Diversity Analysis and Identification of Sources Resistant to Downy Mildew, Shoot Fly and Stem Borer in Wild Sorghums

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CERTIFICATE

This is to certify that the Thesis entitled "**Diversity Analysis and Identification of Sources Resistant to Downy Mildew, Shoot Fly and Stem Borer in Wild Sorghums**", submitted for award of the degree of Doctor of Philosophy in Genetics, Osmania University, is a record of the *bona fide* research carried out by Ms. Kamala Venkateswaran under my supervision, and no part of the Thesis has been submitted for any other degree or diploma.

The assistance and help taken during the course of this investigation and the sources of literature referenced have been fully acknowledged.

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DECLARATION

I hereby declare that the research work presented in this Thesis, entitled "**Diversity Analysis and Identification of Sources Resistant to Downy Mildew, Shoot Fly and Stem Borer in Wild Sorghums**", has been carried out by me at the Dept. of Genetics, Osmania University, Hyderabad and at ICRISAT, Patancheru, under the supervision of Dr. D. Manohar Rao, Associate Professor, Dept. of Genetics, Osmania University. The work is original and no part of the Thesis has been submitted earlier for any other degree or diploma of any University.

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Introduction

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is a significant cereal crop for the sustainable livelihood of the resource poor farmers of the semi-arid tropics. It thrives with less rainfall than is needed for rice or maize and can be grown where no other major cereal can be cultivated. Sorghum, known by several names, such as *jowar, cholam, jonna*, is important as a multi-purpose crop. The grain is a major food in most of Africa, Asia and Central America while it is an important animal feed in the Americas and Australia. Sorghum crop residues and green plants also provide sources of animal feed, building materials and fuel particularly in dry land areas of the semi-arid tropics. Alternative uses include beer, alcohol and syrup production. It is believed to have been domesticated in the northeast quadrant of Africa, an area that extends from the Ethiopia-Sudan border westward to Chad, and to have spread to India, China, the Middle East and Europe soon after its domestication about 8000 years ago (Doggett, 1988; Wendorf *et al.*, 1992).

In India, which is the secondary centre of its diversity, sorghum is third in importance after rice and wheat, and is currently grown on 10.3 million hectares with an annual production of 9 million tonnes (FAO, 2001). It is grown in areas where rainfall ranges from 500 to 1000 mm and temperatures from 26 to 32°C. More than 90% of India's sorghum production comes from the states of Andhra Pradesh, Maharashtra, Rajasthan, Gujarat, Tamil Nadu, Karnataka, Madhya Pradesh and Uttar Pradesh (Anahosur, 1992). Over the past fifteen years, though area under sorghum and its production have declined, average yields have shown a marginal increase from 633 to 873 kg/ha mainly due to the adoption of high yielding hybrids (FAO, 2001). Even so, these yields are much lower than all major grains reported except millet (FAO, 2001). Less than optimum yields are more often a result of biotic and abiotic stresses and inadequate agronomic practices. Sorghum has a high yield potential, comparable to that of rice, wheat and maize and will even out- yield maize when managed well (House, 1985).

Sorghum is an immensely variable genus with 24 species distributed in five sections: *sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum*. The most comprehensively studied section, *sorghum*, includes the cultivated grain and fodder sorghums (*S.bicolor* subsp. *bicolor*), a complex of closely related wild annual taxa (*S.bicolor* subsp. *verticilliflorum*) from Africa, a complex of weedy perennial taxa (*S.halepense*) from southern Europe and Asia, and a perennial wild species (*S.propinquum*) from southern and south-eastern Asia (Doggett, 1988). The other four

sections contain only wild species with distributions ranging from Africa through to Australia across Asia. Immense morphological diversity of the cultivated types has emerged because of variable climate and geographical exposure in which its wild ancestors evolved, coupled with selection pressures imposed by the environment and by man for domestication (Doggett, 1988).

Given the importance of sorghum in the semi-arid tropics, an *ex situ* collection for sorghum was initiated in the 1960s by the Rockefeller Foundation as part of the Indian Agricultural Research Programme, which was transferred to ICRISAT, Patancheru, India in 1974 (Stenhouse *et al.*, 1997). Two decades ago, the danger of genetic erosion in traditional landraces due to the release of new varieties and hybrids increased the collection and conservation efforts throughout the world. Concomitantly, there was a heightened awareness of the importance of wild species in crop improvement, and both National and International gene banks began augmenting their collections with wild relatives. At present the ICRISAT sorghum collection, which is one of the largest, contains 461 accessions of wild sorghums belonging to 19 species besides 35,238 accessions of cultivated sorghum. Wild sorghums in the US sorghum collection include 532 accessions of about 10 species, which is only 1.2% of their entire sorghum collection (Dahlberg and Spinks, 1995). Collections of wild species (~ 370 accessions of 23 species) are also maintained at the Australian Tropical Crops and Forages Genetic Resources Centre, Biloela, Australia (AusPGRIS, 2002).

Notwithstanding the availability of vast germplasm with wide degree of variability for various economic characters within cultivated types, little progress has been made in evolving varieties/hybrids with durable resistance to biotic stresses. Sorghum productivity continues to be constrained by a wide range of pests and pathogens. Over 40 diseases and more than 150 insect pests have been reported to attack the sorghum crop (Jotwani *et al.*, 1980; Frederiksen and Duncan, 1982). The sorghum downy mildew [*Peronosclerospora sorghi* West. and Upp. (Shaw)] among the diseases, and the sorghum shoot fly (*Atherigona soccata* Rond.) and spotted stem borer (*Chilo partellus* Swinhoe) among the insects, cause significant economic losses. Estimated loss in grain yield due to sorghum downy mildew in unprotected over protected plots, ranged from 9.6% to 78.5% in different cultivars (Anahosur and Laxman, 1991), and nearly 32% of the sorghum crop is reportedly lost due to insect pest infestation (Borad and Mittal, 1983). Some improved rabi sorghums (CSH 13R, CSH 14R and CSH 15R) are reported to be resistant to shoot fly and stem borer, but those released for kharif cultivation (CSH 9, CSH 10, CSHV 16, CSV 11 and SPV 462 among others) are quite susceptible (Rana *et al.*, 1999). Further, both irregular plantings and/or delayed monsoons are known to cause heavy shoot fly damage. This is especially critical since sorghum is predomi-

nantly grown in rain-fed marginal lands and low input farming systems so that losses caused by biotic stresses and the cost of their control is often the most limiting factor in profitable production.

Host plant resistance offers a viable economic solution in this situation. Several sources of resistance have been identified among the world collections of sorghum and used in breeding programmes, but improved types are not entirely stable and increased insect pressure often causes breakdown of resistance. Wild species have frequently been used as sources of resistance to pests and diseases in various crops. Several examples are known where genes from wild relatives, both near and distant, have played a key role in salvaging a crop and preventing its failure as a commercial enterprise (Harlan, 1984; Goodman *et al.*, 1987). However, despite the availability of a wide array of wild sorghums their utility in sorghum improvement has not been fully explored. The few isolated reports of utilization of wild species in sorghum breeding include their exploitation as sources of resistance to green bugs (Dixon *et al.*, 1990) and shoot fly (Nwanze *et al.*, 1990).

Ex situ collections are often the starting point for many crop-breeding programmes and enhanced utilisation of these germplasm collections requires a detailed understanding of the diversity and distribution of the accessions. Extensive investigations have been undertaken for the purpose of understanding levels of diversity, taxonomic relations and evolution of *Sorghum*. Characterisation using taxonomy, biogeography, morpho-agronomy and cytology has revealed considerable phenotypic variability (Celarier, 1958, 1959, Harlan and deWet, 1972; Harlan *et al.*, 1973). In addition, several studies have reported on the morphological variation in specific cultivated collections (Appa Rao *et al.*, 1996; Ayana and Bekele, 1999; Grenier *et al.*, 2000).

Interestingly, despite enormous morphological variation, allozyme studies have shown sorghum to be strikingly less variable than other cereals such as maize or barley (Melchinger *et al.*, 1990). Further, studies using molecular markers have revealed varying levels of diversity depending on the marker system used (Menkir *et al.*, 1997; Jordan *et al.*, 1998; Grenier *et al.*, 2000). It is also reported that levels of genetic diversity are lower in cultivated types than in wild germplasm (Tao *et al.*, 1993; Cui *et al.*, 1995; Ahnert *et al.*, 1996).

These assessments of diversity have focussed mainly on cultivated types in the primary genepool and little is known of the extent of variation or the nature of traits available in wild sorghums belonging to the other genepools. Further, taxonomic confusions and lack of evaluation information on traits of interest particularly with reference to resistance to serious pests and diseases seem to have precluded their intensive study and utilisation.

Though databases on phenotypic and genotypic diversity are available for the cultivated sorghums no such descriptions are reported for the wild sorghum collections. The systematic study of wild sorghums, therefore, assumes crucial significance as the availability of new sources of resistance can provide an active means to sustain sorghum improvement particularly when suitable levels of resistance in the cultigens are unavailable and virulent strains of pests and pathogens overcome host plant resistance. An assessment of the levels and patterns of genetic diversity among wild relatives would facilitate the understanding of the evolutionary processes of sorghum domestication and the utility of wild genepools in future plant-breeding programmes.

Thus, with an aim to furthering an understanding of wild sorghums and their potential significance in sorghum improvement the present study was undertaken with the following objectives:

- To characterise germplasm of wild sorghum using morphological traits and molecular markers.
- To screen germplasm of wild sorghum for resistance to sorghum downy mildew [*Peronosclerospora sorghi* West. and Upp. (Shaw)].
- To evaluate germplasm of wild sorghum for resistance to sorghum shoot fly (*Atherigona soccata* Rond.) and to study the mechanisms of resistance.
- To evaluate germplasm of wild sorghum for resistance to spotted stem borer (*Chilo partellus* Swinhoe) and to study the mechanisms of resistance.

Review of Literature

Review of Literature

Taxonomy and Distribution

Sorghum is a small but immensely variable genus comprising 24 species distributed in five sections: *sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum* (Garber, 1950; Harlan and de Wet, 1972; de Wet, 1978; Lazarides *et al.*, 1991). The systematic classification of genus *Sorghum* is presented in Fig 1. By the more widely accepted system of classification, section *sorghum* includes three species; two wild perennials viz., *S.halepense* (2n = 40) and *S.propinquum* (2n = 20) and the economically important annual, *S. bicolor* (2n = 20), which is further divided into three subspecies. Subspecies *bicolor* includes all domesticated grain sorghums, subspecies *arundinaceum* consists of the wild progenitors of grain sorghum and subspecies *drummondii* includes the stabilised derivatives of hybridisation among grain sorghums and their closest wild relatives. Subspecies *arundinaceum* is now called subsp. *verticilliflorum* (Steud) Piper (Doggett and Prasada Rao, 1995). It was divided among three varieties (*arundinaceum, aethiopicum, verticilliflorum*) by de Wet and Huckabay (1967), and a fourth variety (*virgatum*) was added by de Wet *et al.* (1970). These varieties/ecotypes/races grade morphologically and ecologically so completely into one another that they do not deserve formal taxonomic status (Doggett, 1988). *S.halepense*, a native of southern Eurasia, has been introduced to all warm temperate regions where it has come to occupy the status of one of the world's most pernicious weeds. *S.propinquum* occurs in Sri Lanka and southern India and extends eastwards to the Islands of south eastern Asia. This species crosses with introduced grain sorghums to produce obnoxious weeds in some areas in the Philippines (Doggett, 1988). Species of section *sorghum* are distributed through southern Africa to India, south east Asia and the Philippines but did not reach either Australia or America until carried there by man in the past few centuries (Doggett, 1988).

Sections *chaetosorghum* and *heterosorghum* are represented by one species each. *Chaetosorghum* includes *S.macrospermum* which is confined to the Port Darwin - Katherine region of the Northern Territory, Australia whereas *heterosorghum* is represented by *S.laxiflorum* restricted to northern Queensland, Australia, New Guinea, and the Philippine Islands (Garber, 1950, Lazarides *et al.*, 1991). *Parasorghum* includes nine species with a range of distribution extending from south and eastern Africa in a discontinuous arc through India, southern and eastern coastal Asia and the East Indies

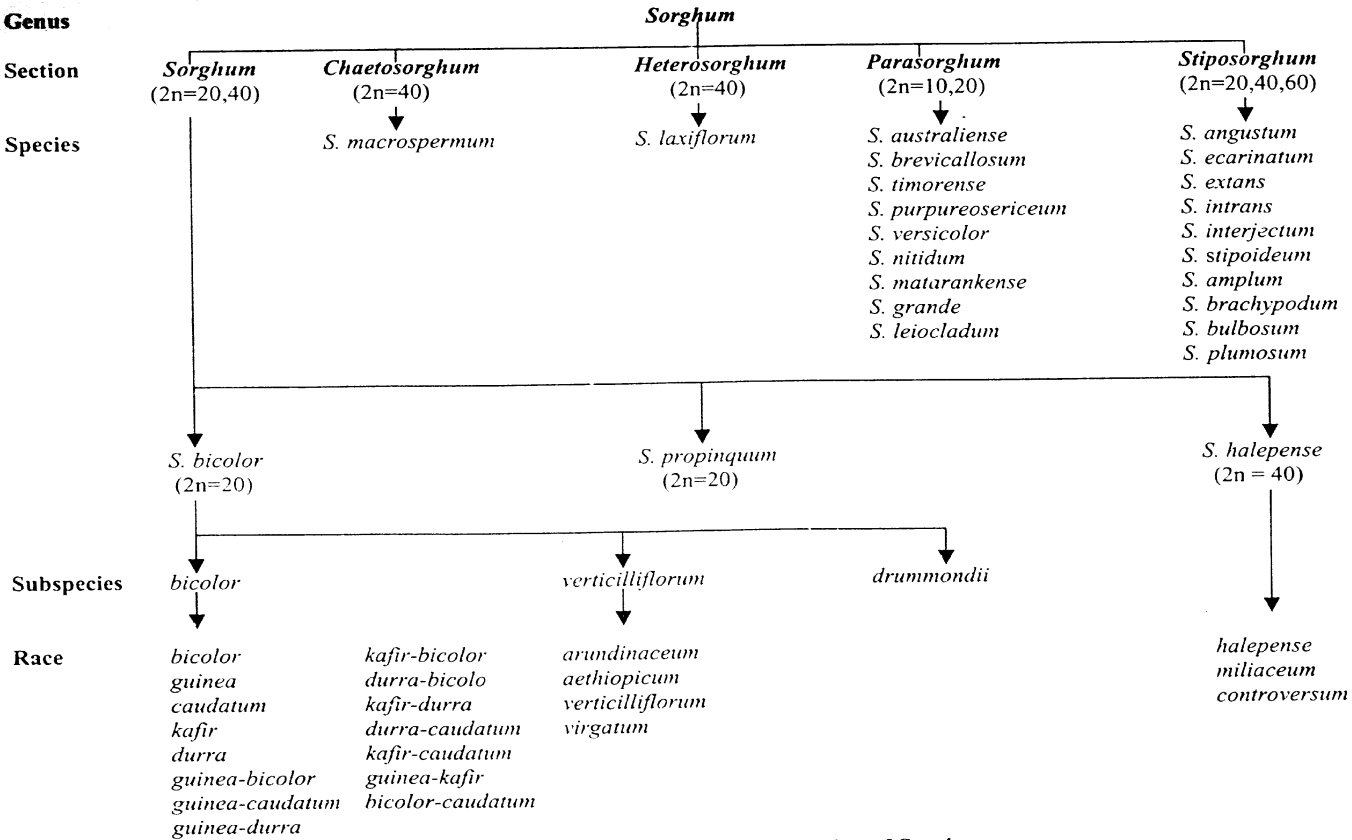


Fig. 1 Systematic Classification of *Sorghum*

to Australia. Five *parasorghum* species are endemic to Australia *(S.australiense, S.brevicallosum, S.grande, S.leiocladum and S.matarankense*); *S.timorense* is found in the Timor Islands and Australia; *S.versicolor* occurs in Africa; *S.purpureosericeum* in Asia and Africa and *S.nitidum* shows a distribution from Asia to Australia. *Stiposorghum* comprises 10 species and the section is reported to be restricted to the Northern Territory, Western Australia and Queensland (Lazarides *et al.*, 1991). In Australia, of the 17 indigenous species distributed in four sections, 14 are endemic.

Sorghum Genepools

Harlan and de Wet (1971) proposed three informal categories to provide genetic-taxonomic descriptions of cultivated plants and to discuss the wide range of crosses that are carried out with the aim of enhancing genetic variation in breeding programmes for crop improvement. Schematic representation of *Sorghum* genepools is shown in Fig. 2. By this classification the primary genepool includes all cultivated sorghums and their wild and weedy relatives with 2n = 20 chromosomes (*S.bicolor* complex) along with the diploid perennial *S.propinquum*. The secondary genepool includes *S.halepense*, which is an autotetraploid species that is reported to have probably arisen from a cross between *S.propinquum* and *S.verticilliflorum* (Doggett, 1988). The tertiary genepool includes members of all other sections of sorghum (2n = 20,40,60) as well as related genera – *Saccharum, Zea, Cliestachne and Sorghastrum*. Nwanze *et al.* (1990), reported that a few F1 seeds were obtained in a cross between *S.dimidiatum* (*parasorghum*) and cultivated sorghum with varying degrees of sterility. Except for this, efforts to cross the members of section *sorghum* with those in other sections have been unsuccessful. Sun *et al.* (1991)and Huelgas *et al.* (1996) attempted hybridisation using *S.bicolor* as female parent with species of other sections and reported strong cross-incompatibility due to pre-fertilisation barriers such as lack of pollen germination or very slow and irregular pollen tube growth. Shivanna and Seetharama (1997) showed that the cross *S.bicolor* x *S.dimidiatum* exhibits strong pre-fertilisation barriers at the level of pollen germination and pollen tube entry into the stigma in both directions.

Utilisation of Wild Germplasm in Crop Improvement

Crop improvement occurs through selection operating on genetic variability and has resulted in major advancements in agricultural productivity. However, continued success in plant breeding can only be realised when new genetic variability is available for selection. Variability provides adaptability, the capacity for genetic change / evolution in response to natural or artificial selection pressures. Crop improvement thus, rests on the cornerstone of genetic diversity.

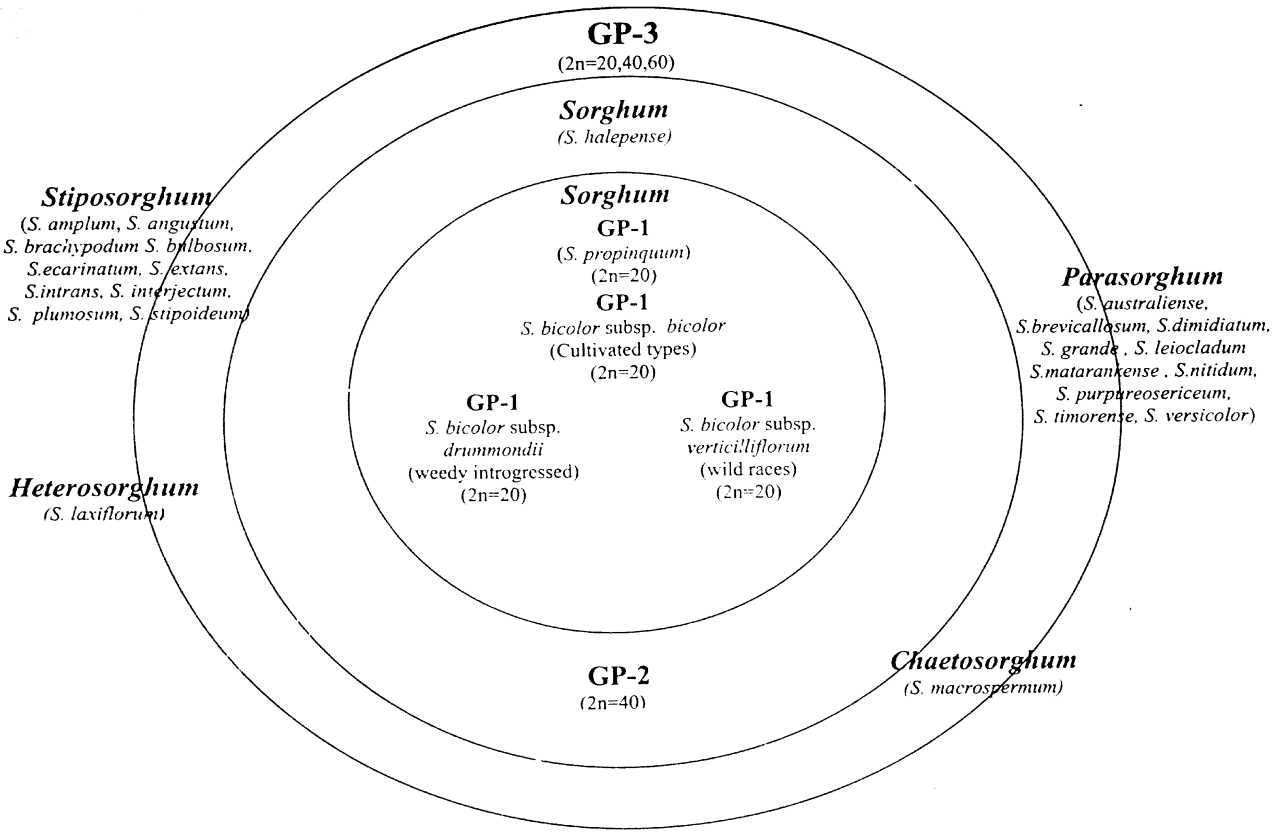


Fig. 2 Genepool Classification of *Sorghum*

Since, plant breeders tend to use favoured cultivars as the basis for generating new ones, it often leads to a progressively narrower genetic base, slower progress (genetic gain) and increased risk of crop vulnerability. Examples of visible consequences of this are the Irish potato famine, during 1845-49 caused by late blight, and more recently the southern leaf blight epidemic in the US maize crop in 1970 (Hawkes *et al.*, 2000).

In an attempt to broaden the genetic base of important crops, plant breeders have traditionally sought for additional diversity either in other species of the concerned genus or among related genera. Wild species have frequently been used as sources of resistance to pests and diseases and several examples are known where genes from wild relatives, both near and distant, have played a key role in salvaging a crop and preventing its failure as a commercial enterprise (Harlan, 1984; Goodman *et al.*, 1987). Notable instances of the successful transfer of alien genes for improvement of cultivated cereals includes that of rust resistance in bread wheat (Knott, 1971), grassy stunt resistance in rice (Khush, 1977), mildew and crown resistance in oats (Browning and Frey, 1969; Aung and Thomas, 1976) and for increased biomass and grain yield in oats, pearl millet and sorghum (Frey, 1983). Using electrophoretic techniques, Nevo *et al.* (1979) showed greater diversity in the wild and weedy barleys (*Hordeum vulgare* subsp. *spontaneum*) in the small country of Israel than in a composite cross of cultivated barleys that included over 6000 cultivars in its parentage. Wild species of genera as *Saccharum, Solanum* section *tuberosum, Glycine, Gossypium, Arachis, Lycopersicon, Hordeum* and *Triticum*, among others, range beyond the ecological amplitudes of their cultivated counterparts and have been profitably exploited by plant breeders. Wild peas were used to extend the crop into warmer, drier regions in the Soviet Union (Drozd, 1965) and winter hardiness in wheat has been improved by the use of *Agropyron* (Kuvarin, 1973). *Secale kuprijanovii* is reported to improve rye with respect to cold tolerance, protein content, rust resistance and yield (Yakovlev, 1972).

Wild sorghums have not been investigated as much as their cultivated counterparts. The expectation that wild sorghums could be used to broaden the adaptability of the crop is based primarily on the greater geographic range of the former. The *arundinaceum* race flourishes in African rain forests where cultivated sorghums are very poorly adapted (Harlan, 1984). Downes (1971) showed such material to be more photosynthetically efficient at low light intensities than cultivated sorghum. Race *virgatum* is known to extend into the fringes of the desert, thriving naturally beyond the range of the crop and could be a source of drought tolerance; seeds of *virgatum* are also known to germinate at very high temperatures, a trait often important to stand establishment in parts of the world (Bramel-Cox and Cox, 1988). Lazarides *et al.* (1991) reported that the indigenous Australian sorghums are extensively distributed in the

monsoonal region, occurring over extensive areas as major components of grassland, woodland and forest communities, and with some exceptions (*S.timorense, S.macrospermum and S.grande* which are habitat specific), are ecologically widely adaptable. Several of the Australian species have also been shown to possess high resistance to the sorghum midge (*Contarinia sorghicola*), a major pest of cultivated sorghums in the Americas, Africa, Asia and Australia (Harris, 1979; Sharma and Franzmann, 2001). Bramel-Cox and Cox (1988) showed the possibility of increasing sorghum yields through transfer of genes from races *virgatum*, *arundinaceum* and *verticilliflorum*. Most of the green bug (Biotype C) resistant hybrids grown in US are reported to be derived from race *virgatum* and best levels of antibiosis to Biotype E were found in *S.halepense* (Duncan *et al.*, 1991).

The collection and study of wild species therefore, assumes crucial significance as the discovery and incorporation of alien genes provides an active means to sustain crop improvement particularly when levels of resistance in the cultigens are low and virulent strains of pests and pathogens overcome host plant resistance. Further, an assessment of the levels and patterns of genetic diversity within and among wild relatives would substantially help in understanding the static and dynamic properties of genetic variation in natural populations, the evolutionary processes of domestication and the utility of wild genepools in future plant breeding programmes. Additionally, critical reviews of the state of diversity within the various genepools of a crop would help to provide a more objective basis for determining the most appropriate way to overcome a suspected bottleneck, and in choosing the most suitable base-broadening approach.

In the following account the status of morphological and molecular diversity in *Sorghum* is reviewed along with available literature on host plant resistance to sorghum downy mildew, sorghum shoot fly and spotted stem borer as relevant to the present investigation.

Morphological Diversity

Sorghum has been extensively investigated for the purpose of understanding the levels of diversity, taxonomic relations and evolution of the crop. Traditionally, diversity studies and inter-relationships in sorghum have been undertaken using morphological and cytological traits and methods of numerical taxonomy. Snowden (1936,1955) was the first to study systematically the extent of morphological variation within the *S.bicolor* species of section *sorghum*. He subdivided the complex into the *Halepensia* and *Arundinacea* groups. The former included four rhizomatous taxa (*S.halepense, S.propinquum, S.miliaceum and S.controversum*) that are widely distributed in the Medi-

terranean region and extend across India and south east Asia to the adjacent Pacific Islands. The *Arundinacea* included 48 taxa: 28 cultivated species, 13 wild species and 7 represented hybrids (introgressed types) between wild and cultivated sorghums. Liang and Casady (1966) studied 21 Snowdenian *Sorghum* species within the section *sorghum*, following the method of Sokal and Michener (1958). Their study separated the wild and cultivated sorghums, with *S.drummondii* included in the wild group. Murty *et al.* (1967) presented an analysis of 905 samples drawn from the available world collection of 1964. They established 63 working groups within the *S.bicolor* complex. De Wet and Huckabay (1967), using the method of Sokal and Michener (1958), published a study using only type specimens and original collections that fitted Snowden's type descriptions in detail. For each of Snowden's 52 taxa, 38 characters were recorded as clearly expressed (positive) or absent (negative). Three groups were decernible: the first included *S.propinquum and S.halepense,* (along with forms *miliaceum, controversum* and *halepense*); the second contained the wild types and the hybrids obtained from the crosses of wild and cultivated, and the third group clearly comprised of the cultivated types. Further biosystematic studies by de Wet *et al.* (1970), de Wet and Harlan (1971) and de Wet (1978) established that all 'Snowdenian' species of group '*Arundinacea*' belong to one biological species, *S.bicolor* with three subspecies corresponding to (i) cultivated, (ii) wild and (iii) weedy types (introgressed material between the wild and cultivated types).

