happens, even the most carefully and wisely chosen blocking and/or covariance analysis may not help to rescue the situation—and they might even prove counter-productive particularly when, as normally is the case, there is no way to *quantify* and *measure* the *effect of lack of care* in different experimental units. Since there are many ways other than local control to reduce experimental error, the principle of local control is sometimes replaced by a broader term *error control*, which includes all techniques, including blocking, to reduce the experimental error.

Replication vs Blocks

Experiments often confuse the terms *replication* and *block*. But they do not represent the same concept. *Replication is a numerical entity. A block, however, is a physical concept related to local control.* Also, the number of replications is *not always* the same as the number of blocks in an experiment. Their numbers may be the same in the case of a complete block experiment. In an incomplete block, or a confounded factorial experiment, the numbers of blocks is more than the number of replications.

Increasing the number of replications is *wrongly* conceived by some as a device to reduce the experimental error. Reduction in the magnitude of experimental error may be achieved only through local/error control. Increased replication provides only a more *stable* (*consistent* in statistical terms) estimate of experimental error.

2b. Good Design Demands Good Planning and ... Innovative Thinking

Design is *only one facet* of the whole process of planning any experiment. *The selection of an appropriate and efficient design is possible only when it is seen as an integrated part of the whole experimental planning process.* The following steps, taken sequentially, can greatly increase the overall success of an experiment. Guidance is also provided in the planning proforma in the Appendix 1 to this paper.

- Step 1.** Identify and state the problem *clearly* and *concisely*.
- Step 2.** State the objective(s) *precisely* in the form of *specific question(s)* to be answered or *specific hypotheses* to be tested, or *specific effects* to be estimated. If you have more than one objective, list them in their order of priority. Do not be too vague, or too ambitious in stating your objective(s).
- Step 3.** Select the treatments *objectively* to ensure that the objective(s) can be met.
- Step 4.** Select the experimental material in accordance with the objectives and the population about which inferences are to be made. The experimental material should adequately represent this reference population.
- Step 5.** Select the experimental design considering the objective(s) and the available resources. Try to choose a simple and operationally feasible design that is likely to provide good precision.

- Step 6.** Select that form and size of experimental unit and that number of replications that are likely to provide good precision. Historical data from similar experiments can be very helpful in making these decisions.
- Step 7.** Decide on measures to control the effects of adjacent experimental units on each other. This can be done by treatment randomization, and other physical measure, such as using border rows.
- Step 8.** Decide on the types of data, and the *manner* and *order* in which they are to be collected. Work out a *data-recording mechanism* that will facilitate *data entry, validation and analysis* on a computer in accordance with appropriately chosen statistical method(s) and computing software. *Give particular attention to the collection of those data that may help explain why the treatments behave as they do.*
- Step 9.** Prepare an outline of statistical analysis, and, if possible, a summary of expected outcomes.
- Step 10.** Review your plan (Steps 1-9) with a statistician and your colleagues. This may reveal important points inadvertently overlooked. Modify your plan if required.

Conduct the experiment as per the above plan, validate the collected data to check for possible errors in data and unexpected values, analyze the validated data and interpret the results, and prepare a complete, correct, and readable report.

In conducting the experiment, use experimental procedures that are free from personal bias. *Make sure* that differences among individuals, or differences associated with the order in which data have been collected do not contribute to experimental error. *Avoid* fatigue in data collection. Immediately *recheck* observations that seem dubious.

Proper data entry and validation of entered data are important parameters to make sure that the results obtained from analyzing the data are free from incorrect/unexpected data values. *Without proper data entry and validation, the entire effort in analyzing the data may well prove to be futile.* Epi Info (version 6) is an excellent software to use for data entry and validation. It enables you to introduce many effective data-check options at the entry stage as well as later for data validation. Analyze all data using appropriate statistical tools. Interpret the results in the light of experimental conditions, hypotheses tested effects estimated, and their relation to previously reported results.

In preparing the report, remember that there is no such thing as *negative* result. If null hypothesis is not rejected, it is *positive* evidence that there may not be real differences among the treatments.

2c. Selecting a Design

An appropriate design for an experiment depends on

- a) The *form* and *magnitude* of extraneous variation present in the experimental material;
- b) The *nature* and *number* of treatments to be tested; and
- c) The *degree of precision* desired.

Normally a design should be chosen based on the principles of replication, randomization and local control *unless* you have very good reasons not to.