Several studies have established the immense variability at the morphological level for various traits in cultivated sorghums. Chantereau *et al.* (1989) studied 157 landraces using 25 agro-morphological traits, and distinguished three groups with different cropping performances: the *durra* race, hardy and adapted to dry zones; the *guinea* and *bicolor* races, hardy and adapted to wet zones; and the high yielding *kafirs* and *caudatums*, adapted to intermediate zones. Appa Rao *et al.* (1996) undertook an analysis of the morphological diversity in sorghum germplasm from India and found considerable diversity for all 13 characters studied, particularly for days to flowering (post rainy: 42-129; rainy: 33-180), plant height (65-655cm), panicle length (5-52cm) and panicle width (1-51cm). Teshome *et al.* (1997) in a study of sorghum landraces from Ethiopia found that accessions of the five most common landraces named by farmers formed dissimilar groups. Primary traits used by the farmers in naming the sorghum landraces included colour of midrib/grain/glume, grain size, glume hairiness and grain shape. Ayana and Bekele (1998) studied geographical patterns of morphological variation in sorghum from Ethiopia and Eritrea and showed high and comparable levels of phenotypic variation between regions of origin and the adaptation zones. Panicle compactness and shape were observed to contribute relatively more to regional differentiation. Grenier *et al.* (2000) analysed the diversity in three core sets of sorghum landraces using a number of

agro-morphological traits and showed that overall diversity was high in the three differently created core subsets (a sample established by a random sampling within a stratified collection-logarithmic strategy; a sample based on morpho-agronomic diversity-principal component score strategy; and a sample based upon an empirical knowledge of sorghum-taxonomic strategy), and did not differ among themselves. However, for individual traits there were differences between the core subsets and the total collection.

Molecular Diversity

Classical methods of estimating genetic diversity and / or relatedness among groups of plants relied upon phenotypic (observable) traits. However, these had two disadvantages: firstly the traits were subject to environmental influences and secondly the levels of polymorphism (allelic variation) that could be looked at were limited. These limitations were significantly overcome by deployment of environment-neutral biochemical markers (isozymes) and protein electrophoresis (Hunter and Markert, 1957) and molecular markers that focus directly on the variation controlled by genes or on the genetic material (DNA) itself. The higher resolution of molecular markers makes them a valuable tool for a variety of purposes, such as fingerprinting and protection of breeders rights, facilitating appropriate choice of parents for breeding programmes, analysing quantitative traits and location and detection of quantitative trait loci (QTLs), gene mapping, marker assisted selection and gene transfer, understanding evolutionary pathways, and for the assessments of genetic diversity of plant germplasm. Hillis (1987) recommended that morphological work on large samples combined with molecular analyses on smaller samples maximise both information and usefulness. Kresovich and McPherson (1992) believed that molecular markers could resolve biological, operational and logistical questions dealing with four broad areas of germplasm characterisation: the determination of the correct identity of an individual (whether it was true to type, duplicate etc.); the estimation of the degree of similarity among individuals; understanding of the hierarchical structure and partitioning of variation among individuals, accessions, populations and species; and identification and detection of the presence of particular alleles in individuals, accessions, populations, chromosomes or cloned DNA segments.

The range of molecular markers that can be relatively easily used on most plant germplasm is quite extensive (Table 1, Mohan *et al.*, 1997; Gupta and Varshney, 2000). Techniques vary from identifying polymorphism in the actual DNA sequence to the use of DNA hybridisation methods to identify RFLPs (restriction fragment length polymorphisms), or the use of PCR-based (polymerase chain reaction) technology to find polymorphisms using RAPD (random amplified polymorphic DNA), SSR (simple sequence repeat) or combination techniques such as AFLP (amplified fragment length

Table 1 Different Molecular Techniques Developed and Used

S.No.	Acronym	Technique / Reference
1	AFLP	Amplified Fragment Length Polymorphism Vos *et al.*, 1995
2	ALP	Amplicon Length Polymorphism Ghareyazie *et al.*, 1995
3	AP-PCR	Arbitrarily Primed PCR Welsh and McClelland, 1990
4	AS-PCR	Allele Specific PCR Sarkar *et al.*, 1990
5	CAPS	Cleaved Amplified Polymorphic Sequence Lyamichev *et al.*, 1993
6	DAF	DNA Amplification Fingerprinting Caetano-Anolles *et al.*, 1991
7	IMP	Inter-MITE (Miniature Inverted-Repeat Transposable Elements) Polymorphism, Chang *et al.*, 2001
8	ISA=ISSR	Inter-SSR Amplification = Inter Simple Sequence Repeat Zietkiewicz *et al.*, 1994
9	MP-PCR	Microsatellite-Primed PCR Meyer *et al.*, 1993
10	RAMS	Randomly Amplified Microsatellite Ender *et al.*, 1996
11	RAPD	Random-Amplified Polymorphic DNA Williams *et al.*, 1990
12	REMAP	Retrotransposon-Microsatellite Amplified Polymorphism Kalendar *et al.*, 1999
13	RFLP	Restricted Fragment Length Polymorphism Botstein *et al.*, 1980
14	SAP	Specific-Amplicon polymorphism Williams *et al.*, 1991
15	SCAR	Sequence Characterised Amplified Region Williams *et al.*, 1991
16	SNP	Single Nucleotide Polymorphism Nikiforov *et al.*, 1994
17	SSCP	Single Strand Conformation Polymorphism Orita *et al.*, 1989
18	SSLP	Microsatellite Simple Sequence Length Polymorphism Rongwen *et al.*, 1995
19	SSLP	Minisatellite Simple Sequence Length Polymorphism Jarman and Wells, 1989
20	SSR	Simple Sequence Repeat Hearne *et al.*, 1992
21	STMS	Sequence Tagged Microsatellite Sites Beckmann and Soller, 1990
22	STS	Sequence Tagged Sites Fukuoka *et al.*, 1994

polymorphism). The different methods differ in their cost, ease of application, type of data generated (whether they provide dominant or co-dominant markers), the degree of polymorphism they reveal, the way that they resolve genetic differences, and in the taxonomic levels at which they can be most appropriately used (Karp *et al.*, 1997).

The application of different techniques to genetic and diversity analyses have been well reviewed (Malyshev and Kartel, 1997; Newbury and Ford-Lloyd, 1997; Westman and Kresovich, 1997; Karp *et al.*, 1998). Assessment of levels of polymorphism and the distribution of polymorphism (usually conceptualised as 'allelic richness' and 'allelic evenness') in a crop permit the sampling and utilisation of genetic resources in a more systematic and efficient manner, and also allows an enhanced understanding of evolutionary relationships both for breeding and conservation. Some applications of diversity analyses using molecular marker tools include: identifying areas of high genetic diversity (Hamrick and Godt, 1990), determining collection priorities and sampling strategies (Schoen and Brown, 1991), guiding the designation of *in situ* or on farm conservation strategies (Bonierbale *et al.*, 1997), monitoring genetic erosion (Robert *et al.*, 1991) or vulnerability (Adams, 1977), guiding the management of *ex situ* collections (Kresovich *et al.*, 1997), maximising the genetic diversity in core collections (Gepts, 1995), comparing agronomically useful regions of the genomes of different crops (Paterson *et al.*, 1995), defining the identity of improved varieties or other plant genetic resources (Lee *et al.*, 1995), monitoring the movement of plant genetic resources (Hardon *et al.*, 1994) and assisting in taxonomic evaluation and enhancing understanding of relationships between crop gene pools (Gepts, 1995), achieving precise, unambiguous and accurate identification of germplasm at the species/subspecies levels (Wang and Tanksley,1989; Virk *et al.*, 1995; Martin *et al.*,1997; Zhu *et al.*, 1998), identifying duplicates within collections particularly in gene banks (Virk *et al.*, 1995).

Restriction Fragment Length Polymorphism (RFLP) markers that provide access to an unlimited number of loci have allowed a thorough analysis of the organisation of genetic diversity within cultivated sorghums. An analysis of 94 accessions, selected on the basis of their geographical origin and racial classification (Deu *et al.*, 1994), using 35 maize probes, well scattered over the maize genome, each with at least one restriction enzyme revealed 50 polymorphic probe-enzyme combinations with 158 individual polymorphic bands. Race *bicolor* appeared highly variable, included many rare markers, did not form a specific group but were scattered among the various clusters. Race *guinea* was divided into three sub groups. *Caudatums, durras* (mainly sampled from central-eastern Africa and Asia) and *kafir* accessions clustered together into one group. Compared to isozymes, the RFLPs have exhibited better relationships between molecular variation and racial differentiation.

Deu *et al.* (1995) analysed 109 cultivated types (subsp. *bicolor*) and 47 wild relatives (subsp. *verticilliflorum* and *S.halepense*) for mitochondrial DNA polymorphisms using 6 wheat mitochondrial probes (heterologous) with two enzymes each. The study confirmed the specificity of *guinea margaritiferum* and demonstrated the presence of two genetic entities in this sub-race. Further, the diversity observed in cultivated forms was found to be encompassed within the wild genepool.

Aldrich and Doebley (1992) surveyed 56 accessions of cultivated and wild sorghums for genetic diversity using 50 low-copy number nuclear DNA sequence probes to detect RFLPs. Greater genetic diversity was noted in wild sorghums including a larger number of alleles per locus. In comparison to the isozyme results of the same accessions, RFLP analysis revealed a greater number of alleles per locus. Correlations between genetic and geographic distances among the accessions were stronger when calculated with RFLP than with isozymes data. Systematic relationships revealed by nuclear and chloroplast restriction site analysis indicate that cultivated sorghum is derived from wild subsp. *verticilliflorum*. The portion of the wild genepool most genetically similar to cultivated sorghum is from central-north eastern Africa. Cui *et al.* (1995) compared the restriction fragment patterns of 53 sorghum accessions from Africa, Asia and the United States including representatives of both subsp. *bicolor* and subsp. *verticilliflorum* using 62 single copy sorghum DNA clones. Greater nuclear diversity was observed in the accessions of wild subsp. than in the cultivated types. Phylogenetic analysis using parsimony separated the subsp. into separate clusters, with one group of intermediate accessions. Accessions classified as the same morphological race tended to group together on the basis of RFLP similarities with a few exceptions for the race *bicolor*.

Tao *et al.* (1993) studied the frequency of DNA polymorphism in 36 accessions of grain sorghums, using 29 oligonucleotide RAPD primers, and reported low polymorphism. But phenetic analysis of band sharing were consistent with current sub groupings of species. The results also indicated that individuals of a similar taxonomic group but different geographic origin may be genetically less identical than previously considered. The study showed the existence of a high level of genetic uniformity within *S.bicolor* subsp. *bicolor*.

Ahnert *et al.* (1996) reported higher levels of polymorphism than Tao *et al.* (1993) among 105 elite sorghum inbred lines using 104 RFLP DNA clones originating from maize genomic, sorghum genomic and maize cDNA libraries. Cluster analysis based on genetic similarity revealed separate groups for R- and B-lines in agreement with parental types.

Menkir *et al.* (1997) analysed 190 accessions of cultivated races of subsp. *bicolor* and found high levels of variation. Partitioning the genetic variation using Shannon's

diversity index revealed that 86% of the total genetic variation occurred among the accessions and 14% among the races. Also, it was seen that 13% of the total genetic variation was attributable to divergence among regions. However, principal component analysis (PCA) failed to separate the accessions into discrete racial or geographic groups. The RAPD markers successfully identified races and regions with maximum genetic diversity. Accessions within races *bicolor* and *guinea* had greater genetic diversity than accessions from race *kafir*; accessions from southern Africa had a lower level of genetic diversity than accessions from the Far and Middle East, central and eastern Africa.

Simple sequence repeats (SSRs), also known as microsatellites, are highly variable DNA sequences that can be used as markers for the genetic analysis of plants. Brown *et al.* (1996) screened a total of 49 sorghum SSR-specific PCR primer pairs using a panel of 17 sorghum and one maize accessions. Length polymorphisms among amplification products were detected with 15 of these primer pairs, yielding diversity values ranging from 0.2 to 0.8 with an average diversity of 0.56. Taramino *et al.* (1997) characterised nine inbred lines using 13 SSR loci in *S.bicolor* and reported a high level of polymorphism. Dean *et al.* (1999) assayed 19 "Orange' accessions of sorghum using 15 SSR primers. Substantial resolution among the accessions was obtained. Average heterozygosity estimates were low and phenetic analysis was generally consistent with known historical relationships among accessions. The molecular variance analysis (AMOVA) showed that 90% of the total genetic variation was partitioned among accessions. Grenier *et al.* (2000) assessed the diversity in three subsets of sorghum landraces constituted from the ICRISAT sorghum collection using 15 SSR primers. The average allelic richness and the genetic diversity for the three subsets were equivalent and comparable. A high percentage of rare alleles was maintained in the three subsets. The global molecular diversity retained in each subset was not affected by the sampling procedure based upon phenotypic characters. Kong *et al.* (2000) developed 38 SSR primers and used them for genotyping of 18 sorghum accessions and the parents of a recombinant inbred (RI) mapping population. Thirty-six loci in 18 accessions and 31 loci between the parents of the RI populations exhibited polymorphism. Subsequently, 17 mapped SSR loci were used for genotyping 190 sorghum accessions. The levels of polymorphism were sufficiently high to be useful in marker assisted selection and further, as few as half a dozen SSR loci are reportedly enough to distinguish the vast majority of sorghum accessions from one another. Dje *et al.* (2000) evaluated the use of microsatellite markers to quantify the genetic diversity within as well as among accessions sampled from the world germplasm collection of sorghum. Considerable variation was found at five loci analysed in the overall sample of 25 accessions. The collection of sorghum was reported to be highly structured with about 17% of the total genetic diversity occurring

among the accessions. However, differentiation among morphologically defined races of sorghum, or among geographic origins accounted for less than 15% of the total genetic diversity.

To compare the diversity of 34 Chinese sorghums, Yang *et al.* (1996) used three marker techniques; RFLPs, RAPDs and Inter-simple sequence repeat (ISSRs). Their studies indicated that different marker techniques for germplasm assessment yield comparable results, but the ISSR technique was relatively rapid, reproducible and inexpensive. Extensive diversity was observed within the Chinese sorghums and all lines could be easily differentiated. Contrary to expectations, improved varieties were found to contain more diversity and to be more different from each other. de Oliviera *et al.* (1996) used RFLPs, RAPDs and ISSRs to analyse the diversity in 82 cultivated and wild sorghums. Both racial classification and geographical origin were correlated with molecular distances. Wild sorghums were shown to have very few novel alleles. The Chinese sorghums used in the study were shown to be a distinct group most closely related to race *bicolor*. Dje *et al.* (1999) studied the level of genetic diversity and population structure of sorghum landraces from northwestern Morocco based on direct field sampling using allozyme and microsatellite markers. Microsatellite markers showed a higher level of polymorphism than allozymes, but relative measures of genetic structure such as Wright's inbreeding coefficient F_{ST}, and Nei's coefficient of genetic differentiation G_{ST} were similar for the two sets of markers.

Besides diversity assessments, molecular markers have been used to identify and characterize QTL associated with several different traits in sorghum including plant height and maturity (Pereira and Lee, 1995), characters associated with plant domestication (Paterson *et al.*, 1995), disease resistance (Gowda *et al.*, 1995) and drought tolerance (Tuinstra *et al.*, 1998). In addition, several sorghum linkage maps (Hulbert *et al.*, 1990; Melake Berhan *et al.*, 1993; Xu *et al.*, 1994; Chittenden *et al.*, 1994; Pereira *et al.*, 1994 ; Lin *et al.*, 1995; Dufour *et al.*, 1996; Boivin *et al.*, 1999) have been generated, but they have not yet been properly integrated to produce a more global and functional map with 10 linkage groups.

Host Plant Resistance

Despite major advances made in sorghum breeding over the past decades, productivity continues to be constrained by a wide range of pathogens and pests. Over 40 diseases and more than 150 insect pests have been reported to attack the sorghum crop (Jotwani *et al.*, 1980; Frederiksen and Duncan, 1982). Among the diseases, the sorghum downy mildew (SDM) causes considerable yield losses. Among the insects, the sorghum shoot fly and spotted stem borer are particularly destructive and cause signifi-

cant economic losses. Nearly 32% of the sorghum crop is reportedly lost due to insect pest infestation (Borad and Mittal, 1983). Host plant resistance (HPR) offers an effective, economical and environment friendly method of pest/pathogen control particularly suitable for a crop like sorghum since it does not involve any additional cash investments by the resource poor farmers.

Sorghum Downy Mildew

Distribution, Description, Biology and Symptoms

Sorghum downy mildew (SDM) caused by *Peronosclerospora sorghi* is particularly destructive, since systemic infection of the host results in a barren inflorescence (Frederiksen *et al.*, 1973). Payak (1975) reported that in parts of India, annual yield losses due to SDM was at least 1.0 x 10^5 metric tonnes. Anahosur and Laxman (1991) estimated yield losses in different cultivars to range from 9.6% in CSV 4 to 78.5% in DMS 652. In USA, incidence of 90% losses have been reported in a single season and SDM epidemic, in grain sorghum in the coastal countries of Texas, caused an estimated loss of US$ 2.5 million (Frederiksen *et al.*, 1969). In Venezuela, crop loss was reported to be so severe in the early 1970s that a national emergency was declared (Frederiksen and Renfro, 1977).

The sorghum downy mildew fungus (*Peronosclerospora sorghi)*, infects both sorghum and maize and is widespread in many tropical and sub-tropical regions of the world where sorghum and maize crops are grown. Butler (1907) was the first to report downy mildew of sorghum. The true taxonomic status of the pathogen as *P. sorghi* was, however, satisfactorily established only much later (Weston and Uppal, 1932; Shaw, 1978). It has caused severe epidemics in both sorghum and maize crops in many countries (Kenneth, 1976; Williams, 1984).

The SDM pathogen has been confirmed in all continents in tropical and sub-tropical areas of the world (Williams, 1984). *Peronosclerospora sorghi* is considered an 'Old World' pathogen, having originated in Africa or Asia (Williams, 1984). It subsequently spread to the Americas in the late 1950s, where it was probably introduced (Frederiksen, 1980).

This fungus produces both asexual conidia and oospores. It is an obligate parasite but has been successfully grown in dual culture with the host tissue on a modified White's medium (Kaveriappa *et al.*, 1980). Most culture maintenance, however, depends on inoculating seedlings of the host with the conidia or oospores and using infected plants as a source of inoculum (Craig, 1976).

Systemic infection can manifest itself at any stage from about one week after seedling emergence. The symptoms initially appear as chlorotic areas emanating from the base of the first leaves showing the infection often covering only half the lamina (the 'half-leaf' symptom). Progressively greater proportions of the lamina of younger leaves show this symptom until the whole leaf becomes chlorotic. In cool, humid weather the asexual reproductive structures of the fungus, i.e., the conidiophores and conidia, form during the night on the leaves, particularly on the abaxial surfaces giving a white down-like appearance to the infected leaves. As the plant ages, white, chlorotic streaks develop from the base of the younger leaves, which turn pale to reddish-brown as the inter-veinal tissue dies and oospores develop. As the streaks turn brown they start to shred into long strips, the lamina disintegrates along the fibro-vascular strands of the leaf thus resulting in typical 'leaf-shredding' symptoms. Plants that are systemically infected as seedlings remain stunted, and often die, while those that survive are upright in habit, with narrow foliage, and are generally barren, although some grain may be produced. Occasionally, a plant may recover and produce healthy, viable grain (symptom remission), but the basis for this phenomenon is unknown (Singh and de Milliano, 1989a). The production of a normal grain-bearing panicle on a systemically infected plant has also been reported (Singh and de Milliano, 1989b). The local lesion phase can occur on any leaf of an infected sorghum plant. Lesions develop as discrete chlorotic areas, variable in size, but generally elongate with parallel edges (1-4mm x 5-15mm). Asexual spores are produced mostly on the abaxial surface of leaves displaying these lesions.

Pathogen Variability

The first indication of pathogenic variability on sorghum was observed in the USA in the late 1970s (Craig and Frederiksen, 1980). A previously resistant hybrid became susceptible to SDM. Subsequently, three distinct pathotypes have been identified in the USA by the differential reaction of the varieties Tx412, Tx430, CS 3541 and QL3 (Craig and Frederiksen, 1983). Other pathotypes have been identified in Brazil (Fernandes and Schaffert, 1983), Honduras (Craig and Odvody, 1992) and Zimbabwe (de Milliano and Veld, 1990). Pawar *et al.* (1985) tested 75 sorghum varieties for their reaction to 16 isolates from different geographic regions and found a differential reaction that identified each isolate as a different pathotype. Those from Africa (Nigeria and Ethiopia) and Asia had greater virulence than those from the Americas.

Disease Status and Host Range

Collateral hosts, common in many areas, where sorghum and maize crops are grown, are known to act as reservoirs of both conidial and oospore inoccula. Several species of

Poaceae from the tribes, *Andropogoneae*, *Maydeae* and *Paniceae*, are reported to be infected with *P. sorghi* (Table 2). Bonde and Freytag (1979) showed that *S.versicolor* from Ethiopia was susceptible to an American isolate of *P. sorghi* in inoculation tests while *S.miliaceum* from India was resistant. Bonman *et al.* (1983) reported that while native *S.nitidum* was fully susceptible, *S.halepense* was resistant in Thailand. Renfro and Shankara Bhat (1981) reviewed the role of wild hosts in downy mildew diseases and reported that the mycelia of *P. sorghi* perennate in underground parts of Johnson grass (*S.halepense*), the only known wild host in Israel but also that the "great majority of clones of Johnson grass are not infected and are considered resistant to SDM". Dange *et al.* (1974) stated that the form of the *P. sorghi* found in Rajasthan is pathogenic to maize and *Heteropogon contortus* but not to sorghum. In contrast, *P. sorghi* in Karnataka

Table 2 Host range of *Peronosclerospora sorghi*

S.No.	Host	Author
1	*Panicum trypheron* Shult.	McRae, 1934
2	*Pennisetum americanum (L.)* Leeke	Castellani,1939
3	*Parasorghum* sp.	Karunakar *et al.*,1994
4	*Sorghastrum rigidifolium* Stapf.	Karunakar *et al.*,1994
5	*Sorghum almum* Parodi.	Tarr, 1962
6	*S. arundinacium (Wild.) Stapf.*	Karunakar *et al.*. 1994
7	*S. bicolor x S. sudanense* (Piper) Stapf.	Futrell and Bain, 1967
8	*S. bicolor* (L.) Moench	Bonde and Freytag,1979
9	*S. controversum* (Steud.) Snowden	Karunakar *et al.*, 1994
10	*S. drummondii* (Steud.) Millsp. & Chase	Karunakar *et al.*, 1994
11	*S. halepense* (L.) Pers.	Frederiksen *et al.*, 1965
12	*S. hewisonii* (Piper) Longley	Bonde and Freytag, 1979
13	*S. lanceolatum* Stapf.	Bonde and Freytag, 1979
14	*S. miliaceum* (Roxb.) Snowden	Karunakar *et al.*, 1994
15	*S. niloticum* (Stapf. Ex Piper) Snowden	Bonde and Freytag, 1979
16	*S. plumosum* (R. Br.) Beauv.	Nagarajan *et al.*, 1970
17	*S. propinquum* (Kunth.) Hitch.	Bonde and Freytag, 1979
18	*S. pugionifolium* Snowden	Bonde and Freytag, 1979
19	*S. purpureosericeum* (A. Rich.) Ashers. & Schwerf.	Karunakar *et al.*, 1994
20	*S. sudanense* (Piper) Stapf.	Nagarajan *et al.*, 1970
21	*S. verticilliflorum* (Steud.) Stapf.	Tarr, 1962
22	*S. controversum* (Steud.) Snowden	Bonde and Freytag, 1979
23	*S. usambarense* Snowden	Karunakar *et al.*, 1994
24	*S. versicolor* Anderss.	Bonde and Freytag, 1979
25	*S. virgatum* (Hack.) Stapf.	Nagarajan, *et al.*, 1970
26	*Zea mays ssp. mexicana* (L.) (Schrad.) Iltis	Uppal and Desai, 1932
27	*Zea mays* (L.)	Bonde and Freytag, 1979

attacks maize and sorghum but not *Heteropogon contotrtus* (Safeeullah, 1976). While the pathogen in Thailand is highly infective on maize but not on sorghum (Schmitt and Freytag, 1977), the American pathotypes seriously affect both maize and sorghum in USA. Wild species in section *sorghum* have been implicated as collateral hosts and are reported to act as reservoirs of infection for both maize and sorghum in different parts of the world: *S.arundinaceum* in South Africa (Storey and McClean, 1930), *S.halepense*, *S.verticilliflorum* and *S.arundinaceum* in Venzeuela (Malaguti, 1976), *S.drummondii* and *S.halepense* in the USA (Williams and Heron, 1974; Warren *et al.*, 1974; White *et al.*, 1978; Partridge and Daupnick, 1979). In Australia the pathogen was reported in maize in 1977 (Reddy, 1979) but the disease is not reported to be present on sorghum (Henzell *et. al.*, 1982).

Sources of Resistance

There are many reports on screening of cultivated sorghum lines for resistance to SDM (Frederiksen *et al.*, 1973; Kumar *et al.*, 1979; Henzell *et al.*, 1982; Williams *et al.*, 1982; Anahosur *et al.*, 1984; Shivana and Anahosur, 1988; Lu *et al.*, 1990). In an attempt to screen sorghum cultivars and to identify stable resistance and differences in pathogen virulence between locations the International SDM Nursery was established in 1976 (Williams *et al.*, 1980). Many of the SDM resistant lines (QL-3, IS 3443, IS 8283, IS 27042) identified in the various screenings have been successfully used to breed SDM resistant varieties and hybrids. Some of the improved lines include SPV-35, SPV-312, CSH-2, CSH-6, SPH-10, SPH-59, SPH-176, DMS 1B, DMS 2219B, CSV-4, PVK-3 and MR-780 (Anahosur, 1992). Reports on screening of wild sorghums are only a few. Nagarajan *et al.* (1970) and Karunakar *et al.* (1994) reported that members of *parasorghum* notably, *S.versicolor*, S.*dimidiatum*, and *S.purpureosericeum* were highly resistant even though their conclusions were based only on small sample sets.

Sorghum Shoot Fly

Distribution, Description, Biology and Symptoms

The shoot fly was first reported and named by Rondani (1871). It is a widespread and damaging pest in practically all the sorghum-growing areas in the semi-arid tropics in Asia, Mediterranean Europe and Africa, but is absent in the Americas and Australia (Taneja and Leuschner, 1985a). Infestations of upto 90% were recorded by different researchers (Hiremath and Renukarya 1966; Rao and Gowda, 1967). Yield loss was directly correlated with infestation, with a proportionate reduction in grain yield for every 1% increase in shoot fly incidence (Rai and Jotwani, 1977). Yield losses of 30.4,

39.5 and 22.4kg/ha were observed in CSH I, CSH 5 and CSV 3, respectively (Srivastava, 1985).

The adult is a small grey - coloured fly that deposits small (2mm), white, cigar-shaped eggs singly on the undersurface of the leaves parallel to the midrib. Mature larvae are yellow and about 6mm long. The larval period lasts for 8-10 days. Pupation takes place either in the plant or in the soil. The pupal period also lasts for 8-10 days. The shoot fly completes its life cycle in 17-21 days (Kundu and Kishore, 1970). The larvae after hatching in 2-3 days crawl along the leaf sheath and move upward to reach the plant whorl. Then it moves downward between the fifth and sixth leaf till it reaches the growing point, and cuts around it.

As a result of larval feeding, the central leaf wilts and later dries up, giving the typical deadheart symptom. The deadheart can be easily pulled out and the base emits a bad smell. The young whitish yellow maggot feeds only on the decaying tissue. Normally, the attack and damage occur from one week to about a month after seedling emergence. If the attack occurs a little later, plants may produce side tillers that may also be attacked. Late sowing during the rainy season increases the likelihood of attack. Shoot fly numbers begin to increase in July and peaks in August - September. Infestations are high when sorghum sowings are staggered due to erratic rainfall. Shoot fly infestations are high in the post-rainy season crop planted in September - October. Temperatures above 35 °C and below 18 °C and continuous rainfall reduce shoot fly survival (Sharma and Nwanze, 1997).

Pest Status and Host Range

Shoot flies have long been known to attack different species of cereals. The damage caused to sorghum seedlings was recognised by Fletcher (1914) and Ballard and Ramachandra Rao (1924). In addition to sorghum it also attacks several wild graminaceous plants in various parts of Africa (Deeming, 1971). *Sorghum verticilliflorum* was reported as a common wild host of *A. soccata* in east Africa (Nye, 1960; Starks, 1970). Ogwaro (1978) reported that *S.bicolor* was markedly preferred in Kenya to other graminaceous species. Davies and Reddy (1981) reared shoot flies on 21 species of gramineae and noticed that *S.halepense* was by far the most important alternate host to *S.verticilliflorum*. *Digitaria sanguinalis* and *S.propinquum* have been reported to be wild hosts from China (Shiang - Lin *et al.*, 1981). Delobel and Unnithan (1981) observed that shoot fly populations are usually higher on wild sorghum, *S.arundinaceum*, than on local cultivated varieties of *S.bicolor*, especially during the dry season. Granados (1972) reported the recovery of adults from *Brachiaria reptans* despite less preferential oviposition but although *Eleusine indica* was preferred over sorghum, the larvae re-

quired more th ~~e plant to complete their devel~~ opment. This indicated that the wild host maintains a small population that does not build up until cultivated sorghum is available. During the off-season, the insect survives on alternate hosts, *Cymbopogon* sp. *Echinocloa colonum*, *E. procera*, *Paspalum scrobiculatum* and *Pennisetum glaucum* and on volunteer or fodder sorghum (Sharma and Nwanze, 1997).

Sources of Resistance

Sharma (1993), while reviewing the host plant resistance (HPR) to insects in sorghum listed several sources of resistance as screened by different workers, and discussed the role of HPR in integrated pest management. Screening of the world sorghum germplasm collection for resistance to the pest began in 1962 by the Accelerated Hybrid Sorghum Project, Indian Agricultural Research Institute and the Rockefeller Foundation (Nwanze, 1997). Studies at the All India Coordinated Sorghum Improvement Project (AICSIP), at ICRISAT, and in East, West, and South Africa (Starks *et al.*, 1970; Jotwani, 1978; Singh and Rana, 1986) have shown that most sources of resistance to shoot fly originate from the post rainy sorghums grown in India under the stored soil moisture. Efforts to breed for resistance have been made at AICSIP and ICRISAT. Cultivars, M 31-1 (IS 1054), IS 2123, IS 2146, IS 4664, IS 2205, IS 5604 and IS 18551 have been widely tested, and were found to possess moderate levels of resistance. Some of the improved varieties, like CSV 5, CSV 6, CSV 7R, Swati (SPV504) and CSV 8R, developed using land races also possess moderate levels of resistance while other improved lines such as ICSV 700,ICSV 705 and ICSV 717, developed at ICRISAT, have a yield potential better than the landraces (Agrawal and Abraham, 1985). No cultivars are reported as being immune or having high levels of resistance. A few reports on screening of wild sorghums show that *S.purpureosericeum* and *S.versicolor*, the members of section *parasorghum* possess very high levels of resistance to the shoot fly (Bapat and Mote, 1982; Mote, 1984; ICRISAT, 1995).

Mechanisms of Resistance

Non preference for oviposition is considered as a primary mechanism for shoot fly resistance in sorghum (Krishnananda *et al.*, 1970; Sharma *et al.*, 1977; Singh and Narayana, 1978; Singh and Jotwani, 1980a; Unnithan and Reddy, 1985). However, under no choice conditions, the resistant and susceptible varieties are equally damaged (Soto, 1974; Taneja and Leuschner, 1985a). Under glasshouse conditions, none of the varieties are highly resistant (Jotwani and Srivastava, 1970), and non-preference is substantially reduced with a high shoot fly density (Singh and Jotwani, 1980a). Antibiosis to shoot fly has been reported by Jotwani and Srivastava (1970), Blum (1972) and Soto (1974). Survival and development were adversely affected when shoot flies were reared

on resistant varieties (Narayana, 1975; Raina *et al.*, 1981) compared to susceptible genotypes (Singh and Narayana, 1978). Growth and development were retarded and the larval and pupal periods were extended by 8 - 15 days on resistant varieties (Singh and Jotwani, 1980b). After the shoot fly kills the main shoot, some sorghum cultivars can produce side tillers that can produce a reasonable yield if the plant is not subsequently attacked (Blum, 1972).

Spotted Stem Borer

Distribution, Description, Biology, Symptoms

Important genera/species of stem borers that have been reported as pests of sorghum, are *Busseola fusca*, the African maize stem borer which is also the most important stem borer on sorghum in Africa, south of the Sahara, *Sesamia cretica* the major pest of sorghum in the Mediterranean and Middle East which also attacks maize, and *Chilo partellus* the spotted stem borer which is the most important lepidopterous stem borer attacking sorghum, maize and millet in the Indian subcontinent and east Africa. Though it is found throughout India, it is a more serious pest in northern and central regions. Stem borers have not been reported from Australia (Harris, 1985).

The stem borer infects/infests the crop two weeks after seedling emergence until crop harvest, and affects all plant parts except the roots. Stem boring by the larvae in young plants (upto one month old) damages the growing point and results in deadheart formation. In India incidence of stem borer ranges from 10% to 75%, with severe infestations that can necessitate resowing of the crop (Pahman 1944, Pradhan and Prasad 1955). Overall losses are estimated to be of the order of 5-10% in many sorghum-areas, especially where early attack causes loss of stand (Harris, 1985). Avoidable grain losses with improved varieties (CSH 1 and Swarna) are estimated to be 55% to 83% (Jotwani *et al.*, 1971, Jotwani and Young, 1972). Trials conducted at Hisar have shown high yields in protected plots and very low yields in the non-protected ones (Singh, 1997).

Adults are nocturnal and live for 2-3 days, during which time each female moth lays about 200 - 500 scale like off-white eggs in overlapping batches of 10 - 80 near the midrib on the under-surface of the leaves. Eggs hatch in 4-5 days, larvae move to the leaf whorl and feed on tender leaves till the second instar. In the third or fourth instar, most of the larvae migrate to neighbouring plants by suspending themselves on silken threads and being easily blown by the wind (Srivastava, 1985). Those larvae that remain, move to the base of the plant and bore into the shoot. Damage to the growing tip results in the production of a typical deadheart. In mature plants, the larvae tunnel inside the stem. The larval development is completed in 2-4 weeks. Pupation takes place inside the stem and the adults emerge in 5 - 12 days (Harris, 1985). During the off-

season, the larvae undergo diapause in plant stalks and stubbles. With the onset of rains, the larvae pupate and the adults emerge in 7 days. In northern India, moth catches in light traps begin to increase during the last week of July and peak during August - September, while in southern India the peak in moth catches has been observed during January - February (Sharma and Nwanze, 1997).

Stem borer infestation is indicated by the appearance of small, elongated windows in young whorl leaves where young larvae have eaten the upper surface of the leaves. Later, the plants present a ragged appearance as the severity of damage increases. The 3rd 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, the leaves dry up as a result of the stem borer damage. Larvae continue to feed inside the stem. Throughout the crop growth, extensive tunnelling in the stem and peduncle leads to drying up of the panicle, to a partially chaffy panicle, or to peduncle breakage. Stem borer infestation starts about 20 days after seedling emergence, and deadhearts appear on the 30 - 40 day-old crop. All parts of the plant are affected except the roots (Sharma and Nwanze, 1997).

Pest Status and Host Range

Chilo partellus has been recorded on other host plants, including Sudan grass *(S.sudanense), Eleusine coracana, Sorghum halepense, Coix lachryma-jobi and Polytoca barbata* (Trehan and Butani, 1949) and *Cenchrus ciliaris, Echinocloa haplochada, Leptrous repens, Panicum maxicum, Pennisetum macrourum, P. purpureum, Sorghum arundinaceum, S.verticilliflorum, Sporobolus marginatus, Cyperus articulata, C. papyrus, Launaea cornuta* (Reddy, 1989).

Sources of Resistance

Resistance to stem borers has been studied in India (Jotwani *et al.*, 1979; Singh and Rana, 1984; Srivastava, 1985; Taneja and Leuschner, 1985b; Jalaluddin *et al.*, 1995, Patel *et al.*, 1996), Brazil (Lara *et al.*, 1979), Kenya (Alghali, 1985; Reddy, 1985), and in southern Africa (Leuslıner, 1989; van den Berg *et al.*, 1990). IS 1055 (BP 53), IS 1044, IS 2123, IS 2195, IS 2146, IS 5469 and IS 18551 show moderate levels of resistance to spotted stem borer. Genotypic resistance is poorly expressed under conditions of low fertility, drought and unfavourable weather. The stage of infestation is most critical to expression of resistance, as a progressive delay in infestation reduces the production of deadhearts (Taneja and Leuschner, 1985b). ICSV 705, SPV 135, CSV 8 R, SPV 104, SPV 238 and SPV 842 are improved genotypes with moderate levels of resistance to spotted stem borer and with a better yield potential than the original sources

of resistance. There are no reports of screening wild sorghums to the spotted stem borer.

Mechanisms of Resistance

Ovipositional non-preference by moths has been reported in several genotypes (Lal and Pant, 1980a; Dabrowski and Kidiavai, 1983; Taneja and Woodhead, 1989; Saxena, 1990; van den Berg and van der Westhuizen, 1997). The main mechanism of stem borer resistance in sorghum is antibiosis (Jotwani, 1978; Singh and Rana, 1984; Saxena, 1992; Singh and Marwaha, 1996; van den Berg and van der Westhuizen, 1997), with high mortality in the early larval stages (Jotwani, 1978; Jotwani *et al.*, 1978) and poor survival of larvae (Lal and Pant, 1980b; Saxena, 1990) in resistant genotypes. Pupal development is also affected adversely and fecundity is reduced (Lal and Sukhani, 1982; Singh and Verma, 1988; Taneja and Woodhead, 1989; Verma *et al.*, 1992). In some selections with severe leaf injury and stem tunnelling yields were not reduced very much by spotted stem borer infestation (Jotwani *et al.*, 1978). Similar results were reported by Dabrowski and Kidiavai (1983) and Alghali (1987) in Kenya.

Concluding Remarks

It is seen from the foregoing account that most of the investigations both for assessments of diversity and searches for host plant resistance have concentrated on the cultivated sorghums in the primary genepool. Wild and weedy species in the secondary and tertiary genepools have not been studied so well and may have much to offer sorghum breeders although they have been little used to date.

Materials and Methods

Materials and Methods

The present investigation was undertaken on *Sorghum* species representing five sections: *sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum* (Table 3). Eighty-five accessions belonging to 17 wild species and the appropriate checks from cultivated *S.bicolor* were selected from the *Sorghum* collection maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The first part of the study dealt with characterisation and analysis of variation using morphological traits and molecular markers. The second component involved evaluation of accessions for host plant responses to identify resistance to sorghum downy mildew, sorghum shoot fly and spotted stem borer. Experiments were conducted at ICRISAT situated at an altitude of 545m above mean sea level, 17°N latitude and 78°E longitude. Details of statistical techniques used are given separately under each section.

Morphological Characterisation

Eighty-five accessions of wild sorghums were morphologically characterised (Table 3). The plants were grown on deep black soil (vertisol) fields. Each accession was sown on a 4m long ridge with inter-row spacing of 60cm and inter-plant spacing of 10cm. The experiment was laid out in an augmented design (Peterson, 1994), with a cultivated variety, Maldandi (IS 1054), as control planted after every 10 accessions. Normal agronomic practices were followed to raise the crop. Observations on selected morphological characters were recorded during post-rainy (rabi) and rainy (kharif) seasons of 1999 and 2000 respectively according to agro-morphological and taxonomic descriptors (IBPGR and ICRISAT, 1993). Data on 9 quantitative and 22 qualitative traits were recorded on ten randomly selected plants from each accession (Tables 4, 5).

Statistical Analysis for Morphological Diversity

Quantitative traits were subjected to descriptive statistical analysis (mean, standard deviation, variance, range). They were also analysed using REML (Restricted Maximum Likelihood or Residual Maximum Likelihood) procedure assuming a fully random model. REML was preferred since it provided unbiased and efficient estimates of treatment effects in unbalanced designs with more than one source of error. It allows optimal combination of information over similar experiments conducted at different times or places. Assuming asymptotic normality, the ratio of the variance component

Table 3 Accessions of *Sorghum* used in the present study

	Species / Subspecies	Race	Acc. ID	Source	Code
Sorghum					
	S. bicolor (L.) Moench				
1	subsp. *bicolor* (cultivated check)		IS 1054	India	s(1054)
2	*S. bicolor* subsp. *verticilliflorum* (Steud.) Piper	*aethiopicum*	IS 27584	Cameroon	s(353)
3			IS 18819	Sudan	s(39)
4			IS 14564	Sudan	s(40)
5			IS 18821	Egypt	s(46)
6			IS 18822	Sudan	s(47)
7			IS 14485	Sudan	s(181)
8			IS 18820	Egypt	s(45)
9		*arundinaceum*	IS 18826	Ivory Coast	s(54)
10			IS 18830	Tanzania	s(58)
11			IS 14571	Kenya	s(155)
12			IS 18883	Ghana	s(171)
13			IS 14211	Angola	s(49)
14			IS 18824	Ivory coast	s(52)
15			IS 18878	Nigeria	s(158)
16			IS 14301	South Africa	s(151)
17			IS 14315	Swaziland	s(168)
18			IS 18882	USA	s(162)
19			IS 14218	South Africa	s(164)
20		*verticilliflorum*	IS 18865	Sudan	s(100)
21			IS 14278	South Africa	s(125)
22			IS 14717	Ethiopia	s(149)
23			IS 14717-1	Ethiopia	s(21)
24			IS 20995	Kenya	s(454)
25			IS 14219	Angola	s(105)
26			IS 18802	Chad	s(18)
27			IS 14357	Malawi	s(134)
28			IS 18797	USA	s(13)
29			IS 14493	Uganda	s(20)
30			IS 18858	Zimbabwe	s(93)
31			IS 18859	Zimbabwe	s(94)
32		*virgatum*	IS 18803	USA	s(23)
33			IS 18805	Egypt	s(25)
34			IS 18808	Egypt	s(28)
35			IS 18813	Egypt	s(33)
36			IS 18817	Sudan	s(37)
37	*S. propinquum* (Kunth) Hitchc.		IS 18933	Philippines	s(287)
38			IS 37358	Taiwan	s(344)
39			IS 37359	Philippines	s(345)
40	*S. halepense* (L.) Pers.	*halepense*	IS 18891	USA	s(182)
41			IS 33712	India	s(487)
42			IS 14299	South Africa	s(75)
43			IS 14212	Angola	s(77)
44			IS 18845	India	s(80)
45			IS 18847	India	s(82)

Contd.

Table 3

	Species / Subspecies	Race	Acc. ID	Source	Code
46			IS 18849	India	s(84)
47			IS 18844	USA	s(79)
48		*miliaceum*	IS 18899	USA	s(195)
49			IS 14263	Angola	s(196)
50		*controversum*	IS 18897	USA	s(189)
Chaetosorghum					
1	*S. macrospermum* Garber		TRC-241162	Australia	cs(24)
Heterosorghum					
1	*S. laxiflorum* Bailey		TRC-243486	Australia	hs(155)
2			TRC-243492	Australia	hs(157)
3			IS 18958	Australia	s(321)
Parasorghum					
1	*S. australiense* Garber and Snyder		IS 18954	Australia	ps(317)
2			IS 18955	Australia	ps(318)
3			IS 18956	Australia	ps(319)
4	*S. brevicallosum* Garber		TRC-243491	Australia	ps(2-1)
5			IS 18957	Australia	ps(320)
6			RN 401	Australia	ps(401)
7	*S.timorense* (Kunth) Buse		TRC-243437	Australia	ps(14-1)
8			TRC-243498	Australia	ps(26-1)
9	*S. purpureosericeum* Hochst. Ex A. Rich.		IS 18944	Sudan	ps(307)
10			IS 18945	Sudan	ps(308)
11			IS 18943	Tanzania	ps(306)
12			IS 18947	India	ps(310)
13			RN 285	India	ps(285)
14			IS 18951	India	ps(314)
15	*S. versicolor* Anderss.		IS 18926	South Africa	ps(279)
16			IS 23177	Tanzania	ps(295)
17			IS 14262	Angola	ps(299)
18			IS 14275	South Africa	ps(301)
19			IS 18940	South Africa	ps(303)
20			IS 18941	Tanzania	ps(304)
21	*S. nitidum* (Vahl) Pers.		TRC-243514	Australia	ps(22-1)
22	*S. matarankense* Garber and Snyder		TRC-243576	Australia	ps(25)
23			RN 341	Australia	ps(341)
Stiposorghum					
1	*S. angustum* S. T. Blake		TRC-243598	Australia	st(9)
2			TRC-243499	Australia	st(10)
3	*S. ecarinatum* Lazarides		TRC-243574	Australia	st(19)
4	*S. extans* Lazarides		TRC-243601	Australia	st(34)
5			TRC-243601-1	Australia	st(35)
6	*S. intrans* F. Muell. ex Benth.		TRC-243571	Australia	st(29)
7			TRC-243602	Australia	st(30)
8	*S. interjectum* Lazarides		TRC-243461	Australia	st(50)
9	*S. stipoideum* (Ewart & Jean White) C. Gardener & C.E. Hubb		TRC-243399	Australia	st(100)

Table 4 Quantitative Traits Recorded

S.No.	Quantitative Trait
1	Days to 50% flowering (from emergence to when 50% plants started flowering)
2	Number of basal tillers at maturity
3	Culm height of main stalk at 50% flowering (cm)
4	Leaf length of 5th leaf at 50% flowering (cm)
5	Leaf width of 5th leaf at 50% flowering (cm)
6	Peduncle exertion at harvest (cm)
7	Panicle length at harvest (cm)
8	Panicle width at harvest (cm)
9	Number of rachis nodes

Table 5 Qualitative Traits Recorded

S.No.	Qualitative Trait	
	Character	**Character State**
1	Longevity of Plants	Annual / Biennial / Perennial
2	Rhizomatus nature	Yes / No
3	Rooting at culm nodes	Yes / No
4	Culm branching	Branched / Unbranched
5	Culm hairiness	Glabrous / Sparse / Dense
6	Culm pigmentation at harvest	Green / Light pigmentation / Deep pigmentation
7	Waxy bloom	Absent / Slight / Medium bloomy / Completely bloomy
8	Nodal hairiness	Absent / Sparse / Dense
9	Nodal pigmentation	Green / Light pigmentation / Deep pigmentation
10	Leaf sheath clasping	Absent / Partial / Complete
11	Leaf shape	Linear / Linear-lanceolate / Ovate-lanceolate / Ovate / Elliptic / Obovate
12	Leaf colour	Light Yellow Green / Light Green / Dark Green / Pigmented
13	Midrib colour	White / Green / Yellow / Brown
14	Leaf blade hairiness	Glabrous / Sparse / Dense
15	Leaf sheath hairiness	Glabrous / Sparse / Dense / Hirsute / Pubescent
16	Ligule form	Unfringed membrane (variously hairy or ciliolate) / Fringed membrane / A fringe of hair / A rim of minute papillae
17	Panicle shape	Open / Contracted
18	Panicle branching	Simple / Compound
19	Pedicilate spikelet	Present / Absent / Reduced to glume
20	Callus (sessile spikelet)	Absent / Short / Conspicuous
21	Nature of callus joint	Linear / cupular
22	Awn length (cm)	Absent / short / medium / long

estimate to its standard error was compared to standard normal deviate, at 5 and 1 percent levels of significance to test the significance of the variance component estimates.

Phenotypic inter-relationships among accessions were assessed using Euclidean distance (Sneath and Sokal, 1973). The resulting phenotypic distance matrix was subjected to non-metric multi-dimensional scaling (MDS) to graphically visualise any evidence of clustering among the accessions in two-dimensional Euclidean space. The evid ence present for inter-relationships among accessions in the MDS plot was hierarchically represented in a dendrogram obtained by subjecting the distance matrix to sequential agglomerative hierarchical non-overlapping (SAHN) cluster analysis using the average-linkage (UPGMA) clustering algorithm. Co-phenetic correlation coefficients were estimated to assess the degree of agreement between the observed proximity matrices and their resultant dendrograms and MDS plots. Statistical analysis was done using the latest (2002) NTSYSpc version 2.11 (Rohlf, 1994).

Molecular Characterisation

Molecular diversity in 17 species of *Sorghum* was studied using (i) RFLPs with maize mitochondrial probes, and sorghum derived resistant gene candidates, (ii) AFLPs and (iii) SSRs. A stratified random sample of 22 accessions of the seventeen *Sorghum* species was selected to study intra-generic variation using three different marker systems (Table 6). Likewise, thirty accessions of *S.bicolor* subsp. *verticilliflorum* were analysed to assess intra-specific variation using AFLPs and SSRs. However, the data analysis was restricted to 21 accessions that uniformly amplified across all primer combinations with both AFLPs and SSRs (Table 7).

DNA Isolation

The CTAB procedure (Murray and Thompson, 1980) was adopted for isolation of DNA with a few modifications as detailed below. Seeds of wild accessions were grown in the green house in 15cm diameter pots with sterilized potting mixture (black soil: sand : FYM :: 2: 1: 1). About 5gm of tender leaves were harvested from 5 to 7 seedlings, frozen in liquid nitrogen and stored at -70°C till extraction. Seeds of Maldandi were germinated in dark at 20°C using the paper towel method (ISTA, 1985). About 5gm of etiolated seedlings were harvested after a week, frozen in liquid nitrogen and stored at -70°C till extraction.

The frozen sample was ground to fine powder with liquid nitrogen using a pre-cooled mortar and pestle. About 100mg of PVP (poly vinyl pyrrolidone) was added during the process to avoid phenol formation. The ground leaf powder without being allowed to thaw was transferred to 50ml polypropylene tube containing 15ml of warm CTAB extraction buffer, mixed by inversion and incubated for 90min at 65°C in a water bath. An equal volume (15ml) of chloroform-isoamyl alcohol (24:1) was added to the

Table 6 Accessions studied for Intra-Generic variation using RFLPs, AFLPs and SSRs

	Species / subspecies	Race	Acc. ID	Source Country	Lane No.	Code
Sorghum						
1	*S. bicolor bicolor*	*durra*	IS 1054	India	1	BIC
2	*S. bicolor* subsp. *verticilliflorum*	*aethiopicum*	IS 14564	Sudan	3	AET
3		*arundinaceum*	IS 18826	Ivory Coast	2	ARU
4		*verticilliflorum*	IS 18865	Sudan	4	VERT
5		*virgatum*	IS 18808	Egypt	5	VIR
6	*S. halepense*		IS 18844	USA	6	HAL
Chaetosorghum						
1	*S. macrospermum*		TRC-241162	Australia	8	MAC
Heterosorghum						
1	*S. laxiflorum*		IS 18958	Australia	7	LAX
Parasorghum						
1	*S. australiense*		IS 18956	Australia	11	AUS
2	*S. brecicallosum*		IS 18957	Australia	12	BRE
3	*S.timorense*		TRC-243575	Australia	13	TIM
4	*S. purpureosericeum*		IS 18944	Sudan	10	DIM
5			IS 18943	Tanzania	9	DEC
6	*S. versicolor*		IS 23177	Tanzania	15	VER
7	*S. nitidum*		TRC-243514	Australia	14	NIT
8	*S. matarankense*		TRC-243576	Australia	16	MAT
Stiposorghum						
1	*S. angustum*		TRC-243599	Australia	17	ANG
2	*S. ecarinatum*		TRC-243574	Australia	19	ECA
3	*S. extans*		TRC-243601	Australia	18	EXT
4	*S. intrans*		TRC-243571	Australia	19	INT
5	*S. interjectum*		TRC-243461	Australia	21	INTER
6	*S. stipoideum*		TRC-243399	Australia	22	STI

tubes containing sample and buffer. They were mixed by gentle inversion for 5min and centrifuged at 8000rpm for 10min at 20°C in RC–5 Sorval centrifuge. The top aqueous phase was transferred to fresh 50ml polypropylene tubes. Chloroform-isoamyl alcohol extraction was repeated, after which, an equal volume of chilled isopropanol was added to the clear supernatant, the solution was mixed by gentle inversion and left at room temperature for 1hr. The DNA was then spooled out with a bent Pasteur pipette, and suspended into 15ml falcon tubes containing 70% ethanol, washed twice with 5ml of 70% ethanol and air-dried. Four ml of $T_{50}E_{10}$ was added and DNA was allowed to dissolve. Eighty µl of RNase (10mg/ml) was then added and this was incubated over-night at room temperature followed by further incubation at 37°C for 1hr. An equal volume (4ml) of chloroform–phenol (1:1) was added, mixed by gentle inversion and

Table 7 Accessions studied for Intra-Specific variation using AFLPs and SSRs

	Race	Acc. ID	Source Country	Lane No. AFLP	Lane No. SSR	Code
S. bicolor* subsp. *verticilliflorum						
1	*aethiopicum*	IS 27584	Cameroon	1	4	aet1cam
2		IS 18821	Egypt	2	5	aet2egy
3		IS 14485	Sudan	3	6	aet3sud
4		IS 18822	Sudan	4	7	aet4sud
5	*arundinaceum*	IS18826	Ivory Coast	8	11	aru4civ
6		IS 18883	Ghana	11	14	aru7gha
7		IS 14211	Angola	5	8	aru1ang
8		IS 18824	Ivory coast	10	13	aru6civ
9		IS 14301	South Africa	6	9	aru2zaf
10		IS 14315	Swaziland	7	10	aru3swi
11		IS 18882	USA	12	16	aru8usa
12	*verticilliflorum*	IS 14278	South Africa	20	26	vert8zaf
13		IS 14219	Angola	18	23	vert6ang
14		IS 18802	Chad	17	22	vert5chad
15		IS 14717	Ethiopia	19	25	vert7eth
16		IS 14357	Malawi	14	19	vert2mwi
17		IS 14493	Uganda	13	18	vert1uga
18		IS 18859	Zimbabwe	15	20	vert3zim
19	*virgatum*	IS 18803	USA	21	28	vir1usa
20		IS 18805	Egypt	22	29	vir2egy
21		IS 18813	Sudan	23	30	vir3sud

centrifuged at 5000rpm for 10min. The clear supernatant was transferred to a fresh tube and the previous step was repeated. An equal volume (4ml) of chilled isopropanol and 200ml of sodium acetate was added to the supernatant, mixed gently by inversion and DNA was allowed to precipitate. The DNA was hooked into 1.5ml Eppendorfs containing 1ml ethanol (70%). The Eppendorfs were centrifuged at 10,000rpm for 5min at 4°C, the ethanol decanted and the DNA air-dried for 30min. Depending on the size of the pellet, 80–300µl of $T_{10}E_1$ was added and the tubes stored at 4°C until further use.

Estimation and Assessment of DNA Quantity and Quality

DNA concentration of all samples was estimated based on the spectrophotometer measurement of UV absorbance at 260nM. The DNA concentration in the sample was

calculated using the relationships of double stranded DNA i.e., 1.0 OD at 260nM = 50µg/ml. The ratio of OD_{260} to OD_{280} was calculated to check the purity. Pure DNA preparation shows an OD_{260} to OD_{280} ratio between 1.8 and 2.0 (Maniatis *et al.*, 1982). To test the quality of DNA, samples were subjected to agarose gel electrophoresis, using 0.8% agarose in TBE buffer and subsequently stained with ethidium bromide. The gel was photographed on an UV trans-illuminator and checked for RNA contamination (RNA usually runs ahead). DNA quality was assessed by comparison with different concentrations of undigested lambda DNA sample.