The selection of a design should be based *not solely* on the number of treatments, as is often advocated in design textbooks. Once the nature and number of treatments are known, the *major* consideration in the selection of an appropriate design *must* be the form and magnitude of extraneous variation in the experimental material if they are fairly known to the experimenter. The reason is very simple: for a given number of treatments and their nature, the right design should be one that effectively controls the extraneous variation, so that the least possible experimental error is obtained and, consequently, the treatment effects are estimated and tested more precisely.

There are two broad categories of designs: complete block design (CBD), and incomplete block design (IBD). The CBDs are useful for experiments in which the experimental material stratifies itself into homogeneous blocks within each of which *all* treatments can appear *together*. The completely randomized design (CRD), randomized complete block design (RCBD), and Latin square design (LSD) are CBDs. Some features of CBDs are:

- a) All treatments appear together in each block;
- b) Analysis of data is simple;
- c) Missing data are easier to cope with; and
- d) They can be used for single-as well as multifactor experiments.

The IBDs are useful in experiments wherein the experimental material stratifies itself into homogeneous blocks within each of which all treatments *cannot* be tested together. Lattice designs and confounded factorials are examples of IBDs. Some features of IBDs are:

- a) All treatments do not appear together in each block;
- b) Analysis of data is more complicated than for CBDs;
- c) Missing data are not easy to handle; and
- d) They can also be used for single as well as multifactor experiments.

Some general guidelines/features for a few commonly used designs are given below where: t = number of treatments, r = number of replications. B = number of blocks, k = number of experimental units per block (ie, block size).

Complete block designs

Complete randomized design (CRD)

- All experimental units are homogeneous.
- Treatments can be (un)equally replicated.
- Main effects and interactions in case of factorial experiments are studied with equal precision.

Randomized complete block design (RCBD)

- Unidirectional gradient in the experimental material.
- Homogeneous blocks each of size $k=t$ are available.
- $r=b$
- Main effects and interactions in case of factorial experiments are studied with equal precision.

Latin square design (LSD)

- Bidirectional gradient in the experimental material.
- Perpendicular blocks of t homogeneous rows and t homogeneous columns are available such that, within each row and within each column, the treatments can appear together.
- $k=r=t$
- Main effects and interactions in case of factorial experiments are studied with equal precision.

Incomplete block design

Balanced lattice design

- t is such that $t = p^2$, eg, $t = 36 = 6^2$, which gives $p = 6$.
- $r = p+1$
- $b = p$
- Equal precision for all comparisons between pairs of treatments.

Partially balanced lattice design

- t is such that $t = p^2$
- $r \geq 2$.
- $b = p$
- Some pairs of treatments will have higher precision than others.

Generalized lattice (or alpha-lattice) design

- t is such that $t = p \times q$, $p \leq q$
- $b = q$, $k = p$
- $r \geq 2$
- Some pairs of treatments will have higher precision than others.

Confounded factorials

- More than two factors involved.
- Accuracy on certain higher-order interactions sacrificed.

In addition to the above, there is a special class of designs – split-plot designs - which are frequently misused or overused by researchers. These designs should be used *only when* there is no other alternative. General guidelines/features for their use are as follows

Split-plot design

- At least two groups of factors under study: this includes the situation where each group has only one factor.
- One group requires larger plots and the other(s) can be tested on smaller plots, the large plots being called main plots and the small plots subplots.
- The group(s) tested on subplots, and their interactions with the group tested on main plots, are studied more precisely.

Strip-plot design

- Two groups of factors under study: this included the situation where the groups have only one factor each.
- Both groups require large plots.
- The interaction between the two groups is studied more precisely.

3. The Analysis

Analysis of variance (ANOVA) is the most widely used (but not always correctly applied) statistical tool in analyzing experimental data. The Restricted Maximum Likelihood (ReML) (Appendix 2) is now widely used as a state-of-the-art statistical tool to analyze data. A *correct* analysis of data using ANOVA re ReML demands clear specification of

- a) The *treatment structure* and the *design* which together comprise the *data structure*;
- b) An equation (a *statistical model*) for every observation *in accordance with the data structure*. The equation, so formulated, expresses an observation as the sum of number of components (*sources of variation*) that are identified in the data structure;
- c) Nature of each source of variation (*fixed, random*) – See Appendix 3;
- d) Nature of variable (discrete, continuous) and its statistical distribution (normal....).

The treatment structure refers to the way a treatment has been made up. In a single-factor experiment, the different levels of the factor constitute the treatments; each level stands in its own right. The different levels may be quantitative in nature; for example, the graded doses of an insecticide, or a fertilizer, or different cultivars of a crop. The levels may sometimes be classified into biologically relevant groups, each group consisting of a number of distinct levels, *this gives rise to a hierarchical structure, and not to cross-classified treatment structures (as sometimes incorrectly interpreted)*. In a factorial experiment, on the other hand, any single treatment is made up of a cross-classified combination of the different levels of the factors involved; there is a main effect for each factor as well as interaction effects of various orders among the factors, which is not the case in a single-factor experiment.