RFLP Analysis using Maize Mitochondrial DNA Probes

Maize clones containing known mitochondrial (mt) DNA genes were obtained for use as probes in Southern blot hybridizations. The $F_1 F_0$ atpase sub-unit 6 (*atp* 6; Dewey *et al.*, 1985), as purified plasmid DNA with the corresponding inserts, was supplied by Dr C. S. Levings III, Department of Genetics, North Carolina State University, Raleigh, NC, USA. Clones of cytochrome oxidase sub unit 1 (*cox* I; Isaac *et al.*, 1985), sub-unit II (*cox* II; Fox and Leaver, 1981), and *atp* α (Isaac *et al.*, 1985) were gifted by Dr C. J. Leaver, Department of Plant Science, University of Oxford, Oxford, U.K. Accessions used in the study are listed in Table 6.

The RFLP technique involved five steps: (1) Restriction endonuclease digestion of the DNA, (2) Separation of DNA fragments by gel electrophoresis, (3) Transferring DNA fragments to a nylon membrane, (4) Hybridization of specific DNA fragments using a radioactively labeled probe, and (5) Auto radiography analysis of results. Details of these are presented below.

Restriction Enzyme Digestion

Genomic DNA (about 15µg) of each accession was separately digested with 3µl of buffer and 60 units each of restriction enzymes, *Bam H* I, *Hind* III and *Xba* I, in a 30µl reaction. The mixture was incubated overnight at 37°C. The reaction was terminated by the addition of 3µl of loading buffer (25% sucrose, 0.5% bromophenol blue and 20mM EDTA). Restriction was confirmed by running the samples on a 0.8% agarose gel in TAE buffer and viewing on a UV trans-illuminator after staining with ethidium bromide. Restricted DNA was seen as a smear.

Gel Electrophoresis

Restricted DNA fragments were separated by electrophoresis for about 16 hrs using 0.8% agarose horizontal slab gels (5 mm thick) in TAE buffer. Gels were prepared

in the same buffer, which was used for electrophoresis. Lambda DNA fragments generated by *Hind* III digestion were used as molecular size markers. The gels were stained in ethidium bromide (0.5µg/ml) for 15min, de-stained for 10min in deionised water and viewed on a UV trans-illuminator.

Southern Blot Hybridisation

DNA fragments from the agarose gels were transferred onto nucleic acid nylon transfer membranes (Hybond N+ Amersham, UK) using the Vacugene blotting apparatus (L B Vacugene XL, Pharmacia). The process of transfer involved destaining of the gel for 30min; followed by depurination, denaturation and neutralization for 20min each. Subsequently, the transfer was allowed to proceed for 1½hrs at 45mbar pressure with 20x SSC. The blots were rinsed in 3x SSC, air-dried and cross-linked using Stratgene UV cross-linker (Stratagene, Germany).

Hybridisation using Labelled Probes

Random primed method of Feinberg and Vogelstein (1983) was used for radio labelling of DNA. Purified insert DNA was denatured by heating at 95°C for 10min followed by immediate cooling on ice for 5 min and labelled with α-^{32}P-deoxyadenosine 5' triphosphate (dATP) using the New England Labs' labelling kit. The probe was labelled in a 50µl reaction mixture containing about 25-50ngm of denatured probe DNA, 1x labelling buffer, 2ml equimolar concentrations of dCTP, dGTP, and dTTP and 1.5 units of Klenow enzyme. The reaction mixture was incubated at 37°C for 1hr. The reaction was terminated by adding 400µl of 200mM EDTA. The labelled probe was again denatured by heating at 95°C for 10min and subsequent snap cooling on ice for 10min. Lambda *Hind* III marker was also labelled similarly and added to the reaction mixture prior to hybridization.

Southern blots were pre-hybridised overnight at 65°C with 25ml of pre-hybridisation solution (7% SDS, 1% BSA, 0.5M Na_2HPO_4 and 20µg/ml sheared and denatured salmon sperm DNA) per two blots (20x15cm) in standard bottles (30x3.5cm). While placing the blots in the bottle, care was taken to remove all air bubbles trapped between the blots and the sides of the bottle. Hybridisation was carried out by adding labelled probe to the pre-hybridisation solution and incubating for 16hrs at 65°C in a hybridisation oven (Hybaid, UK). Following hybridisation, the blots were washed twice in 2x SSC containing 0.5% SDS followed by once in 0.2x SSC with 0.5% SDS. All three washes were at 65°C for 20min each. Blots were dried between sheets of tissue, enclosed in Saran Wrap, and exposed to X-AR film (Kodak, USA) with intensifying

screens at -70°C for varying time periods. The X-ray films were developed with Kodak developer for 2min, washed in a stop bath (3% acetic acid solution) for 1min, fixed with Kodak fixer for 5min, washed in running water and then air dried. The fragment sizes were determined using the lambda *Hind* III standard marker.

RFLP Analysis using Sorghum Resistance Gene Candidates

The Sorghum resistance gene candidate (RGC) probes used were; S8-1, S27-2, S2-2 and S30-5, provided by Dr. Sivaramakrishnan, ICRISAT. The same procedure as outlined for the mitochondrial probes was used for the RGCs except that the blots were washed twice for 10min in a solution containing 2x SSC with 0.5% SDS. Accessions used in the study are listed in Table 6.

AFLP Analysis

Four AFLP primer combinations *viz.*, E ACA - M CTC, E ACT - M CTG, E ACT - M CAT, and E ACT - M CTA were used for the analysis. The wild sorghum accessions studied at the intra-generic and intra-specific levels are listed in Tables 6 and 7.

The AFLP technique involved three major steps: (1) Restriction endonuclease digestion of the DNA and ligation of adapters, (2) PCR amplification of the restriction fragments and selective AFLP amplification, and (3) Gel analysis of the amplified fragments.

Restriction Digestion of Genomic DNA and Ligation of Adapters

About 250ngm of genomic DNA was digested with 1.25 units each of enzymes *Eco* R I and *Mse* I, 2µl of 5x reaction buffer and made to a final volume of 10µl with distilled water in a 1.5ml micro-centrifuge tube. The contents were mixed gently by centrifugation and incubated at 37°C for 2 hours. The mixture was further incubated at 70°C for 15 minutes to inactivate restriction endonucleases. The tube was then placed on ice and the contents were collected after brief centrifugation. To 5µl of the digested DNA, 4µl of adapter ligation solution and 1µl of T4 DNA ligase were added, mixed gently by brief centrifugation and incubated at 20°C for 2 hours.

PCR Amplification of restricted DNA fragments and selective AFLP Amplification

The ligated sample was diluted 10 folds. To 2µl of the diluted ligated DNA sample which was used as the template in a PCR reaction, 8µl of pre-amplification primer mix, 1µl of 10x PCR buffer, 1 unit of Taq DNA polymerase (Amersham, Pharmacia, UK.)

were added along with distilled water to make up the volume to 10µl. The contents were mixed gently and the samples were pre-amplified in a Perkin-Elmer 9600 Thermocycler with the following conditions: 20 cycles were performed at 94°C for 30sec followed by 20 cycles at 56°C for 60sec and finally for 20 cycles at 72°C for 60sec. The preamplified samples were diluted 50 times with TE buffer.

10µl of 5x kinase buffer, 20µl of [α-^{32}P] dATP, and 2µl of T4 kinase were added to 18µl of the selected *EcoR* I primer (E ACA or E ACT), mixed gently by brief centrifugation and incubated at 37°C for 1hr. The enzyme was heat inactivated at 70°C for 10min.

For each primer pair, the amplification was performed by adding 2.5µl of preamplified and diluted DNA, 0.25µl of labelled primer, 2.25µl of *Mse* I primer containing dNTPs, 4µl of sterile distilled water, 1µl of 10x PCR buffer and 1 unit of Taq DNA polymerase. The conditions for PCR were as follows: One cycle was performed at 94°C for 30sec, 65°C for 30sec, and 72°C for 60sec; during the next 12 cycles, the annealing temperature was progressively lowered by 0.7°C; and 23 cycles were performed at 94°C for 30sec, 56°C for 30sec, and 72°C for 60sec.

Gel Electrophoresis

After PCR, an equal volume (10µl) of formamide dye (98% formamide, 10mM EDTA, 0.1% bromophenol and 0.1% Xylene cyanolene cyanol) was added to each reaction. The samples were heated for 3min at 95°C and placed on ice immediately. The fragments were separated using a Model S2 sequencing unit (GIBCO BRL). Six per cent polyacrylamide was poured (20:1:: acrylamide: bis; 7.5M urea; 1x TBE buffer) with 0.4mm spacers and sharks-tooth combs. The gel was pre-electrophoresed at 1500V for 20min. 3µl of the sample was loaded on the gel and electrophoresed at 1500V until xylene cyanol reached two- thirds down the length of the gel. The gel was dried using a Bio-Rad gel drier. The dried gel was exposed to X-ray film at room temperature overnight and developed.

SSR Analysis

Ten SSR primer sets (Table 8) were used for genotyping the same set of 22 and 21 accessions as listed in Tables 6 and 7.

The analysis of simple sequence repeats (SSRs), also known as sequence tagged microsatellites (STMS), involved two steps: (1) PCR amplification of genomic segments flanked by repeats and site specific annealing and (2) Gel electrophoresis.

Table 8 SSR Primer Sets in Genotyping of *Sorghum* Germplasm

S.No.	SSR ID	Composition of repeats	Fragment size (bp)	Linkage group
1	Sb 1-1	$(AG)_{16}$	260-300	h
2	Sb 1-10	$(AG)_{27}$	350-400	d
3	Sb 4-15	$(AG)_{16}$	120-130	e
4	Sb 4-22	$(ACGAC)_4/(AG)_6$	270-300	Not mapped
5	Sb 4-121	$(AC)_{14}$	200-225	d
6	Sb 4-32	$(AG)_{15}$	160-180	e
7	Sb 5-236	$(AG)_{20}$	165-185	g
8	Sb 6-84	$(AG)_{14}$	170-190	f
9	Sb 6-57	$(AG)_{18}$	285-305	c
10	Sb 6-36	$(AG)_{19}$	155-190	c

PCR Amplification and Site Specific Annealing

Each 25µl reaction contained 25ngm of genomic DNA, 1x PCR buffer (50mM KCl, 20mM Tris-HCl (pH 8.4)), 10pmol of each primer, 2mM $MgCl_2$, 200µM each of dCTP, dGTP, dTTP, 50µM of dATP and 1µCi of [α-^{32}P] dATP and 1 unit of Taq DNA polymerase. PCR reactions were carried out in a PTC-100 Thermocycler (MJ Research Inc, USA) using a 65°C to 55°C Touchdown PCR cycle. Denaturation was carried out at 94°C for 30sec and extension was carried out at 72°C for 1min. Annealing was carried out between the denaturation and extension steps using a touch down program: the first cycle at 65°C for 30sec followed by 63°C for 3 cycles, 61°C for 3 cycles, 59°C for 5 cycles, 57°C for 5 cycles and 55°C for 14 cycles. In all, 31 cycles were carried out.

Electrophoresis and Band Scoring

PCR products were electrophoresed on a denaturing polyacrylamide gel (6% acrylamide, 7.5M urea, 1x TBE) at 1500V for two hours. The gel was transferred to Whatman 3 filter paper, covered with Saran Wrap and dried under vacuum for 1hr at 80°C. Autoradiograms were obtained by exposing the gel for varying periods in a cassette with intensifying screen using Kodak X-OMAT film. Fragment sizes were determined using end labelled AFLP marker (30-330bp; Life Technologies, USA). The autoradiogram was manually scored for the presence or absence of a band for each locus for all the accessions.

Statistical Analysis for Molecular Diversity

The molecular data was analysed in two ways. First, using accessions as a unit of analysis to study inter-relationships; and second, using a suitably defined population as a unit of analysis to assess gene diversity and inter-population genetic differentiation.

The first type of analysis was based on binary allelic data and the second was based on allelic frequency data.

Inter-relationships among Accessions

For co-dominant markers (RFLPs and SSRs), similarity coefficient of Nei and Li (1979) was computed as: $S_{ij} = 2M_{xy} / (M_x + M_y)$ where M_{xy} is the number of shared fragments and M_x and M_y are the number of fragments in accessions *x* and *y* respectively. For dominant markers (AFLPs) Jaccard's similarity coefficient (S_{ij}) was computed as: $S_{ij} = M_{xy} / (M_x + M_y - M_{xy})$.

The distance matrix was subjected to non-metric MDS and SAHN cluster analysis using the UPGMA clustering algorithm to graphically visualise the genetic inter-relationships among accessions in two-dimensional Euclidean space.

Gene Diversity

Gene diversity (H_j), alternatively termed as polymorphic information content and expected heterozygosity, was estimated for each individual locus $j = 1,\ldots,n_l$, following Nei (1987) : $H_j = [N/(N-1)](1-\Sigma_i p_{ij}^2)$ $i = 1,\ldots,a_j$ where p_{ij} is the frequency of the i^{th} allele at locus j, N is the sample size, and a_j is the number of alleles at locus j. The average gene diversity (H_j) was estimated as $H = \Sigma_j H_j / n_l$ where n_l is the number of loci.

Population Differentiation

Genetic differentiation among sections, races and geographic regions with respect to allele frequencies was estimated using Wright's F-statistics (Weir and Cockerham, 1984). Fisher's exact tests for population differentiation was also performed to determine if significant differences in allele frequencies existed among sections, races and regions. The Markov Chain Monte Carlo (MCMC) simulation approach was employed to estimate the exact probability of observed differences in allele frequencies. Population distances were computed using Rogers' modified distance (Rogers, 1972). In the dendrograms for Sections, Races and Regions presented with the Results, two numbers are mentioned alongside each node. The number within brackets indicates the percent loci supporting the node and the other number represents the bootstrap P value.

Computations were done using GENSTATpc version 6 (Payne, 2002), the latest (2002) NTSYSpc version 2.1.1 (Rohlf, 1994) and TFPGA version 1.3 (Miller, 1997).

Evaluation for Host Plant Resistance

Accessions of wild sorghums belonging to 17 species were screened for their resistance reaction to the sorghum downy mildew (*Peronosclerospora sorghi)*, sorghum shoot fly (*Atherigona soccata*) and spotted stem borer (*Chilo partellus)*. The experiments on downy mildew were conducted in greenhouse. The screening for both the insects was carried out in field and greenhouse. Appropriate resistant and susceptible checks along with selected improved varieties/hybrids were included in each experiment for comparison.

Screening for Resistance to Sorghum Downy Mildew

Eighty-five accessions of wild sorghums (Table 3) were screened for resistance to SDM. In addition, five cultivated accessions of sorghum, *viz.*, IS 14383 (*guinea-Zimbabwe*), IS 18773 (*kafir-USA*), IS 21812 (*caudatum-Sudan*), IS 12868 (*durra-India*) and IS 611 (*bicolor-USA*) selected at random from the sorghum world collection, to represent the five basic races, and a commercial hybrid, CSH-1, were also screened. Cultivars, DMS 652 (IS 18433) and QL 3 (IS 18757) were included as susceptible and resistant checks respectively. The experiments were conducted in the greenhouse.

The pathogen culture maintained at ICRISAT was used in the screening. The pathogen was multiplied on SDM susceptible cultivar, DMS 652, grown in pots and maintained under glasshouse conditions at 24 ± 2°C and 65 ± 5% relative humidity. Seeds of each accession were pre-soaked for 3hrs and incubated at 25°C in darkness. Wild accessions took two to three days for germination whereas, cultivated types germinated in 24hrs. About 25 sprouted seeds of similar shoot and root lengths from each accession were transplanted in 15cm diameter pots filled with a mixture of black soil and farmyard manure in a ratio of 1:1. All accessions were replicated twice whereas the checks were replicated five times. Leaf pieces from systemically infected sorghum plants were incubated in the dark for 6hrs to 7hrs at 18°C to 20°C and >90% RH for conidial development. Conidia were harvested from the leaves by gently brushing them into cold distilled water (to prevent conidial germination).

Seedlings at the coleoptile-stage to one-leaf-stage were inoculated with conidia (a suspension with $4x10^5$ conidia per ml) as described by Reddy *et al.* (1992). After inoculation, the pots were incubated overnight at 20°C and 95% RH, and transferred to the greenhouse for disease development. Symptoms of systemic infection, with clear chlorosis beginning at the base of the infected leaves, started to appear 8 to12 days after inoculation, and were clearly visible in about 14 days. Counts of total plants and infected plants were recorded at 14 and 21 days after inoculation and the percentage of

diseased plants (disease incidence) was calculated. Accessions that remained disease free were re-inoculated to ensure that these were not escapes. The experiment was conducted in a completely randomised design (CRD). The accessions of the test entries differed in the two years.

Statistical Analysis for Downy Mildew Resistance

Percentage infection data were analysed using REML (Restricted Maximum Likelihood or Residual Maximum Likelihood) procedure assuming a fully random model. The percentage data were also analysed after angular transformation. Since the conclusions with and without transformation remained similar, the results obtained from analysis of original percentage data only are reported. Assuming asymptotic normality, the ratio of the variance component estimate to its standard error was compared to standard normal deviate, at 5% and 1% levels of significance to test the significance of the variance component estimates.

Evaluation for Resistance to Sorghum Shoot Fly

Field screening

Fifty-five accessions of wild sorghums belonging to 17 species along with four cultivated checks were screened for resistance to sorghum shoot fly (Table 9).

Field screening for resistance to sorghum shoot fly, was conducted during 1998 and 1999 rainy seasons. The experiments were laid out in a randomised complete block design (RCBD) with three replications. Normal agronomic practices were followed for raising the crop. Accessions of wild sorghums along with resistant and susceptible checks were planted in 2m long rows with an inter-row spacing of 75cm. The plants were thinned to 20 plants per row, 15 days after seedling emergence.

To ensure high and uniform shoot fly pressure, the interlard fish meal technique of Taneja and Leuschner (1985a), was followed as described below. Four rows of interlards of a susceptible cultivar (CSH 1) were sown after every 24 rows of the test material, 20 days before their sowing. One week after seedling emergence, moistened fishmeal was kept in plastic bags among the interlards to attract the sorghum shoot fly females. One generation of the shoot fly was thus completed on the interlards, and the emerging flies infested the test material. Fishmeal was also spread in plastic bags in the test material, one week after seedling emergence.

Table 9 Accessions Evaluated for Resistance to Sorghum Shoot Fly/Spotted Stem Borer

	Species / subspecies	Race	Acc. ID	Source	Shoot Fly		Stem Borer		
					NC	AB	LC	NC	AB
Sorghum									
1	*S. bicolor* subsp. *verticilliflorum*	*aethiopicum*	IS 27584	Cameroon					
2			IS 18819	Sudan					
3			IS 14564	Sudan		√	√		
4		*arundinaceum*	IS 18826	Ivory Coast		√	√		
5			IS 18830	Tanzania					
6			IS 18883	Ghana					
7			IS 18882	USA					
8		*verticilliflorum*	IS 18865	Sudan		√	√		
9			IS 14278	S. Africa					
10			IS 14717	Ethiopia					
11			IS 20995	Kenya					
12		*virgatum*	IS 18803	USA					
13			IS 18805	Egypt					
14			IS 18808	Egypt		√	√		√
15			IS 18813	Egypt					
16			IS 18817	Sudan					
17	*S. halepense*	*halepense*	IS 18891	USA					
18			IS 33712	India					
19			IS 14299	S. Africa					
20			IS 14212	Angola		√	√		√
21			IS 18845	India					
Chaetosorghum									
1	*S. macrospermum*		TRC-241162	Australia	√				
Heterosorghum									
1	*S. laxiflorum*		TRC-243486	Australia					
2			TRC-243492	Australia	√	√	√		√
3			IS 18958	Australia	√	√	√		√
Parasorghum									
1	*S. australiense*		IS 18954	Australia					
2			IS 18955	Australia	√	√	√		√
3			IS 18956	Australia	√	√	√	√	√
4	*S. brevicallosum*		TRC-243491	Australia					
5			IS 18957	Australia			√		
6	*S.timorense*		TRC-243437	Australia	√	√	√	√	√
7			TRC-243498	Australia					

Contd.

Table 9

	Species / subspecies	Race	Acc. ID	Source	Shoot Fly		Stem Borer		
					NC	AB	LC	NC	AB
8	*S. purpureosericeum*		IS 18944	Sudan	√	√	√	√	√
9			IS 19845	Sudan	√	√	√	√	√
10			RN 285	India	√	√	√	√	√
11			IS 18943	Tanzania	√	√	√	√	√
12			IS 18947	India	√	√	√	√	√
13			IS 18951	India					
14	*S. versicolor*		IS 18926	S. Africa					
15			IS 23177	Tanzania	√	√	√	√	√
16			IS 14262	Angola	√	√	√	√	√
17			IS 14275	S. Africa	√	√	√	√	√
18			IS 18940	S. Africa					
19			IS 18941	Tanzania					
20	*S. nitidum*		TRC-243514	Australia	√	√	√		√
21	*S. matarankense*		TRC-243576	Australia	√	√	√		√
22			RN 341	Australia					
Stiposorghum									
1	*S. angustum*		TRC-243598	Australia					
2			TRC-243599	Australia	√	√	√		√
3	*S. ecarinatum*		TRC-243574	Australia	√	√	√	√	√
4	*S. extans*		TRC-243601	Australia	√	√	√	√	√
5	*S. intrans*		TRC-243571	Australia	√	√	√	√	√
6			TRC-243602	Australia					
7	*S. interjectum*		TRC-243561	Australia	√	√	√	√	√
8	*S. stipoideum*		TRC-243399	Australia	√	√	√		√
Sorghum									
	S. bicolor bicolor								
1			CSH 1* (S)						
2			IS 1054* (MR)						
3			IS 18551* (R)						
4			IS 2146* (R)						
5			ICSV 1** (S)						
6			IS 2205** (R)						
7			ICSV 700*** (IR)						
8			ICSV 708*** (I)						
9			ICSV 743*** (I)						

Notes

* Checks for Shoot Fly screening ** Checks for Stem Borer screening

*** Improved varieties for stem borer screening

(S) Susceptible; (R) Resistant; (MR) Moderately resistant; (IR) Improved resistant cultivar;

(I) Improved cultivar; NC = No choice; AB = Antibiosis; LC = Limited choice;

√ = Accessions selected to study mechanisms of resistance

Observations on shoot fly damage (14 and 21 days after seedling emergence) were recorded in terms of number of plants with eggs, number of eggs/plant, and number of plants with deadhearts.

Evaluation for mechanisms of resistance

Selected accessions were used to study antixenosis and antibiosis components of resistance, (Table 9). Cultivars, IS 18551 and CSH 1 were used as resistant and susceptible checks, respectively. These studies were carried out under greenhouse conditions (temperature 23 ± 2°C, relative humidity 85 ± 5%).

Non preference for oviposition (Antixenosis)

Non-preference for oviposition was studied, on 22 accessions (Table 9), under no choice conditions in the greenhouse using the top-cage technique (Sharma *et al.*, 1992) as described below. The system consisted of two plastic trays (40x30x14cm^3), one for sowing test material and the second fitted with fine wire mesh, which was clamped upside down over the first tray, thus forming a cage. Seeds were sown in the bottom tray in a soil mixture of black soil, red soil and FYM in the ratio of 1:1:0.5. Di-ammonium phosphate (DAP) was applied before sowing @ 20gm per tray. Only one genotype was sown in each tray at an inter-plant spacing of 5cm. The experiment was laid out in CRD. The seedlings, 10 days after emergence, were thinned to 50 per tray. Urea was applied, 10 days after seedling emergence @ 5gm per tray. Seedlings at 4 to 5 leaf stage (15-18 day-old for wild sorghums and 10 day-old for the cultivated types) were infested with shoot fly females (25 flies/cage). Shoot flies, caught in the field in fish-meal-baited traps, were collected in early mornings and/or evenings. *Atherigona soccata* flies were separated from other species, and introduced into the closed cage. Shoot flies were confined with sorghum seedlings for 1 to 2 days. Flies were provided with glucose water in a cotton swab. Observations were recorded for number of plants with eggs, number of eggs/plant and number of deadhearts, one week after the infestation.

Antibiosis

Seedlings of different accessions were raised in plastic trays as described above. 15 to 18 days after seedling emergence each plant was infested artificially by gently placing two shoot fly eggs into the central leaf whorl. The eggs were obtained by allowing gravid shoot fly females to oviposit on susceptible cultivar, CSH-1, placed in a wooden cage fitted with a wire mesh. After oviposition, the eggs were gently dislodged from the leaves with a camel hair brush for infestation of test entries.

Observations on number of plants with deadhearts were recorded, four days after eggs release. The deadhearts were removed from the trays and transferred into plastic jars with moist sand. Subsequently, adult emergence was recorded. Wherever deadhearts were not found, accessions were raised in trays and infested again with shoot fly eggs. Three days after deadhearts were noticed 10 plant per accession were dissected open to check for larval survival.

Evaluation for Resistance to Spotted Stem Borer

Field screening

Fifty-five accessions of wild sorghums belonging to 17 species along with three improved cultivated varieties and two checks were screened for resistance to spotted stem borer (Table 9).

Field screening for resistance to spotted stem borer was conducted in 1998 rainy and 1999 post-rainy seasons. The experiments were laid out in an RCBD with three replications. Normal agronomic practices were followed for raising the crop. Accessions were planted in 2 row plots, 2m long, with an inter-row distance of 75cm. Plants were thinned to 20 per row, 15 days after seedling emergence.

For field infestation, a modified version of the Bazooka applicator was used (Sharma, 1997). About 500 black-head-stage egg masses, along with 85gm of poppy seeds (*Papaver* sp.) were kept overnight in a plastic jar with a tightly fitted lid. In the morning, the 1st-instar larvae were gently mixed with the carrier (poppy seeds) and transferred into the plastic bottle of the Bazooka applicator.

Field infestation

The seedlings were infested 18 to 20 days after emergence. The nozzle of the Bazooka applicator was placed close to the leaf whorl and each plant was infested with 5 to 7 larvae. Generally, 5 to 7 larvae per plant are sufficient to cause >50% leaf feeding and >90% deadhearts in a susceptible genotype. The crop was infested in the morning between 08.00 to 11.00hrs to avoid larval mortality due to high temperature. The Bazooka applicator was rotated after every 10 strokes to ensure uniformity in larval distribution. The whorl was gently tapped before infestation to avoid drowning of the larvae in water retained in the leaf whorl. A selective insecticide (cypermethrin) was used to control shoot fly interference without causing any residual effect on stem borer establishment.

One week after artificial infestation, stem borer damage was recorded as percentage of total number of plants showing leaf-feeding symptoms, and leaf-feeding (on a 1-9 scale, with 1 = low and 9 = high). Plants showing deadhearts were recorded 21 days after artificial infestation (Sharma *et al.*, 1992).

Mechanisms of resistance

Selected accessions were used to study antixenosis and antibiosis components of resistance under greenhouse conditions (Table 9). Cultivars, IS 2205 and ICSV-1, were used as resistant and susceptible checks, respectively.

Non-preference for oviposition

Non-preference for oviposition was studied using both the limited-choice test and the no-choice test. Under limited-choice test, the moths were given a choice between 10 to 12 accessions (including the two checks ICSV-1 and IS 2205) for oviposition. For these studies, the test entries, raised in pots, were placed in a wooden cage (80 x 70 x 60 cm^3). The wooden framed cages were covered with a wire mesh screen on three sides, and with a glass door in the front. The front door had a 20cm diameter cloth bag attachment for introducing the moths. The base of the cage was made of wood, while the top was covered with a glass pane. The test accessions were grown in pots in the greenhouse as described above. Plants were thinned, 10 days after seedling emergence, to five in each pot. Pots with 18 day-old plants were placed inside the cage at random along with two checks. Fifty to sixty pairs of newly emerged adult moths were released into each cage. *Chilo partellus* moths were cultured on artificial diet in the insect rearing laboratory following the procedure of Taneja and Leuschner (1985b). Moths were provided with water in a cotton swab. After releasing the moths into the cage, they were allowed to oviposit on the plants for three nights. To avoid predation by ants, Tanglefoot glue was smeared on all the four legs of the cages. The experiment was carried out in three sets of 10, 11 and 12 accessions to accommodate the 27 test entries along with one each of resistant and susceptible checks. Accessions in each experiment were replicated thrice, changing the position of the pots every day to avoid any possible position effect.

Antixenosis, under no-choice conditions was studied by keeping each of the 14 test accessions (Table 9) along with the resistant and susceptible checks, IS 2205 and ICSV-1, respectively individually inside an oviposition cage. In this test, the moths had only one accession for oviposition. Accessions were grown in pots in the greenhouse as described earlier with five plants per pot. The oviposition cages were arranged on a table in the greenhouse in a completely randomised design. Ten pairs of newly emerged

adult moths were released inside each oviposition cage. Moths were provided with water in a cotton swab. Moths were allowed to oviposit on the test entries as well as checks for three nights. To avoid predation by ants, Tanglefoot glue was applied to all four legs of the wooden table.

In both the limited- and no-choice experiments, observations were recorded on the number of egg masses on each plant and the position of the egg masses (on adaxial or abaxial surface). The number of eggs in each egg mass was counted under 40x simple microscope.

Antibiosis

The effect of different wild sorghum accessions on establishment and development of *Chilo partellus* was studied under greenhouse conditions. The experiment was laid out in a completely randomised design with 23 accessions (Table 9), each replicated thrice. The plants were raised in trays in the greenhouse at 23 ± 5°C and 65 ± 5% RH. Ten days after seedling emergence, 20 plants were retained in each tray. Urea @ 10gm per tray was applied after thinning. The plants were infested artificially with 10 first-instar larvae per plant using a camel hairbrush, 20 days after seedling emergence. Observations were recorded on deadhearts, larvae survival and adult emergence.

Statistical Analysis for Shoot Fly and Spotted Stem Borer Resistance

Count and binomial percentage data, as necessary to meet the ANOVA assumptions, were transformed to log (x+1) and angular scales, respectively, and subjected to analysis of variance (ANOVA). The results from transformed and untransformed scales were similar. Therefore, results from untransformed scales only are presented. Significance of treatment differences was tested using least significant difference (LSD) at 5% level of significance.

Results

RESULTS

Morphological Characterisation

The genus *Sorghum* is traditionally classified into five sections, *sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum*. Eighty-five accessions of *Sorghum* representing 18 species selected from the 461 collections maintained at the ICRISAT gene bank were characterised based on morphological descriptors to confirm their taxonomic identity and study the variation. Details of passport data and traits recorded are given in Tables 3 to 5. An overview of the wild sorghums in the experimental field is presented in Fig. 3.

Qualitative Traits

Distinguishing characteristics of the five sections of *Sorghum* are given in Table 10. Main diagnostic traits include nodal hairiness and inflorescence characters like shape of callus at the base of the sessile spikelet and its corresponding mode of articulation at the apex of the peduncle or apex of the rachis internodes, nature of pedicelled spikelet, panicle branching, raceme joints, awn length and glume size (Fig. 4.)

Nodal Indumentum

The presence or absence of nodal hair is one of the key taxonomic features in identification of the five sections. Members of sections *sorghum* and *chaetosorghum* were characterised by glabrous to finely pubescent culm nodes, whereas *heterosorghums* were characterised by sparsely pubescent or bearded nodes. *Parasorghum* and *stiposorghum* differed from the other three sections in having bearded rather than glabrous or sparsely pubescent culm nodes. Two types of indumentum were observed on the nodes of the culm: (a) a band of short, appressed pubescence covering the node itself, and (b) a ring of spreading rather stiff hair immediately below the pubescent band, the length of which was variable. While bearded nodes are usually a diagnostic trait for the *para-* and *stiposorghums*, sometimes the bearded ring was absent in the specimens examined, especially from the lower nodes of *S.australiense*, *S.matarankense* and *S.brevicallosum*. The nodes were entirely glabrous in *S.extans* of *stiposorghum*. *S.brevicallosum* of *parasorghum* had pubescent and not bearded culm nodes. Differences in nodal hairiness were also observed between accessions of the same species; two accessions of *S.australiense* (*parasorghum*), had stiff spreading nodal hair while

the third, had only appressed pubescence. The other species of *para-* and *stiposorghums* had bearded nodes with the hair of varying lengths (2 mm to 10mm).

Inflorescence

Members of section *sorghum* comprising three species were characterised by a compound/divided panicle with racemes that basically possess 1 to 5 nodes. Awns were absent in the cultivated types while the wild races and weedy types had characteristically small awns (<2.5cm). *Chaetosorghum* represented by *S.macrospermum* had simple panicles with 9-12 pairs of spikelets per raceme with awns of 3.8 cm. *Heterosorghum* represented by *S.laxiflorum,* and members of *stiposorghum* had 1 to 3 jointed racemes. Members of *parasorghum* had 3 to 6 pairs of spikelets in the raceme. With the exception of section *sorghum,* members of the other four sections had well developed awns that were variable in length. Awns of *S.laxiflorum* (*heterosorghum*) averaged 4.2cm, among *parasorghums* the awns varied in length from 1.5cm in *S.nitidum* to 5.8cm in *S.australiense*, and among the *stiposorghums* awn lengths varied from 4.2cm in *S.intrans* to 8.8cm in *S.angustum*. *Parasorghums* and *stiposorghums* differed from one another primarily in the nature of the callus and the articulation joint. *Stiposorghums* were characterised by an elongated, pungent, curved callus, which was reflected in a linear acutely oblique articulation joint. In the other sections, the callus was minute, obtuse and straight, and the joint was cupular and horizontal. Members of sections *sorghum* and *parasorghum* were characterised by well-developed pedicillate spikelets, which were usually staminate but sometimes neuter. Pedicillate spikelets in *S.macrospermum* and *S.laxiflorum* were reduced to glumes while in *S.angustum* they were absent.

Accessions of all five sections showed nodal tillering. Members of section *sorghum* were usually rooted at the basal node or the lower 2 to 5 nodes of the culm with a few exceptions in each race. Among the *para-* and *stiposorghums* rooting at the basal nodes was observed in *S.australiense, S.timorense, and S.angustum* but not in *S.brevicallosum, S.ecarinatum, S.purpureosericeum and S.versicolor.* Except for *S.halepense,* none of the other species showed the presence of rhizomes. Varying amounts of white powdery bloom, especially on the leaf sheaths and near the culm nodes was a characteristic feature of most accessions of section *sorghum* but not of *hetero-* or *stiposorghum.* Pruniosity was absent or slightly present in members of *para-* and *chaetosorghum* Nodal pigmentation varied from green in members of section *sorghum* to purple in other sections though there were a few exceptions in each. Midrib colour was mostly white across all the accessions though a few were yellow /dull. The ligule

was either an unfringed or fringed membrane in members of section *sorghum* while in the other sections it was mainly a fringed membrane with varying degrees of hairiness.

Within section *sorghum*, the four races of subsp. *verticilliflorum* were differentiated from each other mainly based on the nature of inflorescence; typical race *verticilliflorum* was distinguished from the other wild races by its large and open inflorescences with spreading but not pendulous branches. Race *arundinaceum* was similar to race *verticilliflorum* except for the branches which were pendulous at maturity. Race *virgatum* was shorter and had narrow, linear leaf blades compared to the broader leaves of the other races, with its inflorescence branches being more erect rather than spreading. Race *aethiopicum* was characterised by open panicles but was easily distinguished from the others by its large ovate-lanceolate, densely tomentose sessile spikelets.

Quantitative Traits

The REML analysis for nine quantitative morphological traits showed significant genetic differences among the accessions in respect of all traits studied for both the rainy (kharif) and post rainy (rabi) seasons (Table 11). When grouped by sections, the pooled REML analysis showed that the sections were significantly different ($p<0.001$) for all traits except for number of rachis nodes. Seasonal effects were non-significant for leaf width, peduncle exertion, panicle length and number of rachis nodes. Season x section interactions were non-significant except for days to flowering and leaf length. The heritability for the different traits ranged from 0.48 for leaf length to 0.84 for leaf width.

The mean, range and CV estimates for different characters are given in Table 12. A fairly wide range of variation was observed for all the traits and sections. In general, for all sections except *stiposorghums,* it was observed that, in kharif, flowering took longer than in rabi and plants grew to a greater height. Leaves were longer in the kharif than in rabi but leaf and panicle width did not differ in the two seasons.

Inter-Relationships among Accessions

The MDS clustering (Fig. 5) of the 85 genotypes based on the kharif data revealed cultivated Maldandi as a distinct cluster. The wild taxa of the four sections *chaeto-*, *hetero-*, *para-* and *stiposorgums* clustered together in a loose manner. This cluster also included three accessions of *S.propinquum* [s(287), s(344) and s(345)] belonging to the section *sorghum*. Members of section *sorghum* comprising accessions of *S.halepense* and the four wild races of *S.bicolor* subsp. *verticilliflorum* formed a second loose cluster. The MDS clustering (Fig. 6) for the rabi data also showed the cultivated Maldandi as distinct, but, there was no evidence of grouping with others.

The UPGMA grouping (Fig. 7) of the kharif data revealed the hierarchical nature of the different clusters observed in the MDS plot. There were three basic groups. Group 1 comprised the members of the four sections *chaeto-*, *hetero-*, *para-* and *stiposorghum* distributed in several subgroups. Group 2 was sub-divided into two groups - Group 2a and Group 2b. Group 2a comprised members of section *sorghum* including the four wild races of *S.bicolor* subsp. *verticilliflorum* and *S.halepense* and Group 2b contained Maldandi - the cultivated check. There was a third group, Group 3, comprising three members of *parasorghum* [ps(279), ps(299) and ps(301)] clustered together with three members of section *sorghum* [s(25), s(45) and s(46)]. *S.nitidium* (*parasorghum*) remained separate from all groups.

The cophonetic correlation between the observed distances and the dendrogram and the MDS was r = 0.69 and r = 0.97 respectively indicating that the observed distances were better represented by the MDS than the dendrogram.

UPGMA clustering of the rabi data (Fig. 8) exhibited more or less the same pattern as that of the kharif data. All accessions of the wild taxa of *chaeto-*, *hetero-*, *para-* and *stiposorghum* were distinctly separate in Group 1. Group 2a comprised wild taxa of section *sorghum* except for seven accessions that clustered in Group 1 and one accession of race *arundinaceum* [s(171)] that remained separate. Group 2b comprised Maldandi, the cultivated check. A high cophenetic correlation was obtained between the observed distances and the MDS (r = 0.97) compared to the dendrogram (r = 0.69).

Fig. 3 Experimental Field with Wild Sorghum Germplasm

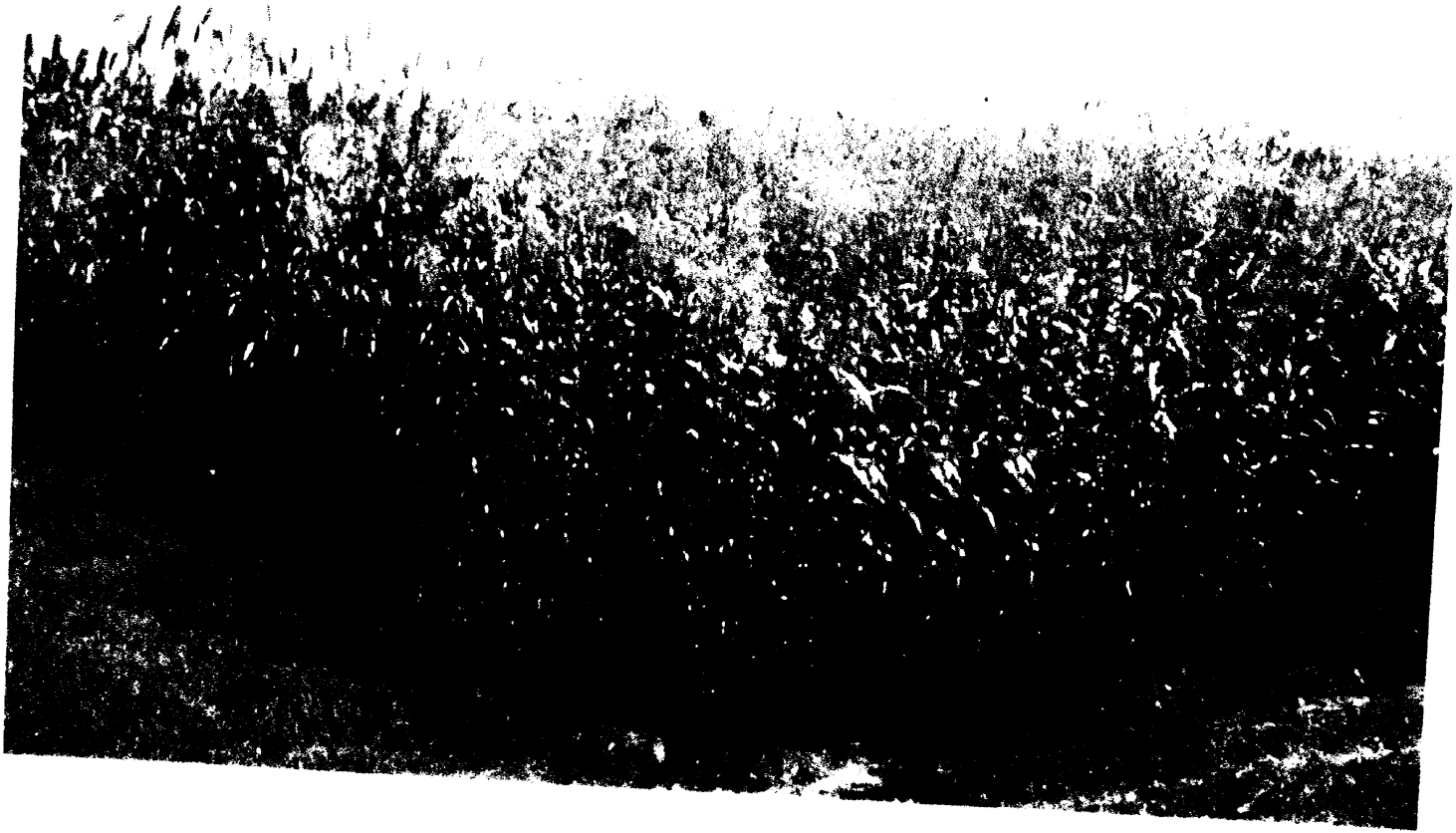


Table 10 Distinguishing Characteristics of the five sections of *Sorghum*

Section	*Sorghum*	*Chaeto-sorghum*	*Hetero-sorghum*	*Para-sorghum*	*Stipo-sorghum*
Duration	Mostly annual; perennial	Annual	Annual	Mostly perennial	Mostly annual
Racemes	1-5 jointed	9-21-jointed	Usually 2-jointed	Usually 3-6(-10) Jointed	1(-3) jointed
Callus	Minute, obtuse	Minute, obtuse	Minute, obtuse	Minute to small, blunt to subacute	Usually elongated and pungent
Awn length	0.6-2.5cm / absent	3.3-5.2 cm	2.5-4.3 cm	1-6.5 cm / absent	5-15 cm
Nodes	Glabrous	Glabrous / slightly pubescent	Pubescent	Usually with a ring of appressed / spreading hair	Usually with a ring of appressed / spreading hair
Pedicellate spikelet	Staminate, neuter	Reduced, neuter	Reduced, neuter	Developed / staminate / neuter	Developed (rarely suppressed), neuter / staminate
Glumes	Equal when present	Slightly unequal	Unequal	Subequal	Subequal
Panicle	Divided	Usually simple	Divided	Simple or divided	Usually simple
Articulation joint	Cupular, horizontal	Cupular, horizontal	Cupular, horizontal	Usually cupular, horizontal	Usually linear, oblique
Culm nodes	Glabrous	Glabrous / pubescent	Glabrous/ bearded / pubescent	Pubescent / bearded / rarely glabrous	Pubescent / bearded / glabrous
Pruniosity	Present	Absent	Present	Absent or sometimes present	Usually present

Fig. 4 Diagnostic Traits in Morphological Characterisation

a. Maldandi (*S. bicolor*), the cultivated check.

b. Differing awn lengths of sessile spikelets.

c. Nodal hairiness, typical of sections *para-* and *stiposorghums*.

d. Glabrous nodes, typical of section *sorghum*.

c

d

Table 11 Variability and Heritability of Quantitative Traits

Source of Variation \ Season	Post Rainy (Rabi)	Rainy (Kharif)	Pooled	Post Rainy (Rabi)	Rainy (Kharif)	Pooled	Post Rainy (Rabi)	Rainy (Kharif)	Pooled
	Variance Components								
	No. of Basal Tillers			Days to 50% Flowering			Plant Height		
Accession	57.92***	45.37***	30.49***	578.47***	449.32***	188.15***	4437.09***	2086.1***	1,465.9***
Season			2.68^{NS}			45.82^{NS}			461.50^{NS}
Accession x Season			20.99***			322.65***			1,754.7***
Error	0.93	0.72	0.83	0.24	1.59	0.95	54.95	405.40	222.80
h^2			0.74			0.54			0.62
	Wald Statistic P - Value								
Section			< 0.001			< 0.001			< 0.001
Season			0.01			< 0.001			< 0.001
Section x Season			0.61			0.00			0.17
	Variance Components								
	Leaf Length			Leaf Width			Peduncle Exertion		
Accession	251.26***	106.33***	59.72**	1.77***	1.45***	1.25***	74.89***	57.28***	43.61***
Season			15.64^{NS}			0.02^{NS}			2.69^{NS}
Accession x Season			118.11			0.31***			21.64***
Error	1.44	20.22	10.42	0.14	0.16	0.15	3.63	0.72	2.14
h^2			0.48			0.84			0.79
	Wald Statistic P - Value								
Section			< 0.001			< 0.001			< 0.001
Season			0.01			0.66			0.11
Section x Season			0.00			1.00			0.88
	Variance Components								
	Panicle Length			Panicle Width			No of Rachis Nodes		
Accession	123.12***	58.31***	51.47***	122.91***	53.06***	59.13***	4.77***	3.17***	2.01***
Season			1.85^{NS}			1.77^{NS}			0.00^{NS}
Accession x Season			37.97***			27.42***			1.89***
Error	0.87	2.39	1.75	0.77	0.30	0.54	0.48	0.38	0.43
h^2			0.72			0.81			0.63
	Wald Statistic P - Value								
Section			< 0.001			< 0.001			0.132
Season			0.14			0.04			0.44
Section x Season			0.16			0.19			0.16

Notes		
	h^2: Heritability	***: Significant at P = 0.001
	^{NS}: Not significant	**: Significant at P = 0.01

Table 12 Section-wise Seasonal Summary Statistics for Quantitative Traits

Statistic \ Season	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)
	No. of basal tillers		Days to 50% flowering		Leaf length	
Sorghum						
Mean	8.47	6.35	86.15	86.20	45.74	45.43
Minimum	1.97	2.42	43.25	57.14	13.88	19.01
Maximum	27.54	21.42	152.01	119.91	79.60	64.04
Variance	24.16	12.86	607.54	262.68	207.35	75.14
Std.Dev.	4.92	3.59	24.65	16.21	14.40	8.67
Std. Error (SE_m)	0.72	0.51	3.63	2.32	2.12	1.24
CV (%)	58.00	56.46	28.61	18.80	31.48	19.08
IS 1054						
Mean	2.84	4.18	75.07	83.90	61.01	59.26
Chaetosorghum						
Mean	11.72	10.00	120.02	115.93	31.48	42.11
Heterosorghum						
Mean	16.73	10.13	97.10	123.90	28.73	50.59
Minimum	11.91	6.65	89.04	107.96	26.71	44.21
Maximum	20.18	14.72	111.22	131.87	29.79	55.73
Variance	18.49	17.22	150.61	190.64	3.07	34.29
Std.Dev.	4.30	4.15	12.27	13.81	1.75	5.86
Std. Error (SE_m)	2.48	2.40	7.09	7.97	1.01	3.38
CV (%)	25.70	40.96	12.64	11.14	6.10	11.57
Parasorghum						
Mean	17.23	13.93	75.80	105.23	28.32	43.31
Minimum	4.02	5.96	43.05	65.11	12.99	30.26
Maximum	29.71	26.04	122.71	157.78	53.06	71.94
Variance	58.93	33.44	544.87	543.87	145.42	117.88
Std.Dev.	7.68	5.78	23.34	23.32	12.06	10.86
Std. Error (SE_m)	1.60	1.21	4.87	4.86	2.51	2.26
CV (%)	44.55	41.51	30.80	22.16	42.58	25.07
Stiposorghum						
Mean	20.65	20.29	102.09	109.28	25.72	41.40
Minimum	13.49	9.21	84.04	81.05	11.59	22.37
Maximum	30.02	30.28	120.02	131.87	39.63	57.41
Variance	43.82	51.19	136.54	249.22	109.11	102.43
Std.Dev.	6.62	7.15	11.69	15.79	10.45	10.12
Std. Error (SE_m)	2.50	2.38	4.42	5.26	3.95	3.37
CV (%)	32.05	35.26	11.45	14.45	40.61	24.44

Contd.

Table 12

Statistic \ Season	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)
	Leaf width		Peduncle exertion		Plant height	
Sorghum						
Mean	2.47	2.78	23.58	20.93	219.38	229.35
Minimum	0.60	0.71	3.80	6.64	86.30	111.43
Maximum	4.77	4.04	44.82	32.20	341.15	327.23
Variance	0.90	0.57	64.79	28.81	4,295.50	1,760.61
Std.Dev.	0.95	0.76	8.05	5.37	65.54	41.96
Std. Error (SE_m)	0.14	0.11	1.19	0.77	9.67	5.99
CV (%)	38.40	27.27	34.14	25.65	29.87	18.29
IS 1054						
Mean	7.24	6.58	4.28	5.35	198.19	250.03
Chaetosorghum						
Mean	1.81	2.19	11.24	12.86	249.29	272.18
Heterosorghum						
Mean	0.94	1.05	22.21	26.20	142.94	175.01
Minimum	0.70	0.84	20.88	13.36	133.72	154.13
Maximum	1.07	1.20	23.26	38.05	152.48	216.09
Variance	0.05	0.04	1.48	153.15	88.14	1,265.84
Std.Dev.	0.21	0.19	1.22	12.38	9.39	35.58
Std. Error (SE_m)	0.12	0.11	0.70	7.14	5.42	20.54
CV (%)	22.69	17.94	5.48	47.24	6.57	20.33
Parasorghum						
Mean	1.06	1.14	17.16	14.99	151.24	214.71
Minimum	0.37	0.71	8.10	7.63	80.38	151.20
Maximum	4.77	1.79	34.33	37.26	253.24	298.42
Variance	0.73	0.10	50.39	51.88	2,435.40	1,590.41
Std.Dev.	0.85	0.31	7.10	7.20	49.35	39.88
Std. Error (SE_m)	0.18	0.07	1.48	1.50	10.29	8.32
CV (%)	80.35	27.65	41.37	48.04	32.63	18.57
Stiposorghum						
Mean	0.71	0.90	13.03	8.10	152.06	204.60
Minimum	0.37	0.43	5.04	3.48	95.19	152.04
Maximum	1.53	1.56	21.74	13.75	193.97	248.74
Variance	0.19	0.10	37.12	9.54	1,456.32	1,148.27
Std.Dev.	0.44	0.32	6.09	3.09	38.16	33.89
Std. Error (SE_m)	0.16	0.11	2.30	1.03	14.42	11.30
CV (%)	61.37	35.84	46.76	38.13	25.10	16.56

Contd.