The design structure refers to the way local control is (physically) exercised in an experiment. For example, in an experiment laid out in a randomized complete block design (RCBD), local control is exercised through blocks. The blocks are structured in a way that every treatment appears in each block at least once. In a split-plot experiment, local control is exercised at two levels. The field is divided into blocks within each of which the main-plot treatments are randomly allocated. Each main plot within every block is subdivided into subplots to which subplot treatments are randomly assigned.

Consider an RCBD with t treatments and b blocks. Let the t treatments represent t different methods of cultivating groundnut. A plot-mean-observation y_{ij} (corresponding to the plot receiving treatment i in block j), as per the treatment and design structures, is here the sum of the following four components:

- a) A general averaged (μ) about which the observations are presumed to be fluctuating.
- b) A component (τ_i) due to the treatment I applied to the plot.
- c) A component (β_j) due to the block j in which the plot falls, it represents the effect of the environmental factor(s) which the experimenter may have been able to properly identify and which the design subsequently permits him/her to isolate.
- d) A residual component (ε_{ij}), representing all other factors that influence the observation; this component constitutes the “experimental error”.

The above components put together make up the *statistical model* for any plot observation y_{ij} in an RCBD that can be written as:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij}$$

If there are t treatments and b blocks in the experiment, the $i = 1, 2, \dots, t$; and $j = 1, 2, \dots, b$; there being a total of $n = tb$ observations corresponding to the $n = tb$ experimental plots. The ANOVA partitions the total variation among the n observations into as many components as are *identified* and *included* in the statistical model. The statistical test commonly used in ANOVA to test the *significance/existence* of treatment differences is the *variance ratio* (called F if normal distribution assumption is met). This is calculated from the relevant mean squares (MS) as $F = \{\text{Treatment MS/Error MS}\}$.

To carry out a *valid* F-test, it is important to identify and use the appropriate composition of mean squares in its numerator and denominator. This identification is done on the basis of the *expected values of the mean squares*. The structure of these expected values is governed by the assumptions made regarding the nature (*random and/or fixed*) of treatments and other sources of variation. *Lack of awareness/understanding of these basic concepts on the part of experiments often leads to the use of invalid F-tests and hence to invalid inferences.*

The calculated value of $F = \{\text{Treatment MS/Error MS}\}$, say F_c , is compared with its corresponding theoretical (called by the “tabulated”) F value to decide *whether or not to reject the (null) hypothesis of no treatment differences* and, accordingly, whether or not to declare the observed treatment differences as significant. The theoretical F values for different significance levels of P have been tabulated and can be found, for example, in Appendix E of the book by Gomez and Gomez (1984). *A significance level is the probability of rejecting a true null hypothesis of no treatment differences.*

To illustrate this, assume $t = 4$ and $b = 5$ in an RCBD. There will be $f_1 = (t-1) = 3$ degrees of freedom for treatment MS, and $f_2 = (t-1)(b-1) = 12$ degrees of freedom

for error MS. A reference to the above Appendix shows that for $f_1 = 3$ and $f_2 = 12$, the theoretical F-value is 3.49 at $P = 0.05$ and 5.95 at $P = 0.01$. If the calculated F-value is $>3.49 < 5.95$, the observed treatment differences will be declared significant at $P < 0.05$. A similar inference will be drawn at $P < 0.01$ if $F_c > 5.95$.

Most statistical software packages have now obviated the need to refer to F-tables in order to arrive at conclusions in the above manner. Instead, they compute and report the P -value for any calculated F-value in the ANOVA table. The P -value is the probability of getting F-value as large, or larger, than the calculated F-value under the condition that the null hypothesis is correct. If the reported P -value, say P_c , is such that $0.01 < P_c < 0.05$, the observed treatment differences are declared significant at $P < 0.05$. If $P_c < 0.01$, a similar statement can be made at $P < 0.01$. However, if desired, the reported value of P_c can itself be quoted to indicate the level of significance. The smaller P_c value, the stronger is the evidence that significant treatment differences exist.

The *reliability* of an estimated treatment effect is usually indicated by its *standard error* (SE). The SE can be estimated from ANOVA and may be used to set limits within which the true (unknown) value falls with *any* specified degree of confidence. These limits are called *confidence limits*. For example, if the estimated treatment mean is m and its SE is s , the 95% confidence limits for the treatment effect are $(m - 1.96s)$ and $(m + 1.96s)$; this means that the true value of the treatment effect will fall within these limits in 95 out of 100 such experiments that could have been conducted under similar conditions.