Table 12

Season / Statistic	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)	Post Rainy (Rabi)	Rainy (Kharif)
	Panicle length		Panicle width		No. of rachis nodes	
Sorghum						
Mean	33.78	32.94	20.59	15.94	9.51	8.92
Minimum	10.76	18.44	1.57	4.06	4.45	4.62
Maximum	60.57	47.54	47.19	27.50	13.53	13.11
Variance	89.69	41.53	79.38	27.08	3.57	2.82
Std.Dev.	9.47	6.44	8.91	5.20	1.89	1.68
Std. Error (SE_m)	1.39	0.92	1.31	0.74	0.27	0.24
CV (%)	28.03	19.57	43.27	32.64	19.87	18.82
IS 1054						
Mean	17.22	17.36	6.08	6.23	8.73	8.79
Chaetosorghum						
Mean	21.24	23.43	4.85	5.34	7.18	7.13
Heterosorghum						
Mean	11.29	17.19	1.97	3.64	8.03	8.07
Minimum	9.17	13.44	1.57	2.85	7.00	6.90
Maximum	12.60	19.11	2.57	4.63	8.81	9.28
Variance	3.44	10.53	0.28	0.82	0.87	1.41
Std.Dev.	1.85	3.24	0.53	0.91	0.93	1.19
Std. Error (SE_m)	1.07	1.87	0.30	0.52	0.54	0.69
CV (%)	16.42	18.88	26.69	24.88	11.61	14.74
Parasorghum						
Mean	18.75	24.65	2.76	3.82	8.24	8.59
Minimum	8.48	14.11	0.68	1.82	1.73	6.30
Maximum	29.83	35.25	9.62	12.10	11.72	12.49
Variance	33.34	31.04	3.78	5.12	5.63	3.22
Std.Dev.	5.77	5.57	1.94	2.26	2.37	1.79
Std. Error (SE_m)	1.20	1.16	0.41	0.47	0.49	0.37
CV (%)	30.80	22.60	70.39	59.22	28.80	20.87
Stiposorghum						
Mean	19.44	25.21	4.54	5.59	8.07	9.41
Minimum	15.68	19.11	1.57	2.15	5.00	6.60
Maximum	22.48	33.13	10.22	16.77	10.27	10.98
Variance	7.55	18.59	10.98	25.69	3.47	2.68
Std.Dev.	2.75	4.31	3.31	5.07	1.86	1.64
Std. Error (SE_m)	1.04	1.44	1.25	1.69	0.70	0.55
CV (%)	14.14	17.10	73.01	90.67	23.08	17.42

Fig. 5 MDS Plot of 85 Wild Sorghums using Quantitative Traits in Kharif (r = 0.762)

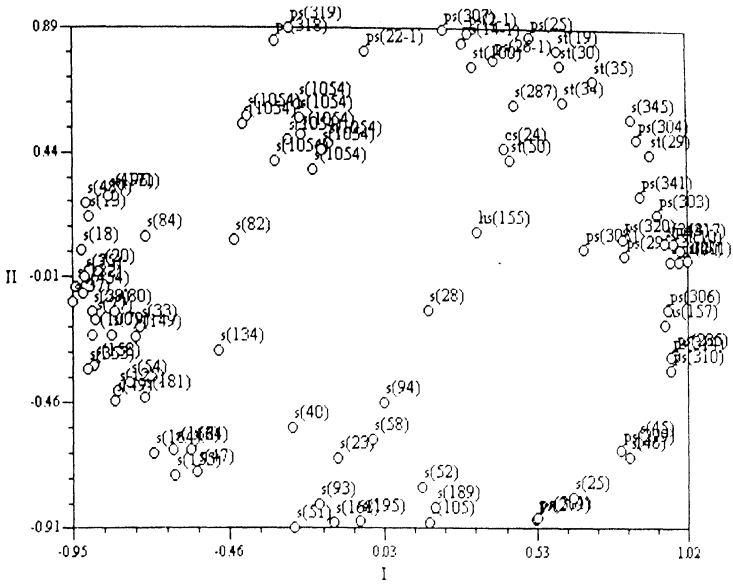


Fig. 6 MDS Plot of 85 Wild Sorghums using Quantitative Traits in Rabi (r = 0.748)

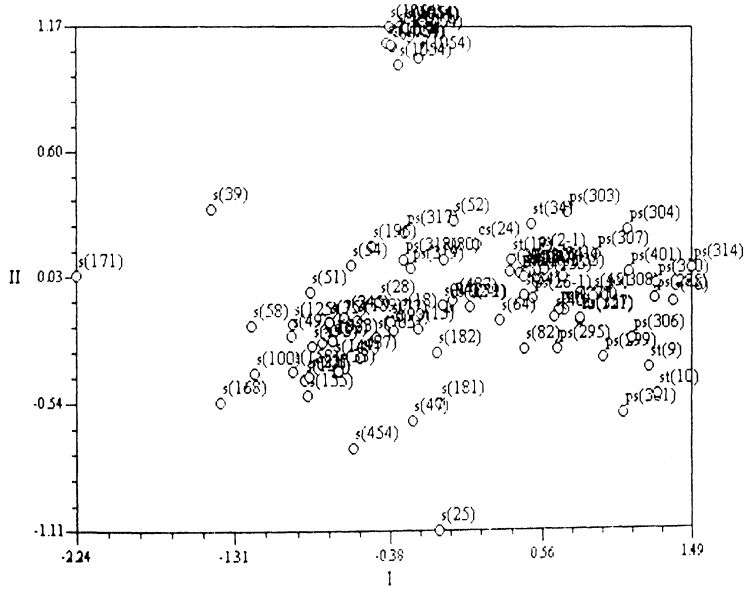


Fig. 7 UPGMA Dendrogram of 85 Wild Sorghums using Quantitative Traits in Kharif (r = 0.762)

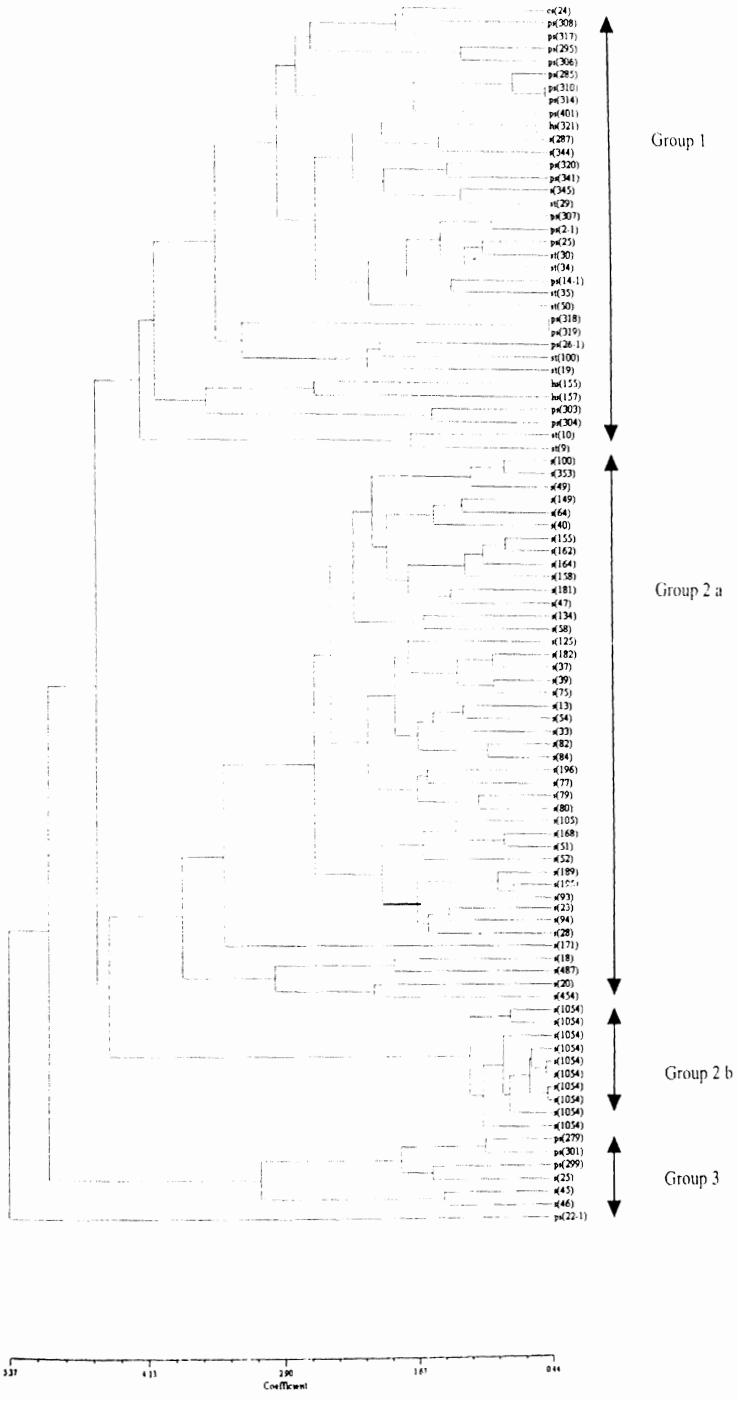


Fig. 8 UPGMA Dendrogram of 85 Wild Sorghums using Quantitative Traits in Rabi (r = 0.752)

Molecular Characterisation

Intra-Generic Diversity in *Sorghum* using RFLPs, AFLPs and SSRs

RFLP Analysis with Maize Mitochondrial DNA Probes

Four maize mitochondrial DNA probes (*cox* I, *cox* II, *atp* 6 and *atp* α) in three restriction enzyme combinations hybridised to 22 accessions, representing 17 *Sorghum* species in five taxonomic sections. A high level of polymorphism was detected among the various accessions by the 12 enzyme-probe combinations (Table 13). Representative hybridisation patterns are shown in Figs. 9a - 9f. The *Bam*H I-*atp* α combination generated the maximum number of 25 hybridisation bands ranging in size from 0.5 to 23.0 kb. The *Xba* I-*cox* I combination was least polymorphic yielding only 6 bands ranging from 4.4 to 16.0 kb. One of the bands generated by *cox* II with each of the three different enzymes was common to accessions of all species.

With high levels of polymorphism detected, the different enzyme-probe combinations were able to uniquely distinguish 20 of the 22 accessions. Within section *sorghum*, races *aethiopicum* and *virgatum* were indistinguishable, as also the races *S.bicolor* and race *verticilliflorum*.

Number of unique banding patterns/haplotypes ranged from 7 for *Xba* I-*cox* I to 17 for *Xba* I - *atp* α and *Bam H* I - *atp* α (Table 14). The number of bands per pattern generally ranged from one to three, though relative intensities of some bands with *atp 6* and *atp* α differed. More hybridising restriction fragments were revealed for sections *para-* and *stiposorghum* (80 and 52 respectively) than for section *sorghum* (19). The majority of patterns were unique to a section though a few were common to two or three out of the five sections. The probe *atp* α in combination with *Hind* III and *Bam H* I were the only combinations in which none of the 15 and 17 patterns generated were shared between any of the sections. Sections, *chacto-* and *heterosorghums* gave unique patterns for all combinations except for patterns with *Xba* I-*cox* I and *Bam H* I-*cox* II that were shared with accessions of *stiposorghum*.

Inter-Relationships among Accessions

Pair-wise similarities (S_{ij}) among the 22 accessions ranged between 0.12 to 1.00 with an average value of 0.29 ± 0.2. Taxa within section *sorghum* were highly similar (S_{ij} = 0.96 ± 0.04) compared to either *stiposorghum* (S_{ij} = 0.37 ± 0.15) or *parasorghum* (S_{ij} = 0.29 ± 0.13). Further, the latter two sections were more similar to each other (S_{ij} =

0.31 ± 0.14) than individually to section *sorghum*. Distances for sections *chaetosorghum* and *heterosorghum* were not computed as there was only one member from each of these sections.

The MDS plot (Fig. 10) revealed one distinct cluster that comprised the 6 accessions of section *sorghum*: *S.bicolor* subsp. *bicolor*, the four wild races of *S.bicolor* subsp. *verticilliflorum* and *S.halepense*. No other major grouping was visible.

The UPGMA dendrogram (Fig. 11) revealed the hierarchical structure of the 22 accessions. Group 1, clearly seen in the MDS plot included all members of section *sorghum*. Within the group, *S.bicolor* subsp. *bicolor* and race *verticilliflorum* of subsp. *verticilliflorum* grouped together; races *aethiopicum* and *virgatum* clustered together and race *arundinaceum* and *S.halepense* formed separate individual subgroups. Group 2 comprised 10 Australian species belonging to both *para-* and *stiposorghum*. Group 3 comprised three *parasorghum* species: *S.versicolor* and *S.purpureosericeum* from Africa and *S.nitidium* from Australia. Groups 4 and 5 included one species each corresponding to *S.macrospermum* (*chaetosorghum*) and *S.laxiflorum* (*heterosorghum*).

RFLP Analysis using Sorghum Resistance Gene Candidates (RGCs)

All the four RGCs, S8-1, S2-2, S27-1 and S30-5, hybridised with members of section *sorghum*, with the highest polymorphism (5-6 alleles) detected by S8-1 and S2-2 (Table 15). Three (S8-1, S2-2 and S30-5) of the four RGCs also hybridised to sections *hetero-* and *chaetosorghums*. S8-1 and S2-2 gave weak signals with members of sections *para-* and *stiposorghums*. Representative hybridisation patterns are shown in Fig. 12.

AFLP Analysis

Twenty two accessions of 17 species were analysed for AFLPs using four primer pair combinations: EACT - M CTA, E ACT - MCTC, E ACA - M CTA and E ACT - M CTG. Representative AFLP profiles are given in Fig. 13. The accession, *S.stipoideum*, for which DNA partially amplified, was excluded from analysis. A total of 1451 scorable bands were detected at 251 loci across the remaining 21 accessions. All bands that could be reliably read on the autoradiagrams were treated as individual dominant loci and scored as 1. The alternative form of an allele was scored as 0. Four loci out of 251 were monomorphic for the presence of the allele, one each for the two primer pair combinations, E ACT - M CAT and E ACT - M CTG and two for the primer combination E ACA - M CTA. Primer pair combination E ACT - M CTC was polymorphic at all 63 loci scored.

Polymorphism and gene diversity in three sections of *sorghum* are given in Table 16. *Parasorghum* was most diverse (0.19), in comparison to section *sorghum* (0.11) or *stiposorghum* (0.12). Markov Chain Monte Carlo (MCMC) simulations showed that, with regard to allelic frequencies, *para-* and *stiposorghums* were highly differentiated with respect to section *sorghum* ($P<0.0001$), with 26% and 30% of the loci significantly differing between the two sections and section *sorghum* respectively ($P<0.05$). Sections *parasorghum* and *stiposorghum* overall were not significantly differentiated ($P>0.05$), but differed significantly ($P<0.05$) at 7 (3%) loci. Genetic differentiation between the three sections, as measured by the F_{ST} value, was 0.49 ± 0.03, with 95% Bootstrap confidence interval (CI) of 0.44 to 0.55. UPGMA clustering of the three sections based on Roger's modified distance, grouped *para-* and *stiposorghum* closer together at a distance of 0.24 while section *sorghum* was joined with the two sections at a distance of 0.46 (Fig. 14).

When classified by region, the Afro-Asian population had a mean gene diversity of 0.22 compared to 0.15 for the Australian population (Table 16). The MCMC simulation showed that, with respect to allelic frequency, the African and Australian populations were significantly differentiated at 27% of the loci ($P<0.05$). Genetic differentiation among these regional populations, as measured by Wright's F_{ST}, was 0.32 ± 0.03 over all loci with a 95% bootstrap CI of 0.27 to 0.38.

Inter-Relationships among Accessions

Jaccard's pair-wise similarity coefficient (S_{ij}) for the 21 accessions ranged from 0.10 to 0.84 with an average of 0.31 ± 0.18. Inter- and intra-sectional similarities are given in Table 17. *Parasorghum* was most diverse ($S_{ij} = 0.47 \pm 0.31$) in comparison to section *sorghum* ($S_{ij} = 0.79 \pm 0.16$) or *stiposorghum* ($S_{ij} = 0.69 \pm 0.24$). Sections *parasorghum* and *stiposorghum* were more similar to each other ($S_{ij} = 0.37 \pm 0.15$) than they were to section *sorghum* ($S_{ij} = 0.18 \pm 0.24$; $S_{ij} = 0.17 \pm 0.03$) respectively. Between *parasorghum* and *stiposorghum*, least similarity was seen between *S.purpureosericeum* (IS 18944) and *S.angustum* ($S_{ij} = 0.14$), while *S.matarankense* and *S.ecarinatum* were most similar ($S_{ij} = 0.71$). Between *stiposorghum* and *sorghum*, *S.halepense* and *S.angustum* were least similar ($S_{ij} = 0.11$) while *S.halepense* and *S.interjectum* were most similar ($S_{ij} = 0.20$). Between *parasorghum* and section *sorghum*, races *arundinaceum* and *S.australiense* were least similar ($S_{ij} = 0.13$), while race *S.verticilliflorum* and *S.nitidum* were closest ($S_{ij} = 0.23$). At the intra-sectional level, within section *sorghum*, maximum similarity occurred between races *arundinaceum* and *virgatum* ($S_{ij} = 0.84$), whereas races *aethiopicum* and *S.halepense* showed least similarity ($S_{ij} = 0.50$). Within *parasorghum*, *S.purpureosericeum* (IS 18944) and *S.australiense* were least similar ($S_{ij} = 0.17$), while

S.australiense and *S.timorense* were most similar (S_{ij} = 0.56). Within *stiposorghum*, *S.angustum* and *S.ecarinatum* were least similar (S_{ij} = 0.14), while *S.extans* and S.*interjectum* were most similar (S_{ij} = 0.70).

The MDS plot (Fig. 15) grouped the 21 accessions into four distinct clusters. The hierarchical structure of these clusters was revealed in the UPGMA based dendrogram (Fig. 16). The six members of section *sorghum* clustered as one major group (Group-1) in which races *arundinaceum* and *virgatum* formed one subgroup along with race *verticilliflorum*; *race aethiopicum* grouped by itself, and *S.bicolor* subsp. *bicolor* and *S.halepense* formed their own individual subgroups. Sections *heterosorghum* (*S.laxiflorum*) and *chaetosorghum* (*S.macrospermum*) formed the second group (Group-2). The African species of *parasorghum* formed the third group (Group-3), where the two *pupureosericeums* clustered in one subgroup and *S.versicolor* remained separate. All the Australian species of both *para-* and *stiposorghum* clustered together in Group 4. *S.angustum* (*stiposorghum*) and *S.nitidum* (*parasorghum*) were the most divergent lines and formed individual subgroups within Group 4, while *S.australiense, S.timorense* and *S.brevicallosum* (*parasorghum*) formed one subgroup, and *S.matarankense* and *S.ecarinatum* (*parasorghum* and *stiposorghum* respectively) clustered together. *S.extans, S.intrans* and *S.interjectum* (*stiposorghum*) also clustered together within Group 4.

SSR Analysis

The ten microsatellite amplified alleles in all accessions of the section *sorghum* and in *hetero-* and/or *chaetosorghum*. High polymorphism was observed within section *sorghum*, with 1 to 6 alleles amplified across the 6 accessions tested. However, only two SSRs amplified alleles in any of the *para-* and *stiposorghums*. Sb 6-14 amplified five alleles across seven species: *S.laxiflorum* (*heterosorghum*), *S.macrospermum* (*chaetosorghum*), *S.nitidum* and *S.versicolor* (*parasorghum*) and *S.angustum, S.ecarinatum and S.stipoideum* (*stiposorghum*). Sb 1-10 amplified three alleles across five species: *S.laxiflorum* (*chaetosorghum*) *S.purpureosericeum, S.australiense and S.nitidum* (*parasorghum*) and *S.angustum* (*stiposorghum*).

Fig. 9a Southern Blot of Wild Sorghums using Maize mt DNA Probes

The following are the enzyme probe combinations

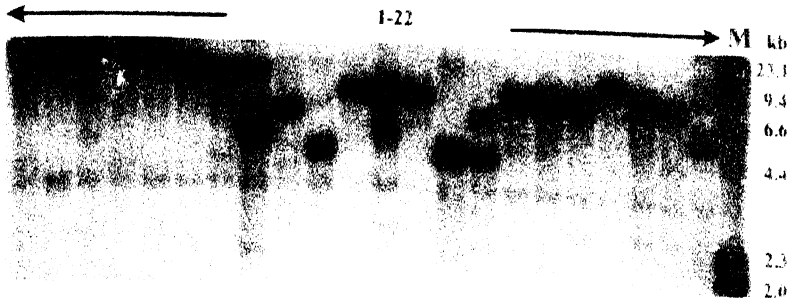
a. *Xba* I - *Cox* I

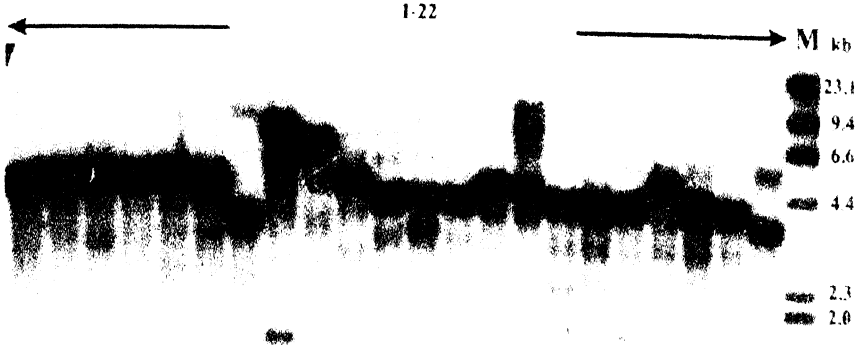
b. *Hind* III - *Cox* I

c. *Hind* III - *Cox* II

M is the marker *Hind* III λ DNA; Fragment sizes are given in kb.

The accessions in the gel from L to R are as listed in Table 6.


1-22
M
kb
23.1
9.4
6.6
4.4
2.3
2.0


1-22
M
kb
23.1
9.4
6.6
4.4
2.3
2.0

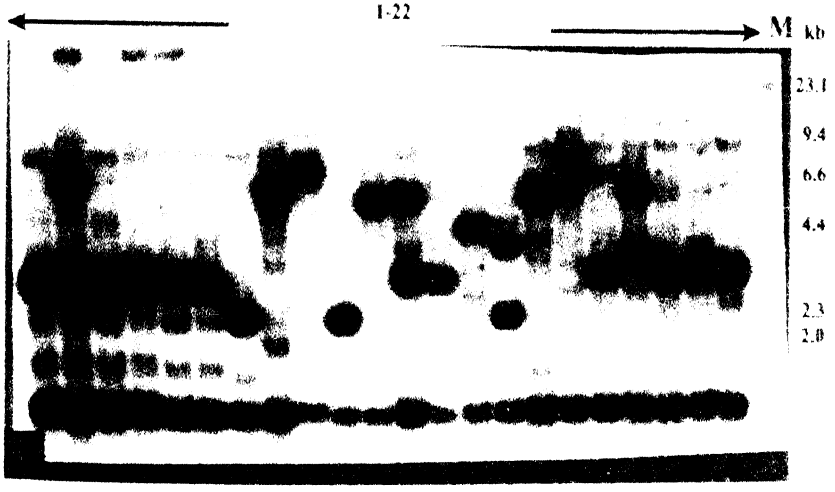

1-22
M
kb
23.1
9.4
6.6
4.4
2.3
2.0

Fig. 9b Southern Blot of Wild Sorghums using Maize mt DNA Probes

The following are the enzyme probe combinations

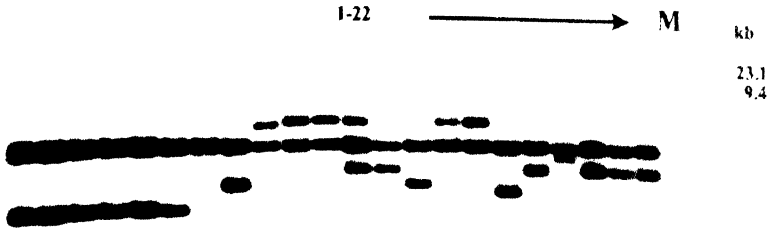
d *Yba* I - *Cox* II

e *Xba* I *atp* α

f *Hind* III - *atp* α

M is the marker *Hind* III I DNA, Fragment sizes are given in kb

The accessions in the gel from L to R are as listed in Table 6



e

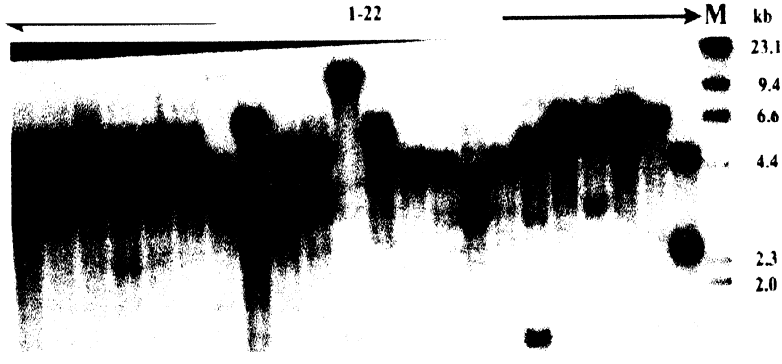


Table 13 Polymorphism in *Sorghum* species with Maize Mitochondrial Probes

Enzyme - probe combination	No of bands	Size (kb) of bands
Hind III - *atp* α	16	7.7 to 14.4
Bam H I - *atp* α	25	0.5 to ~ 23.1
Xba I - *atp* α	17	3 to ~ 23.1
Hind III - *atp* 6	16	2 to 9.2
Bam H I - *atp* 6	12	1.8 to ~ 23.1
Xba I - *atp* 6	15	4 to ~ 23.1
Hind III - *cox* I	10	3.4 to 11
Bam H I - *cox* I	8	2 to 12
Xba I - *cox* I	6	4.4 to 16
Hind III - *cox* II	12	1.8 to 8.8
Bam H I - *cox* I	9	2.5 to 14.8
Xba I - *cox* II	8	1.8 to 6.5

Table 14 Banding Patterns in five sections of *Sorghum*

Enzyme-probe combination	*Sorghum*	*Para-sorghum*	*Stipo-sorghum*	*Chaeto-sorghum*	*Hetero-sorghum*	No. of unique patterns across sections
	No of unique banding patterns					
Hind III - *atp* α	2	6	5	1	1	15 (none shared between sections)
Bam H I - *atp* α	1	8	6	1	1	17 (none shared between sections)
Xba I - *atp* α	3	8	4	1	1	17 (1 shared between *para* - and *stipo* - *sorghums*; 1 shared between *sorghum* and *stiposorghum*)
Hind III - *atp* 6	2	8	3	1	1	15 (3 patterns shared between *para* - and *stiposorghums*)
Bam H I - *atp* 6	2	6	5	1	1	15 (1 pattern shared between *para* - and *stiposorghums*)
Xba I - *atp* 6	1	7	3	1	1	13 (3 patterns shared between *para* - and *stiposorghums*)
Hind III - *cox* I	1	4	1	1	1	8 (2 shared between *para* - and *stiposorghums*)
Bam H I - *cox* I	1	4	1	1	1	8 (1 shared between *para* - and *stiposorghums*)
Xba I - *cox* I	1	4	2	0	0	7 (1 shared between *para* - and *stipo* - *sorghums*; 1 shared between *stipo* - and *heterosorghum*; 1 shared between *stipo* - and *chaetosorghum*)
Hind III - *cox* II	2	7	2	1	0	12 (1 shared between *para* - and *hetero* - *sorghum*; 1 shared between *para* - and *stiposorghum*)
Bam H I - *cox* I	2	6	2	0	0	10 (1 shared between all sections; 1 shared between *chaeto* - and *stiposorghum*; 1 shared between *hetero* - and *stipo* - *sorghum*; 1 shared between *chaeto* - and *heterosorghum*)
Xba I - *cox* II	1	4	2	1	1	9 (1 shared between *para* - and *stipo* - *sorghum*)

Fig. 10 MDS Plot of 22 *Sorghum* Accessions using maize mt DNA probes (r = 0.843)

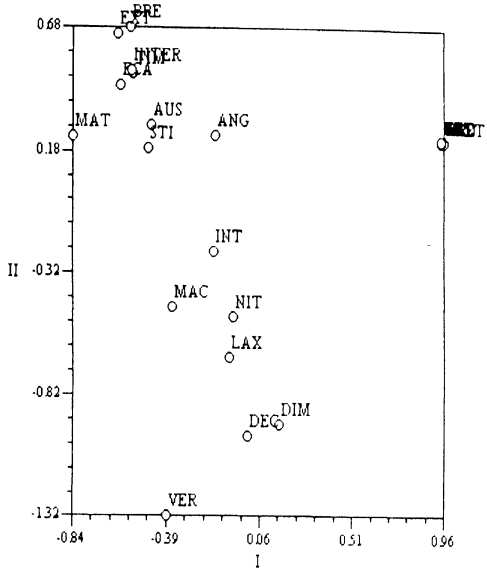


Fig. 11 UPGMA Dendrogram of 22 *Sorghum* Accessions using maize mt DNA probes (r = 0.981)

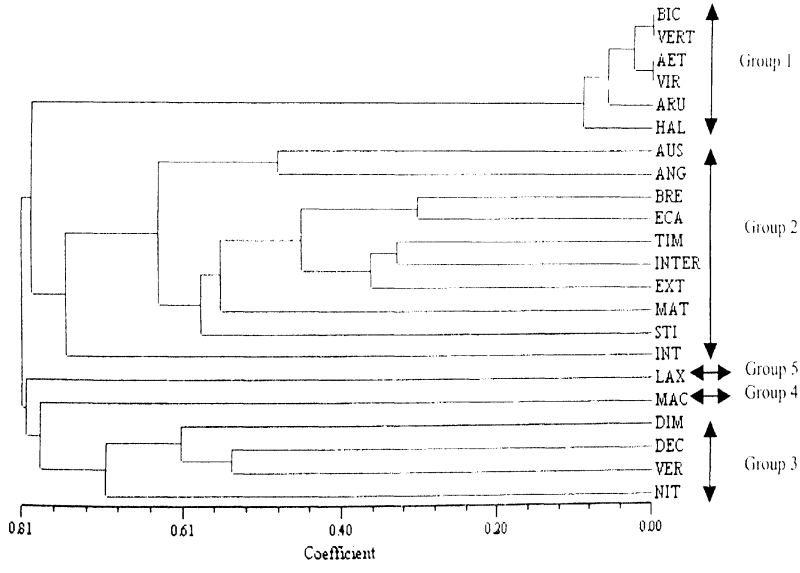


Table 15 RGC Polymorphism in five sections of *Sorghum*

Enzyme - Probe Combination	*Sorghum*	*Chaeto-sorghum*	*Hetero-sorghum*	*Para-sorghum*	*Stipo-sorghum*
S8-1-*Hind* III	6 bands (5 patterns)	1	1	Faint bands	Faint bands
S8-1-*Xba* I	4 bands (4 patterns)	-	-	-	-
S27-2-*Hind* III	2 bands (2 patterns)	-	-	-	-
S27-2-*Xba* I	2 bands (2 patterns)	-	-	-	-
S2-2-*EcoR* I	5 bands (3 patterns)	1	1	Faint bands	Faint bands
S2-2-*Xba* I	1 band	2	1	-	-
S30-5-*Xba* I	2 bands	1	1	-	-
S30-5-*Hind* III	3 bands (2 patterns)	1	1	-	-
S30-5-*Bam H* I	3 bands (2 patterns)	1	1	-	-

Fig. 12 Southern Blot of Wild Sorghums using *Sorghum* RGCs

Enzyme – probe combinations are:

a. *Hind* III – S8 – 1

b. *BamH* I – S30 – 5

c. *EcoR* I – S2 – 2

The accessions in the gel from L to R are listed in Table 6.