In general, the appropriate multiple (eg, 1.96 in the above case) of the s to be subtracted from and added to the estimated treatment effect, depends not only on the specified degrees of confidence (eg, 95% in the above case) but also upon how accurately the s has been estimated. Tables such as the t-tables—eg, Appendix C in Gomez and Gomez (1984)—furnished the values of the appropriate multiple for s in determining the confidence limits. In a t-table, possible inaccuracy in the SE is indicated by its degrees of freedom, which is the effective number of observations used in the estimation of s .

3a. ANOVA Assumption

The assumptions of ANOVA, which the experimental data should fulfill for the resulting analysis to be valid, are:

- a) The observation can be represented by a *linear additive* model, eg, the model (1) in case of an RCBD;
- b) The errors ε (see equation 1) behave like a *normal distribution*;
- c) The errors ε are *independent* of one another; and

- d) The error ε in observations should not be affected by the nature of observations, which is equivalent to saying that the errors should exhibit the *same degree of variation* for each of the experimental treatments.

Failure to meet an assumption affects the significance level as well as sensitivity of the F- and t-tests. The true significance level, as a result, is usually larger than the apparent one. This leads to getting too many significant results. Loss of sensitivity occurs, accompanied by a loss of accuracy in the estimates of treatment effects, in the sense that a more powerful test than the ANOVA F-test, and a more accurate estimate of treatment effects, could be devised if the correct model were known. As a result, the significance levels and confidence limits *must* be considered as *approximate* rather than exact. Also, for similar reasons, the *rigid* application of 5% or 1% significance levels, to divide the treatment effects as real and unreal, appears to be hardly justified.

The nonconsistency of error variance (assumption d) can be the most serious problem. This may happen when:

- a) Certain treatments are erratic in their expression; and
- b) The error obeys a highly skewed distribution that is very different from the normal distribution.

In situation (a), the problem may be overcome by dividing the Error SS into parts, each of which is homogeneous. In situation (b), the error variance for any treatment tends to be some function of the treatment mean, which may be detected by plotting the variance against the mean on graph paper. A suitable transformation, depending on the nature of the functional relation between variance and mean, may make the error variance nearly constant.

The nonadditivity of treatment and environmental effects could be turned into additivity by a suitable transformation. Fortunately, such transformations may also bring the distribution of errors closer to normality. In most cases, the requirement of independence of errors may normally be met by the physical act of randomization.

Usually, moderate deviations, from the assumptions will not unduly affect the validity of the analysis. For example, an approximate normal distribution of errors will give comparable significance levels. Slight heterogeneity in error variation from treatment to treatment will have little effect on the confidence limits. However, where large deviations from the assumptions occur, quite misleading results may be obtained from ANOVA.

3b. Missing Data

When data on certain experimental units are missing, either due to accident or gross error in recording the analysis, its interpretation becomes involved. The correct way to analyze an experiment with missing data is to construct a model for all data that are *available* and use ReML to analyze data.

There are two *problems* in using ANOVA when there are missing values. The Treatment SS is always larger than the correct Treatment SS. This overestimation is unlikely to be large unless an appreciable number of data values are missing. The second problem is that it may not give proper t-tests because, in the analysis of so-to-say complete data, r replications are ascribed to the treatment that contains the missing data value, whereas there are only $(r-1)$ replications. Use of ReML obviates both problems.

4. A word of caution

There goes a saying that it is possible to prove anything by statistics. This may appear to be true for *bad* statistics; but exactly the *converse* is valid for *good* statistics. Use of a statistical design, followed by a proper analysis of data, does *not* provide absolute proof of the effectiveness of experimental treatments; it *only* enables you to *estimate* the *likelihood or reliability* of their continued effectiveness *at the level indicated by the experiment*. This is all statistics can do. Remember that *there is always a probability, even if small, of the results being wrong*. Therefore, avoid jumping to conclusions on the basis of your statistically significant results if they do not make sense. *Statistical significance may not necessarily mean biological significance. Similarly, a statistically nonsignificant result should not be rigidly interpreted as biologically unimportant.*

If you had one more day to live...

*Choose to go spend it doing Biometrics.
That way it would seem twice as long...*

References

- Desu MM** and **Raghavarao D.** 1990. Sample size methodology. Boston, USA: Academic Press.
- Gomez KA** and **Gomez AA.** 1984. Statistical procedures for agricultural research. Singapore: John Wiley & Sons.
- Theoni H.** 1983. Optimale Aufteilung des Stichprobenumfang zum Vergleich von s Standard- mit t Testbehandlungen. EDV in Medizin und Biologie 14:95–97.

Appendix 1: Proforma for Planning an Experiment

(The term “plot” is synonymous with “experimental unit” in item 09, 10, 16.)

- 01. Experiment title:**
- 02. Objective(s):** (If >1, classify as **major** and **minor**; avoid vagueness)
- 03. Details of experimental treatments**
Experimental factor(s):
Levels of factor(s):
Total number of treatments/treatment-combination: $t =$
Relevant information on (nature of) treatments/factors:
(eg, checks, require larger plots, interplot interference, etc.)
- 04. Observations (within units) to be recorded:**
(Indicate their order and manner)
- 05. Treatment effects/differences to be estimated:**
- 06. Hypotheses to be tested:**
- 07. Details of experimental site/material**
(If >1 site, give information by site)
Site/field label:
Dimensions:
Any (suspected) gradient(s)?:
Direction of gradient(s):
Other relevant information about experimental site(s)/material:
- 08. Extraneous factors likely to distort treatment effects:**
- 09. Plot size and shape required to facilitate experimental operations:**
Plot size : Gross: Net:
Plot shape :
Total number of plots : $n =$
Number of plots in a row : $n_{\text{row}} =$
Number of plots in a column : $n_{\text{col}} =$
- 10. Plot orientation**

11. **Block size:** $k =$ **Block shape:**
12. **Block orientation:**
13. **Number of blocks:** $b =$
14. **Number of replications:** $r =$
15. **Experimental design:**
(fully randomized/partially randomized/non-randomized)
16. **Plot sampling plan:**
17. **Outline of statistical analysis;**

Appendix 2: Restrict Maximum Likelihood (REML).

REML is a statistical method that can be used

- To analyze *unbalanced data* that ANOVA cannot handle due to some plots (missing plots) in any design;
- To analyze complete as well as incomplete block designs. For PB1BDs particularly, REML can optimally combine information from more than one stratum to provide more precise estimate effects;
- To estimate variance components;
- To obtain best linear unbiased predictions (BLPs) for random effects; and
- To analyze a series of similar experiments combined over time and/or space.

The most crucial decision for implementation of REML is the “*correct formulation of a model*” with a conscious and careful specification of random and fixed effects. Also, the variable being analyzed should be fairly normally distributed.

Appendix 3: Deciding whether a model term should be taken

As fixed or random (Baker 1996)

Step 1: From Research Point of view

- Initially declare the term to be random effect;
 - Now ask two questions
- a) Is it physically possible for the used factor levels to be repeated at some future time or in some other place?

- b) If answer to (a) is YES, would it be reasonable in the context of this research for you or someone else to choose the same levels for repetition of this research at some future time or in some other place?
- If the answers to questions(a) and (b) are BOTH YES, declare the term as a fixed effect, otherwise as a random effect.

Step 2: From Statistical Point of View

If fixed-effect factor involves a large number of levels (say >10) and there is no structure among those levels, it might perhaps be best to declare the factor effect as random and use BLP to perfect mean values.

- The basis for this recommendation is: With a large number of unstructured levels, it is likely that the extremely low or high means will partially reflect a fortuitous combination of random error effects and should therefore be shrunken toward the trial mean.
- If a random-effect factor involves too few levels (say if the factor represents an uninteresting nuisance factor in the trial), and if comparisons among levels of this factor provide no information about other factors of interest (no inter-class information), then declare the factor effect as fixed. This will ease the computing demands and provide identical results for the factors of interest.

NOTE 1: Whatever the type of model (Fixed, Random or Mixed), μ is always taken as fixed, and ε_{ij} , de random allocation of treatments to plots, is always taken as random. What makes a model Fixed, Random or Mixed then depends on the nature of the remaining terms in any model.

WHAT IS A FIXED-EFFECTS MODEL (*Model 1*)? Under the proviso of NOTE 1, if all other model terms represent fixed effects, the model is said to be a fixed-effects model.

WHAT IS RANDOM-EFFECT MODEL (*Model 2*)? Under the proviso of NOTE 1, if all other model terms represent random effects, the model is said to be a random-effects (or a random) model.

WHAT IS A MIXED-EFFECT MODEL (*Model 3*)? Under the proviso of NOTE 1, if some of the remaining model terms are fixed effects and some are random effects, the model is defined to be a mixed-effects (or a mixed) model.

Reference

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About ICRISAT



ICRISAT
Science with a human face

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, non-political organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 644 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Centers of the Consultative Group on International Agricultural Research (CGIAR).

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