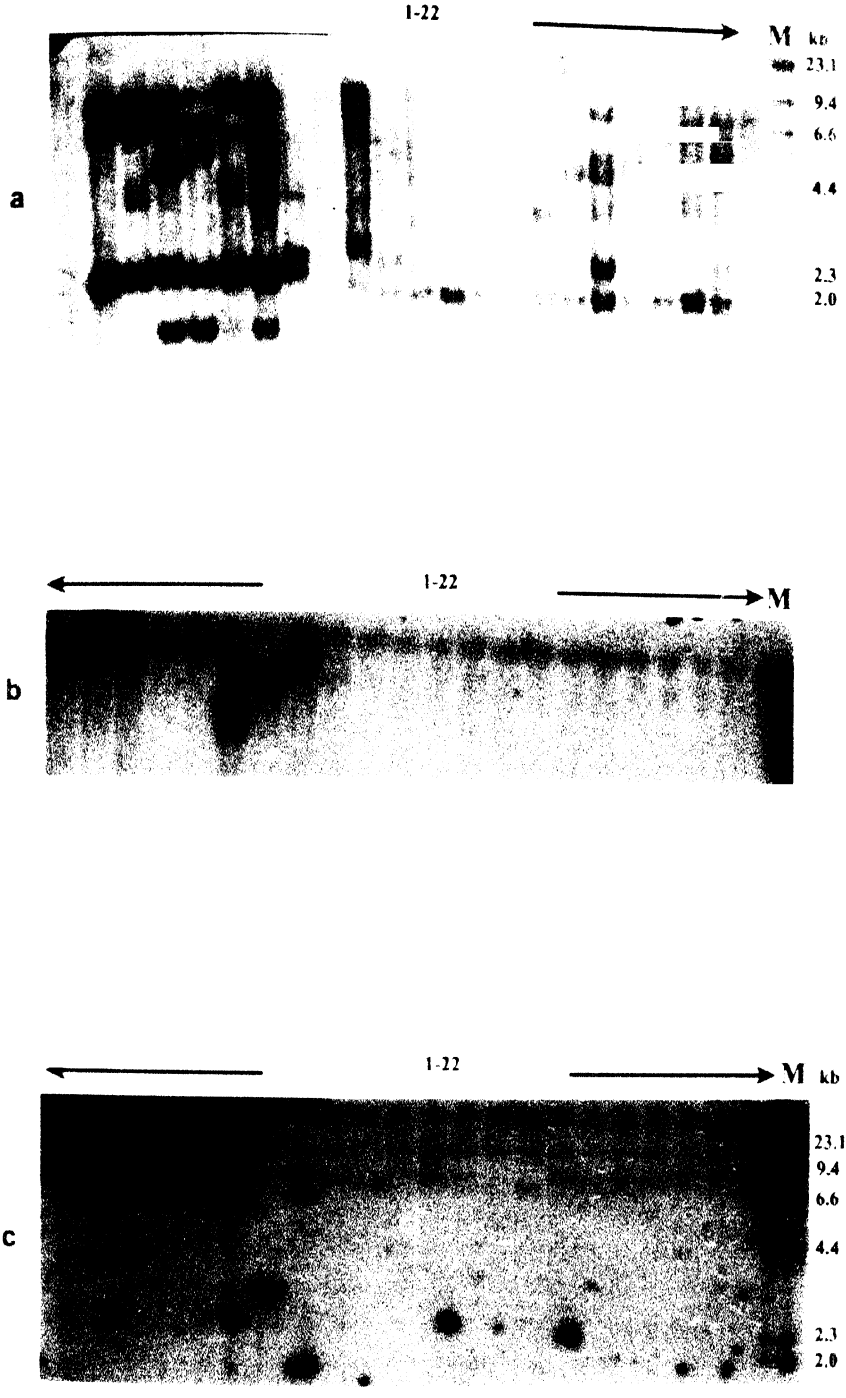

a
1-22
M kb
23.1
9.4
6.6
4.4
2.3
2.0
b
1-22
M
c
1-22
M kb
23.1
9.4
6.6
4.4
2.3
2.0

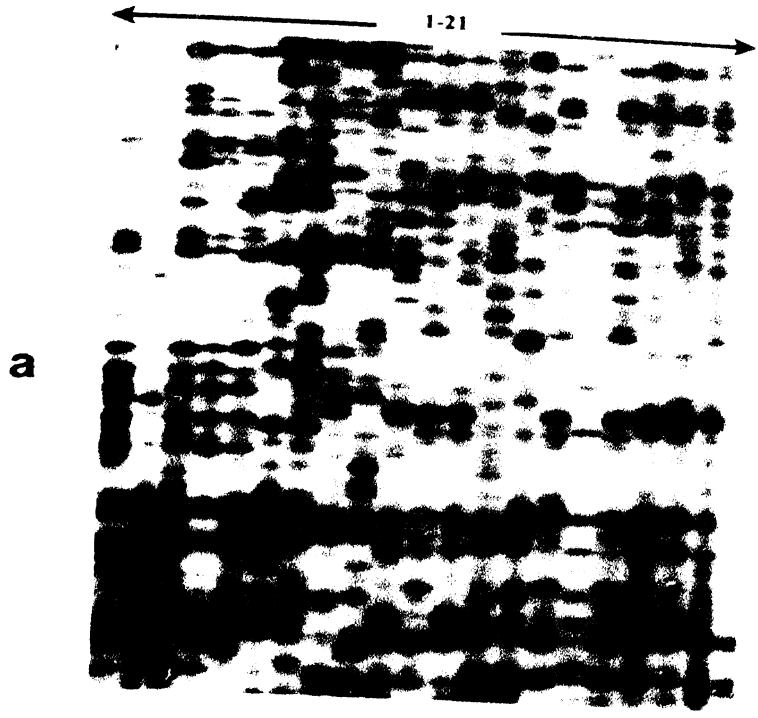
Fig. 13 Representative AFLP profiles of Wild Sorghums

The primer combinations are :

a. *E ACA - M CTA*

b. *E ACT - M CAT*

The accessions in the gel from L to R are listed in Table 6.



a

b

Table 16 Gene Diversity and Population Differentiation in *Sorghum* using AFLPs

	Sorghum	*Para-sorghum*	*Stipo-sorghum*	Overall	Afro- Asia	Australia	Overall
Polymorphism (%)	27	68	33	72	66	37	72
Gene Diversity (H)	0.11	0.19	0.12	0.23	0.21	0.15	0.23
F_{st}				0.50 ± 0.03			0.32 ± 0.03
				(0.44 - 0.55)*			(0.27 - 0.38)*
*: Bootstrap - based 95% Confidence Interval							

Table 17 Intra- and Inter-Section similarities in *Sorghum* using AFLPs

Section	*Sorghum*	*Parasorghum*	*Stiposorghum*
Sorghum	0.79 ± 0.16		
	(0.51 - 0.84)*		
Parasorghum	0.18 ± 0.24	0.47 ± 0.31	
	(0.13 - 0.23)*	(0.17 - 0.56)*	
Stiposorghum	0.17 ± 0.03	0.37 ± 0.15	0.69 ± 0.24
	(0.11 - 0.20)*	(0.14 - 0.71)*	(0.40 - 0.70)*
*: Bootstrap - based 95% Confidence Interval			

Fig. 14 UPGMA Dendrogram of three sections of *Sorghum* using AFLPs

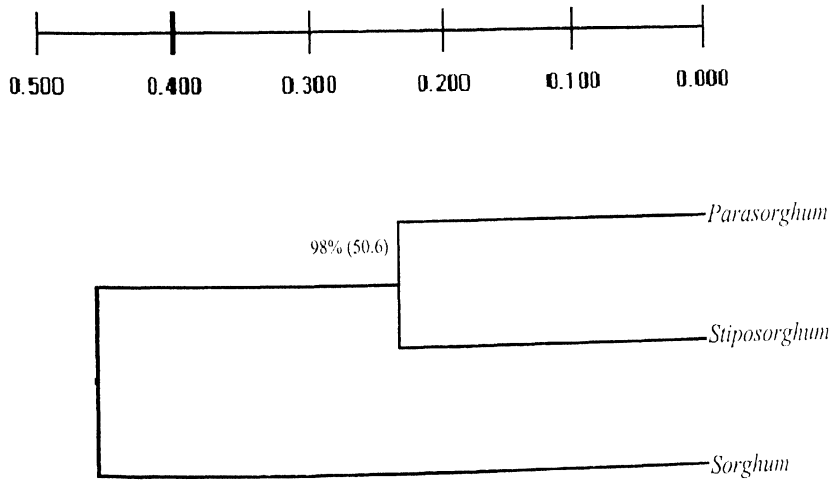


Fig. 15 MDS Plot of 21 *Sorghum* Accessions using AFLPs
(r = 0.890)

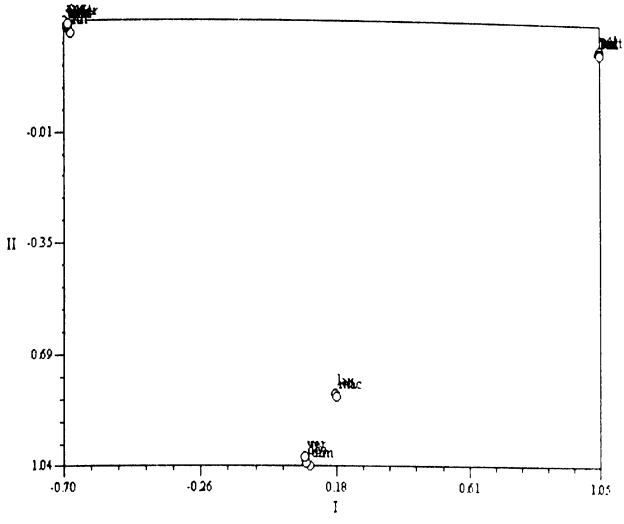
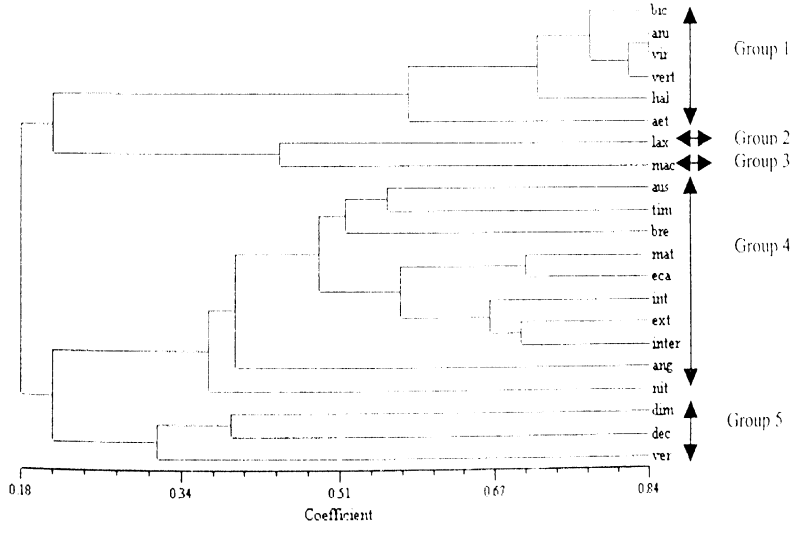


Fig. 16 UPGMA Dendrogram of 21 *Sorghum* Accessions using AFLPs
(r = 0.980)



Intra-Specific Diversity in *S.bicolor* subsp. *verticilliflorum* using AFLPs and SSRs

AFLP Analysis

A total of 1242 scorable bands were generated at 240 loci among the 21 accessions of *S.bicolor* subsp. *verticilliflorum*, using four primer pair combinations: E ACT - M CAT, E ACA - M CTC, E ACA - M CTA, and E ACT - M CTG. Representative AFLP profiles are given in Fig. 17. Band scoring was done in the same manner as described earlier. Out of the 240 loci, alleles at 146 loci (61%) were polymorphic. Monomorphism for both forms of the alleles across the four primers ranged from 80% for E-ACT-M-CTG to 60% for E-ACA-M-CTC.

Average gene diversity over all loci was 0.15. Race-wise, average gene diversity was observed to be 0.13 for races, *aethiopicum* and *virgatum*, and 0.12 for races, *arundinaceum* and *verticilliflorum* (Table 18). MCMC simulation results showed that, with respect to allele frequencies, the genetic differentiation between the races was non-significant, although the race *aethiopicum* was significantly differentiated from races *arundinaceum* and *verticilliflorum* at 4 loci, while race *arundinaceum* significantly differed from races *verticilliflorum* and *virgatum* at 3 loci. Racial differentiation as estimated by F_{ST} over all loci was 0.17 ± 0.03 with a 95% bootstrap CI of 0.12-0.23. UPGMA clustering of the races based on Roger's modified distance, grouped races *arundinaceum*, *verticilliflorum* and *aethiopicum* at a distance of 0.19 while race *virgatum* was separated from the others at a distance of 0.24 (Fig. 18).

When accessions were classified by region, east African accessions were the most diverse (H = 0.16) while west African accessions were the least (H = 0.11) (Table 18). Overall differentiation among regions based on allele frequencies was not significant but east African and south African accessions were significantly differentiated from west African accessions at 4 loci. Regional differentiation as estimated by F_{ST} was 0.08 ± 0.02 overall loci with a 95% bootstrap CI of 0.04 to 0.12.

UPGMA clustering of the five regions based on Roger's modified distance revealed two groups: the first containing east Africa, south Africa and central Africa, and the second group having western Africa and USA (Fig 19).

Inter-Relationships among Accessions

Jaccard's similarity coefficient (S_{ij}) for the 21 accessions ranged between 0.41 to 0.74 with a mean of 0.56 ± 0.06. Intra- and inter- racial similarities for the four races are given in Table 19.

No clear racial or geographical separation was visible in the MDS plot (Fig 20). The absence of hierarchical structure was also evident in the UPGMA based dendrogram (Fig 21). Three groups were observed. Six accessions of *verticilliflorum* clustered together, but, were distributed in Group 1 and Group 2; the seven *arundinaceums* were distributed in the three groups; of the three *virgatums*, two remained in individual groups while one grouped with *verticilliflorum* in Group 1. Four accessions [one of *verticilliflorum*, one of *aethiopicum* and two of *virgatum*] formed their individual groups.

SSR Analysis

Twenty-one races belonging to *S.bicolor* subsp. *verticilliflorum* were analysed using 10 SSR primers. Representative SSR patterns are given in Fig. 22. All ten primers showed high levels of polymorphism and distinguished the 21 accessions. Five loci were 100% homogeneous for the 21 accessions. The heterogeneity at the other five, varied from 9.5% at locus Sb1-1 to 29% at locus Sb4-22. For Sorghum, an often cross-pollinated crop, heterogeneity within an accession could arise in three ways: heterozygosity at a locus, seed mixture/contamination, or a mixture of individuals homozygous for different alleles. In the present study it would be difficult to distinguish between these three possibilities since 5 to 7 plants were pooled to obtain adequate DNA for analysis. Hence, only homogeneous accessions have been considered for analysis. A total of 76 alleles were detected, with an average allelic richness of 7.6 alleles per locus (Table 20). Number of alleles at a locus ranged from 2 at locus Sb4-22 to 10 at loci Sb1-10, Sb6-36, Sb6-84. Gene diversity was generally high, ranging between 0.66-0.91 except for locus Sb4-22 that had a gene diversity of 0.33. Average gene diversity was 0.77 over all the loci.

When classified by race, gene diversity was highest for the *verticilliflorums* (0.68) followed by race *virgatum* (0.67) and race *arundinaceum* (0.63) (Table 21). Race *aethiopicum* was the least diverse (0.45). Number of polymorphic loci varied with the different races. Race *arundinaceum* was polymorphic at all ten loci, while races *verticilliflorum* and *virgatum* were monomorphic at loci Sb6-57 and Sb4-22 respectively. Race *aethiopicum* was monomorphic at three loci (Sb4-22, Sb4-32, Sb6-36). The number of observed alleles was highest for the *verticilliflorums* (40), followed by the *arundinaceums* (36), *virgatum* (26) and *aethiopicum* (21). The two races, *verticilliflorum* and *virgatum*, had similar gene diversity values despite the former having a larger number of 40 alleles compared to 26 in the latter.

Classification by geographic region gave mean gene diversity values of 0.72 for eastern Africa, 0.67 for western Africa, 0.62 for southern Africa and 0.60 for central Africa (Table 22). Allelic richness was higher for the eastern African accessions (with 41 alleles) compared to west African accessions (with 33 alleles), southern African accessions (with 26 alleles) and central African accessions (with 22 alleles). The locus,

Sb4-22 showed low gene diversity values for both eastern and western African accessions (0.53 and 0.36 respectively) and was monomorphic for central and southern Africa.

Overall, the four races were significantly differentiated from each other ($P<0.0001$) at all marker loci. Three loci (Sb1-10, Sb5-23, Sb6-84) differentiated races *verticilliflorum* and *virgatum* while seven loci differentiated races *arundinaceum* and *virgatum*. However, genetic differentiation was non-significant, as estimated by F_{ST} (0.07 ± 0.06) with a 95% bootstrap CI of -0.02 to 0.20. UPGMA clustering, based on Roger's modified distance grouped races *arundinaceum* and *virgatum* together at a distance of 0.41, with race *verticilliflorum* joining at a distance of 0.46. Race *aethiopicum* remained separate, joining the other three at a distance of 0.53 (Fig 23).

The regions, like the races, were also significantly differentiated ($P < 0.05$) based on the allelic frequency tests. Five loci differentiated the eastern African population from the western African population, while the number of loci that differentiated the other regions varied between two for central Africa and USA, and six for central Africa and western Africa. Genetic differentiation as estimated by F_{ST} was non-significant (0.01 ± 0.03) with a 95% bootstrap CI of -0.04 to 0.07. UPGMA clustering based on Roger's modified distance grouped eastern and western Africa together at a distance of 0.39, with central and southern Africa joining the cluster at a distance of 0.49. USA was grouped separately and joined all others at a distance of 0.55 (Fig 24).

Inter-Relationships among Accessions

Pair-wise similarity for the 21 accessions ranged from 0 to 0.61 with a mean of 0.9 ± 0.1. Mean intra-racial similarities for the four races varied from 0.12 ± 0.13 for *virgatum* to 0.31 ± 0.17 for *aethiopicum*. Races *aethiopicum* and *arundinaceum* were most similar (0.24 ± 0.09) while races *aethiopicum*, *verticilliflorum* and *virgatum* were most dissimilar (0.16 ± 0.09). The MDS clustering showed the 21 accessions to be almost randomly spread over two-dimensional Euclidean space (Fig 25). Except for a few pairs of individuals that grouped together, no clear racial or regional grouping pattern was apparent. Cluster analysis using UPGMA algorithm revealed a similar trend. Three clusters were formed with members of the four races distributed in all the groups (Fig. 26). The first major group had 10 members, distributed in three subgroups; one with three *aethiopicums*; the second with three *verticilliflorums*; and the third with two *arundinaceums* along with one *aethiopicum* and one *virgatum*. The second major group with nine members also comprised of three subgroups: the first with a single *arundinaceum*, the second with one *arundinaceum*, one *virgatum* and one *vercilliflorum*, and the third with three *arundinaceums* and two *verticilliflorums*. The third major group comprised of a *verticilliflorum* from South Africa and a *virgatum* from Sudan. The cophenetic correlation indicated that the observed distances were better represented by the MDS ($r = 0.93$) rather than the dendrogram ($r = 0.75$).

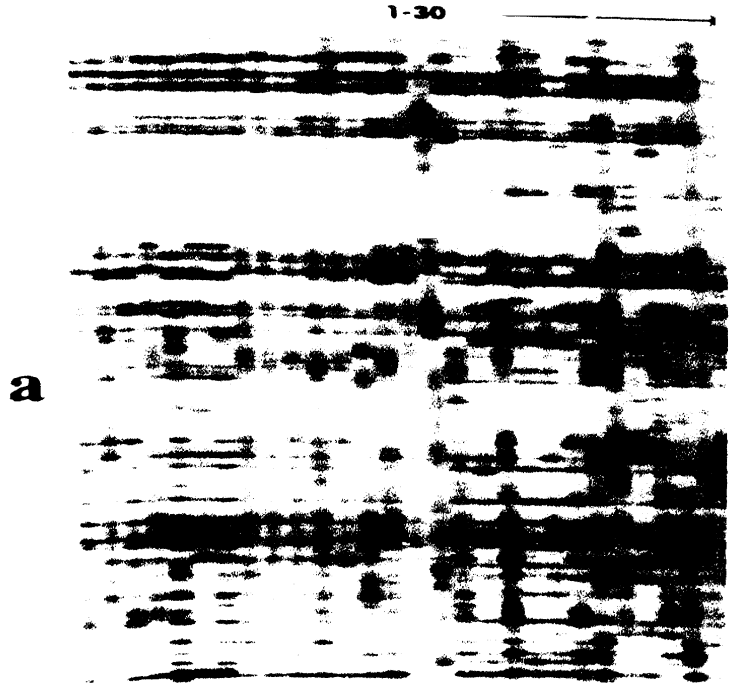
Fig. 17 Representative AFLP profiles of Races of *S. bicolor* subsp. *verticilliflorum*

The primer combinations are :

a. E ACT - M CAT

b. E ACT - M CTG

The accessions in the gel from L to R are listed in Table 7.


1-30
a

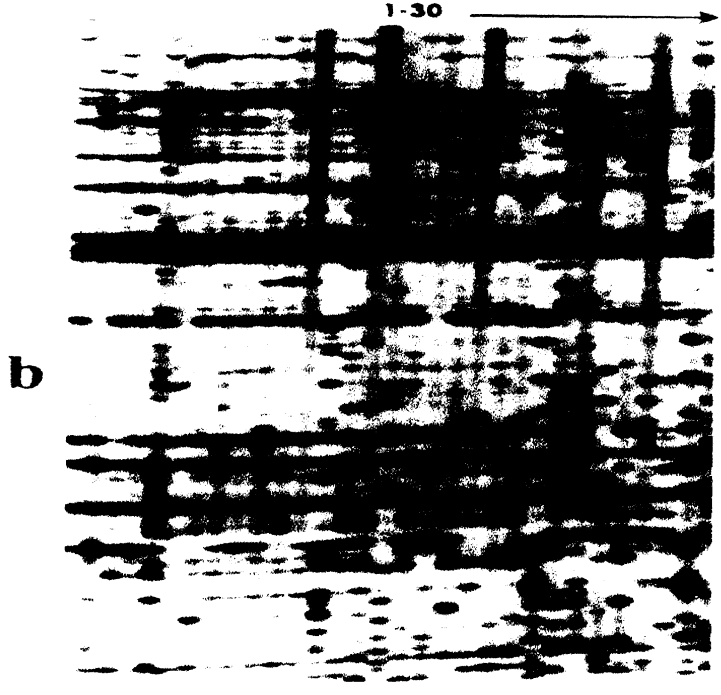

1-30
b

Table 18 Gene Diversity and Differentiation in subsp. *verticilliflorum* with AFLPs

	Polymorphism (%)	Gene Diversity (H)	F_{ST}
Overall	60%	0.15	
Race			
aethiopicum	36%	0.13	0.17±0.03
arundinaceum	44%	0.12	(0.12-0.23)*
verticilliflorum	40%	0.13	
virgatum	33%	0.13	
Region			
Central Africa	31%	0.12	0.08±0.02
Eastern Africa	63%	0.16	(0.04-0.12)*
Southern Africa	33%	0.13	
Western Africa	35%	0.11	
*: Bootstrap - based 95% Confidence Interval			

Table 19 Intra- and Inter-Racial similarities in subsp. *verticilliflorum* with AFLPs

Race	*aethiopicum*	*arundinaceum*	*verticilliflorum*	*virgatum*
aethiopicum	59 ± 0.05			
	(0.55 - 0.68)*			
arundinaceum	0.56 ± 0.06	0.61 ± 0.08		
	(0.46 - 0.64)*	(0.48 - 0.74)*		
verticilliflorum	0.57 ± 0.05	0.57 ± 0.04	0.61 ± 0.05	
	(0.48 - 0.68)*	(0.49 - 0.62)*	(0.54 - 0.72)*	
virgatum	0.49 ± 0.06	0.5 ± 0.05	0.56 ± 0.07	0.53 ± 0.01
	(0.41 - 0.59)*	(0.43 - 0.61)*	(0.46 - 0.67)*	(0.52 - 0.54)*
*: Bootstrap - based 95% Confidence Interval				

Table 20 Polymorphism and Gene Diversity in subsp. *verticilliflorum* with SSRs

S. No.	SSR Locus ID	Fragment Size (bp)	No.of Alleles	Gene Diversity
1	Sb 1-1	230 - 280	9	0.848
2	Sb1-10	240 - 300	10	0.892
3	Sb 4-15	120 - 140	9	0.846
4	Sb 4-22	300 - 330	2	0.331
5	Sb 4-32	170 - 230	9	0.892
6	Sb 4-121	210 - 235	4	0.688
7	Sb 5-236	170 - 200	9	0.902
8	Sb 6-36	150 - 190	10	0.715
9	Sb 6-57	290 - 310	4	0.660
10	Sb 6-84	180 - 220	10	0.911
mean			7.6	0.77
% polymorphism			100	

Fig. 18 UPGMA Dendrogram of Races of subsp. *verticilliflorum* using AFLPs

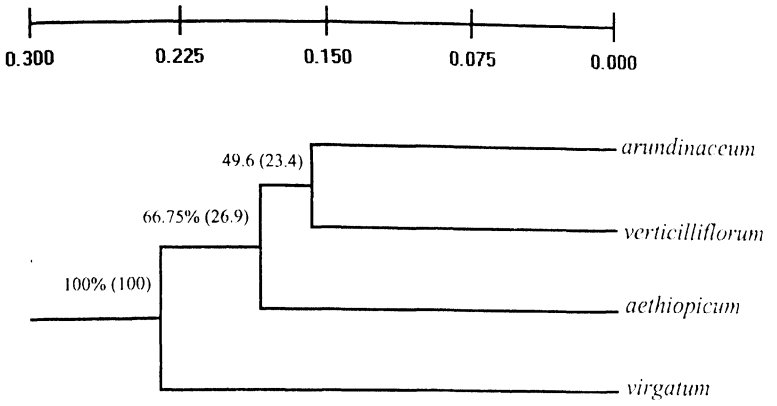


Fig. 19 UPGMA Dendrogram of Regional Populations of subsp. *verticilliflorum* using AFLPs

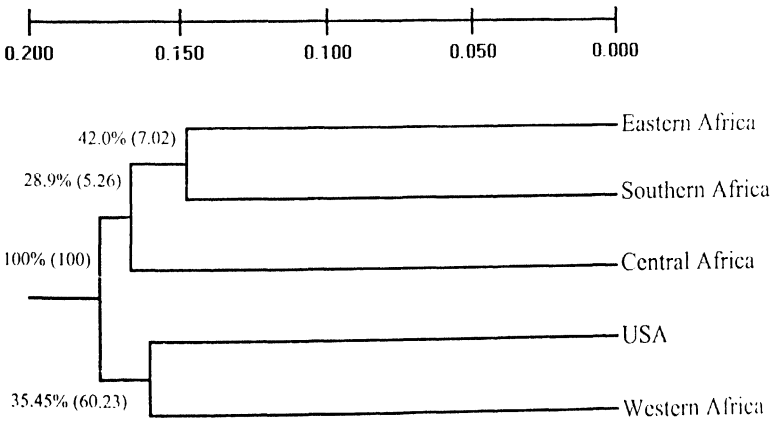


Fig. 20 MDS Plot of 21 Accessions of subsp. *verticilliflorum* with AFLPs (r = 0.928)

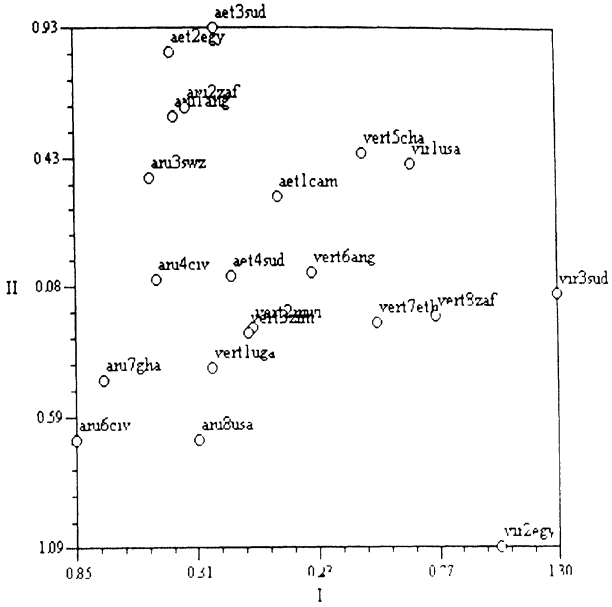


Fig. 21 UPGMA Dendrogram of 21 Accessions of subsp. *verticilliflorum* with AFLPs (r = 0.747)

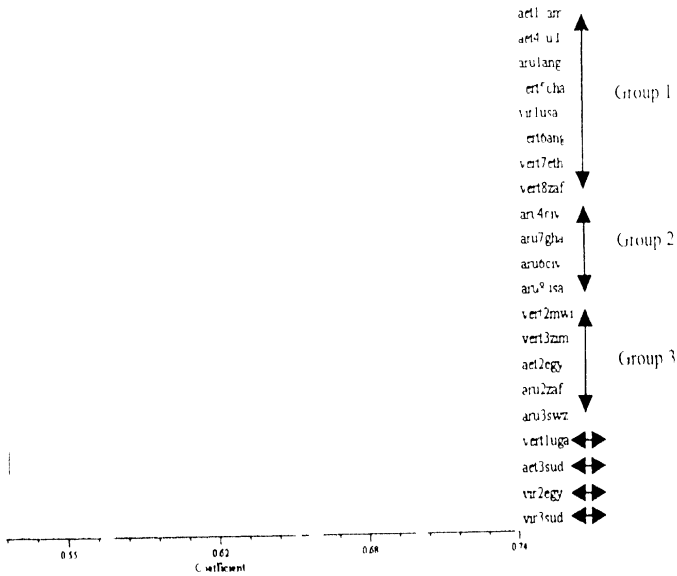


Fig. 22 SSR profiles of Races of *S. bicolor* subsp. *verticilliflorum*

The primer combinations are :

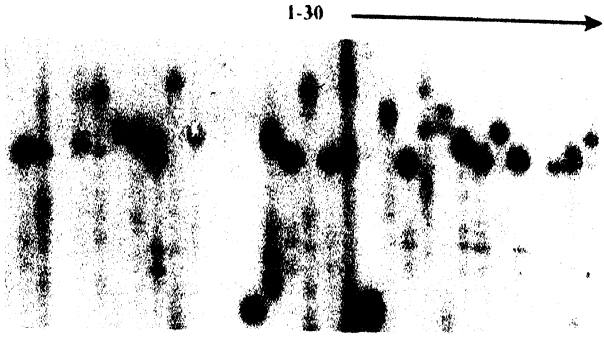
a. SSR Sb 1 - 1

b. SSR Sb 4 - 22

c. SSR Sb 6 - 57

d. SSR Sb 4 - 121

The accessions in the gel from L to R are listed in Table 7.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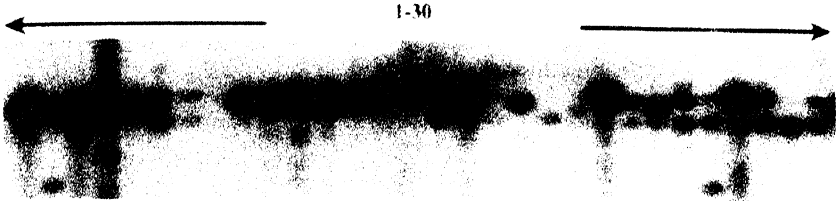
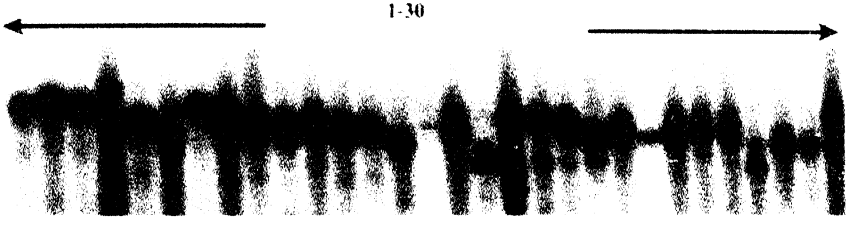

1-30


1-30


1-30

Table 21 Racial Gene Diversity in subsp. *verticilliflorum* with SSRs

S. No.	SSR Locus ID			Race							
				aethiopicum		*arundinaceum*		*verticilliflorum*		*virgatum*	
		No of alleles	H*	No of alleles	H*	No of alleles	H*	No of alleles	H*	No of alleles	H*
1	Sb 1-1	9	0.85	2	0.53	3	0.70	5	0.85	3	0.80
2	Sb 1-10	10	0.89	4	0.85	4	0.84	5	0.79	3	0.80
3	Sb 4-15	9	0.86	3	0.71	3	0.66	4	0.75	3	0.80
4	Sb 4-22	2	0.33	1	0.00	2	0.36	2	0.48	1	0.00
5	Sb-4-32	9	0.89	1	0.00	4	0.86	7	0.92	3	0.80
6	Sb-4-121	4	0.69	2	0.43	3	0.66	3	0.66	3	0.80
7	Sb5-236	9	0.90	2	0.57	5	0.89	4	0.79	3	0.80
8	Sb 6-36	10	0.72	1	0.00	6	0.88	5	0.79	2	0.53
9	Sb 6-57	4	0.66	3	0.71	2	0.26	1	0.00	2	0.53
10	Sb 6-84	10	0.91	2	0.67	4	0.80	4	0.79	3	0.80
	Total	76		21		36		40		26	
	Avg. H		0.77		0.45		0.63		0.68		0.67
Polymorphism (%)			100%		70%		100%		90%		90%

*H: Gene Diversity

Table 22 Regional Gene Diversity in subsp. *verticilliflorum* with SSRs

S. No.	SSR Locus ID			Region							
		C. Africa		*E. Africa*		*S. Africa*		*W. Africa*		*USA*	
		No of alleles	H*	No of alleles	H*	No of alleles	H*	No of alleles	H*	No of alleles	H*
1	Sb 1-1	2	0.67	5	0.85	3	0.71	4	0.80	2	0.67
2	Sb 1-10	2	0.53	6	0.88	2	0.57	5	0.89	2	0.67
3	Sb 4-15	3	0.80	5	0.84	3	0.71	2	0.36	2	0.67
4	Sb 4-22	1	0.00	2	0.53	1	0.00	2	0.36	1	0.00
5	Sb-4-32	2	0.67	4	0.86	3	0.80	3	0.71	2	0.67
6	Sb-4-121	3	0.80	4	0.75	2	0.57	2	0.53	2	0.67
7	Sb5-236	2	0.53	5	0.85	4	0.86	3	0.80	2	0.67
8	Sb 6-36	3	0.80	1	0.00	4	0.86	4	0.80	2	0.67
9	Sb 6-57	2	0.53	4	0.79	2	0.57	3	0.62	1	0.00
10	Sb 6-84	2	0.67	5	0.89	2	0.53	4	0.80	1	0.00
	Total	22		41		26		32		17	
	Avg. H		0.60		0.72		0.62		0.67		0.47
Polymorphism (%)			90%		90%		90%		100%		70%

*H: Gene Diversity

Fig. 23 UPGMA Dendrogram of Races of subsp. *verticilliflorum* with SSRs

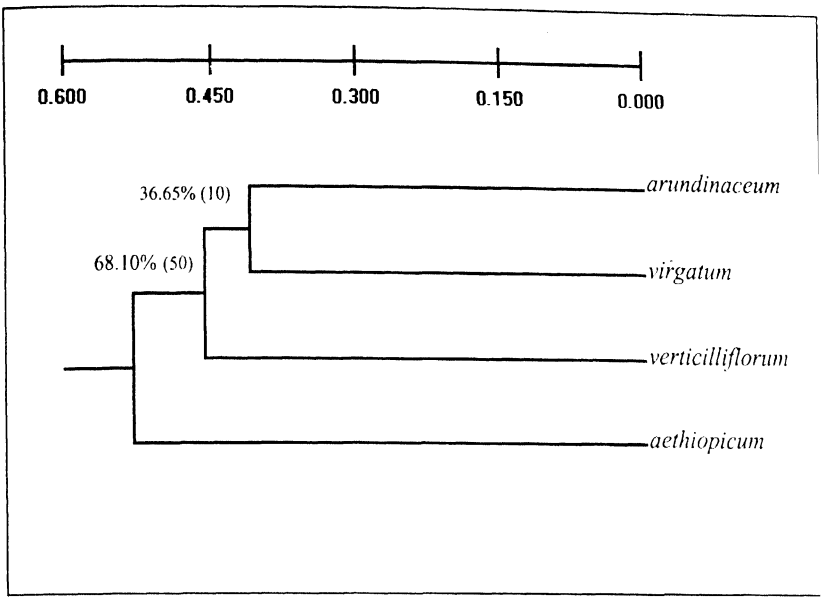


Fig. 24 UPGMA Dendrogram of Regional Populations of subsp. *verticilliflorum* using SSRs

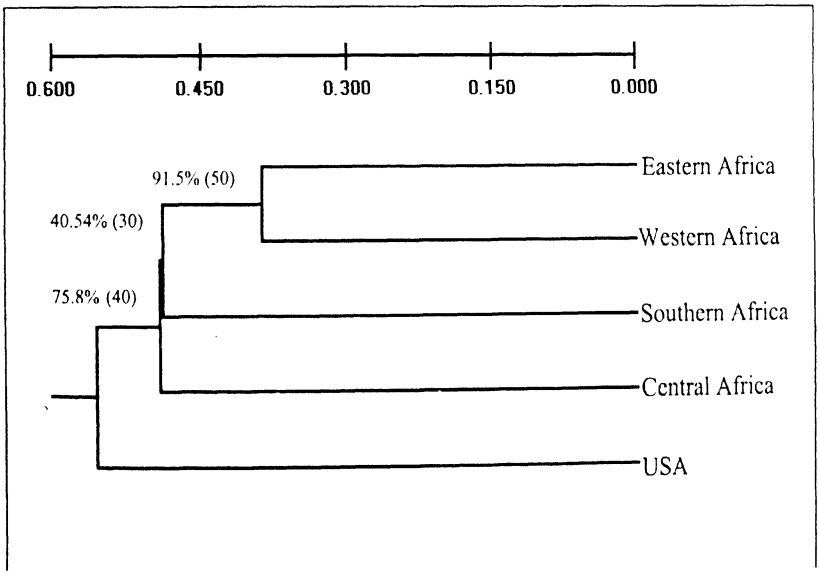


Fig. 25 MDS Plot of 21 Accessions of subsp. *verticilliflorum* using SSRs (r = 0.823)

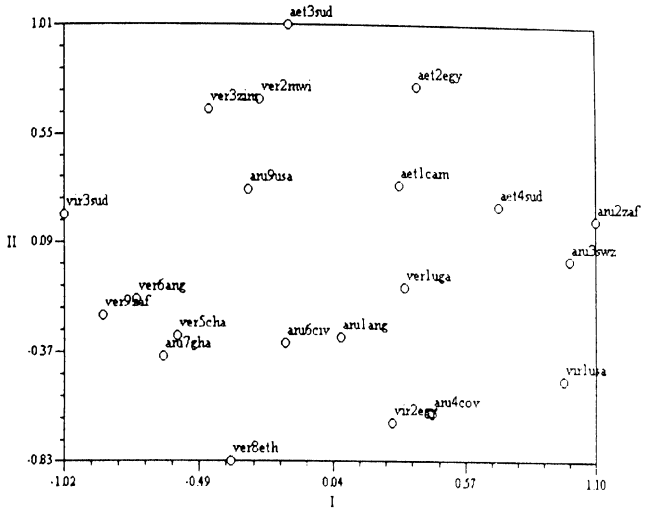
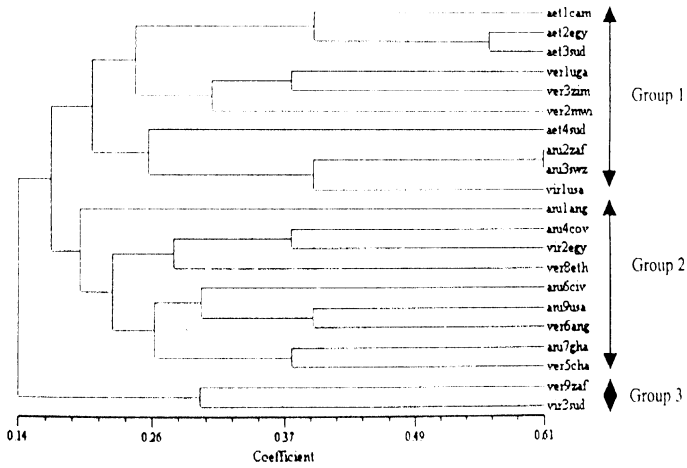


Fig. 26 UPGMA Dendrogram of 21 Accessions of subsp. *verticilliflorum* with SSRs (r = 0.609)



Evaluation of Wild Sorghums for Host Plant Resistance

Evaluation for Resistance to Sorghum Downy Mildew

The responses of different accessions to the pathogen, *Peronosclerospora sorghi*, studied under green house conditions using spray-inocculation techniques, are presented in Table 23 and Fig. 27. The disease symptoms are given in Fig. 28. There were significant differences in resistance to downy mildew among the 85 accessions of wild sorghums belonging to 17 *Sorghum* species and the 6 accessions of cultivated sorghum. Thirty six accessions belonging to sections, *heterosorghum, chaetosorghum, stiposorghum* and *parasorghum* did not show any downy mildew infection except for two accessions belonging to *parasorghum*, [IS 18951 - *S.purpureosericeum* and IS 23177 - *S.versicolor*], which showed about 3% infection. Accessions of *hetero-, chaeto-* and *stiposorghum* were all from Australia, while the *parasorghums* were from Africa, Asia and Australia. Among the wild accessions of section *sorghum*, two accessions, [one each in races *aethiopicum* (IS 18821) and *arundinaceum* (IS 18882), and one weedy accession of *S.halepense* (IS 33712)] were also free from downy mildew. Accessions of the race *verticilliflorum* showed the greatest susceptibility (31.4% - 92.4%). Disease incidence in different accessions of races *aethiopicums, arundinaceums* and *virgatum* varied from 0% to 100%.

The six accessions of cultivated sorghums tested showed high levels of disease incidence (43.5% to 90%) except for IS 14383, that remained downy mildew free in successive inoculation tests. However, when 42 plants of the accession IS 14383 were screened by the sandwich test, two plants developed downy mildew symptoms, but the seedlings recovered within 15 days. This cultivated resistant guinea sorghum from Zimbabwe grew to a height of 225-300cm, flowered in 58-60 days, and produced lustrous grain (Table 24).

Fig. 27 Screening of Wild Sorghums for Resistance to Sorghum Downy Mildew

a. Conidial spray inoculation on seedlings, in humid chamber.

b. Resistant and susceptible accessions in green house.

b

Table 23 Reaction of *Sorghum* Accessions to *Peronosclerospora sorghi*

	Species / Subspecies / Race	Source Country	Acc ID	SDM incidence (%)
Sorghum				
	S bicolor subsp ***bicolor***			
1	race *guinea*	Zimbabwe	IS14383	0 0
2	race *kafir*	USA	IS 18773	43 5
3	race *caudatum*	Sudan	IS 21812	71 5
4	race *durra*	India	IS12868	87 0
5	race *bicolor*	USA	IS 611	90 0
6	*hybrid*	India	CSH1	70 7
	S bicolor subsp ***verticilliflorum*** race *aethiopicum*			
1		Egypt	IS 18821	0 0
2		Cameroon	IS 27584	10 9
3		Sudan	IS 18819	30 7
4		Egypt	IS 18820	40 8
5		Sudan	IS 14504	51 1
6		Sudan	IS 18822	75 5
7		Sudan	IS 14485	100 0
	race *arundinaceum*			
1		USA	IS 18882	0 0
2		Ivory Coast	IS 18826	3 1
3		Tanzania	IS 18830	13 4
4		Ivory Coast	IS 18824	15 9
5		Nigeria	IS 19878	3 4
6		South Africa	IS 14501	15 0
7		Angola	IS 14211	60 7
8		Ghana	IS 18883	72 2
9		Kenya	IS 14571	100 0
10		Swaziland	IS 14315	100 0
11		South Africa	IS 14218	100 0
	race *verticilliflorum*			
1		Kenya	IS 20935	31 4
2		South Africa	IS 14278	34 4
3		Malawi	IS 14357	35 1
4		USA	IS 15797	50 5
5		Chad	IS 18802	70 0
6		Zimbabwe	IS 18859	70 4
7		Zimbabwe	IS 18858	72 4
8		Sudan	IS 18865	72 5
9		Angola	IS 14219	76 2
10		Uganda	IS 14493	0 5
11		Ethiopia	IS 14717	91 0
12		Ethiopia	IS 14717	97 4
	race *virgatum*			
1		Egypt	IS 18815	1 7
2		Egypt	IS 18808	2 1
3		Sudan	IS 18817	42 1
4		Egypt	IS 18805	65 0
5		USA	IS 18803	100 0

Cont

Table 23

	Species / Subspecies / Race	Source Country	Acc. ID	SDM incidence (%)
	S. halepense			
1		India	IS 33712	0
2		India	IS 18849	16.8
3		USA	IS 18897	21.1
4		India	IS 18847	28.5
5		USA	IS 18899	31.7
6		Angola	IS 14212	44.0
7		India	IS 18845	49.5
8		Angola	IS 14263	57.3
9		USA	IS 18891	62.4
10		South Africa	IS 14299	94.2
Chaetosorghum				
1	***S. macrospermum***	N. Territory, Australia	TRC-241162	0.0
Heterosorghum				
1	***S. laxiflorum***	N. Territory, Australia	TRC-243486	0.0
2		N. Territory, Australia	TRC-243492	0.0
3		Australia	IS 18958	0.0
Parasorghum				
1	***S. australiense***	Australia	IS 18954	0.0
2		Australia	IS 18955	0.0
3		Australia	IS 18956	0.0
1	***S. brevicallosum***	N. Territory, Australia	TRC-243491	0.0
2		Australia	IS 18957	0.0
3		Australia	RN401	0.0
1	***S. matarankense***	N. Territory, Australia	TRC-243576	0.0
2		Australia	RN341	0.0
1	***S. nitidum***	Queensland, Australia	TRC-243514	0.0
1	***S. purpureosericeum***	India	RN285	0.0
2		Tanzania	IS 18943	0.0
3		India	IS 18947	0.0
4		India	IS 18951	3.1
1	***S. purpureosericeum***	Sudan	IS 18944	0.0
2		Sudan	IS 18945	0.0
1	***S. timorense***	N. Territory, Australia	TRC-243437	0.0
2		N. Territory, Australia	TRC-243498	0.0
1	***S. versicolor***	South Africa	IS 18926	0.0
2		Angola	IS 14262	0.0
3		South Africa	IS 14275	0.0
4		South Africa	IS 18940	0.0
5		Tanzania	IS 18941	0.0
6		Tanzania	IS 23177	3.2
Stiposorghum				
1	***S. angustum***	Queensland, Australia	TRC-243598	0.0
2		Queensland, Australia	TRC-243499	0.0
1	***S. ecarinatum***	N. Territory, Australia	TRC-243574	0.0
1	***S. extans***	N. Territory, Australia	TRC-243601	0.0
1	***S. extans***	N. Territory, Australia	TRC-243601-	0.0
1	***S. intrans***	N. Territory, Australia	TRC-243571	0.0

Contd.

Table 23

	Species / Subspecies / Race	Source Country	Acc. ID	SDM incidence (%)
2	*S. intrans*	N. Territory, Australia	TRC-243602	0.0
1	*S. interjectum*	Australia	TRC-243461	0.0
1	*S. stiposorghum*	W. Australia. Australia	TRC-243399	0.0
Sorghum				
1	*S. bicolor* (SC)		DMS 652	85.0
1	*S. bicolor* (RC)		QL 3	0.0
		SE_d		3.7

Estimated Variance Components	
Source of Variation	Variance Component
Accession	509.36 ± 71.17
Year	3.77 ± 8.39
Accession x Year	0.00 ± 7.81

Notes: SC=Susceptible check; RC=Resistant check

Table 24 Characteristics of the Cultivated IS 14383 identified as resistant to SDM

	Character	Description
1	Plant Height (cm) rabi / Kharif	225 / 300
2	No. of Basal tillers	2
3	Days to 50% flowering rabi / kharif	58 / 60
4	Peduncle exertion (cm)	14
5	Panicle length (cm)	20
6	Panicle width (cm)	8
7	Seed size (mm)	2
8	100 seed weight (g)	2.09
9	Nodal tillering	Present
10	Seed lustre	Lustrous
11	Subcoat	Absent
12	Mid rib colour	Dull green
13	Earhead shape	Semi-loose stiff branches
14	Glume colour	Straw and Purple
15	Glume covering	Half grain covered
16	Grain colour	White
17	Endosperm texture	Mostly corneous
18	Threshability	Partly threshable

Fig. 28 Sorghum plants with symptoms of Downy Mildew

a. Pale chlorotic streaking of systemic infection.

b. Leaf shredding in older plants.

c. Comparison of resistant and susceptible cultivars.

1. QL 3 (Resistance check)
2. IS 14383 (Identified resistant)
3. DMS 652 (Susceptible check)
4. CSH 1 (Improved susceptible cultivar)
5. ICSV 112 (Improved susceptible cultivar)


a


b


1
2
3
4
5
c

Evaluation for Resistance to Sorghum Shoot Fly

Field Screening

Fifty five accessions of wild sorghums were screened for resistance to sorghum shoot fly in the field using the interlard fish meal technique (Fig 29). There were highly significant differences among the accessions for response to *Atherigona soccata* infestation during the rainy seasons of 1998 and 1999 (Table 25). Percentage of plants with eggs and deadheart incidence ranged from 0 to 100%. Average number of eggs per plant varied from 0 to 1.2 in 1998 and from 0 to 3.2 in 1999. Percentage of plants with eggs and deadhearts during 1998 and 1999 were 98.3% and 96.7% in the susceptible check CSH-1, while the resistant checks IS 18551 had 48.2% and 31.4%, and IS 2146, 46.0% and 31.4% respectively. Within section *sorghum*, all the four races showed high levels of susceptibility to shoot fly, as did *S.halepense*. Maximum oviposition and deadheart formation in the two years ranged from 86.4% to 99.2%, and 70.7% to 98.6% respectively. In comparison, the lone accession of *chaetosorghum* (*S.macrospermum*) showed 15.7% oviposition and 6.7% deadheart formation, while in *heterosorghum* (*S.laxiflorum*) the values ranged from 0 to 3.3% for both oviposition and deadheart formation. There was no oviposition on the *stiposorghums*. Average number of eggs per plant was zero for *stiposorghum*, and ranged from 1 to 2.1 for section *sorghum*. Among the cultivated sorghums, the moderately resistant cultivar Maldandi (IS 1054) showed 88.5% plants with eggs and 66.4% plants with deadhearts. Accession x year interaction was significant for all three variables i.e., percentage of plants with eggs, deadhearts % and average number of eggs per plant.

When the accessions were grouped into wild species (*hetero-, chaeto-, para- and stiposorghum* and *S.halepense* of *sorghum*), wild races (*S.bicolor* subsp. *verticilliflorum*), resistant checks (IS 18551 and IS 2146), and susceptible check (CSH 1), there were significant differences between the groups for percent plants with eggs and average number of eggs per plant, and shoot fly deadhearts. Group x year interaction effects were non-significant for percentage of plants oviposited and deadheart formation, but significant for number of eggs per plant (Table 26).

When the data were analysed to compare taxonomic sections, the differences among sections, years, and section x year interactions were highly significant (Table 27). Comparisons among years for sections *hetero-, para-* and *stiposorghums*, were however non-significant based on the interaction LSD values. Section *sorghum* showed significant differences in both percentage of plants oviposited and number of eggs per plant but not for deadheart formation. Section *chaetosorghum* showed significant interaction effect only for percentage of plants with eggs but such effects were non-significant for both average number of eggs per plant and deadheart formation. The four sections,

namely, *chaeto-*, *hetero-*, *para-* and *stiposorghum*, were significantly different from section *sorghum* for all the three variables.

Mechanisms of Resistance

Non-Preference for Oviposition

Under no-choice conditions in greenhouse, overall there were significant differences in oviposition, deadheart formation and eggs per plant among the 22 wild accessions (Table 28). Differences between resistant and susceptible checks were significant for deadheart formation but not for either plants oviposited or average number of eggs per plant. *Sorghum macrospermum* of *chaetosorghum* was not significantly different from resistant check for any of the three variables but was significantly different from susceptible check for deadheart formation. Accessions of *stiposorghum* were significantly different from both the resistant and susceptible checks for percentage of plants oviposited, deadheart formation and average number of eggs per plant. There was no oviposition on *S.extans* and *S.stipoideum*. Accessions of *heterosorghum* showed significantly less number of deadhearts compared to the resistant check IS 18551 and had significantly less number of eggs compared to the susceptible check CSH 1. Accessions of *parasorghum* showed variable responses in comparison to the susceptible and resistant checks. All accessions showed significantly less deadheart formation in comparison to the resistant check IS 18551 and, except for the two accessions, *S.timorense* (TRC-243498) and *S.purpureosericeum* (IS 18943), also showed significantly less number of plants with eggs. However, based on number of eggs per plant, only six out of the 13 accessions were significantly less oviposited in comparison to resistant check IS 18551. There was no oviposition on *S.matarankense*. *S.versicolor* (IS 23177) exhibited only 6.2% deadhearts in spite of 51.7% oviposition, whereas when tested under field conditions it showed no oviposition (Table 23). Similar results were also obtained with a few accessions from other sections. Response of *S.interjectum* and *S.purpureosericeum* to shoot fly infestation is given in Fig. 30.

Antibiosis

When the plants were infested artificially with shoot fly eggs in the greenhouse, significant differences were observed among all test accessions for percentage deadheart formation and adult emergence (Table 29).

Shoot Fly Deadhearts

Stiposorghums showed the least deadheart formation ranging from 0 to 5.4%. *Sorghum extans* and *S.stipoideum* did not show any deadhearts. Among the *parasorghums*, no deadhearts were recorded in *S.matarankense* (TRC-243576) and *S.purpureosericeum*

(IS 18944) whereas, percentage deadheart incidence ranged from 12.7% to 88.9% among other accessions. The two accessions of *heterosorghum* showed 31.2% and 50.8% deadhearts. The accession TRC-243492 of *heterosorghum* exhibited higher incidence of deadhearts (31.2%) when seedlings were artificially infested with shoot fly eggs in the greenhouse compared to deadhearts obtained either under artificial fly infestation (no-choice conditions) in greenhouse (7.4%; Table 28) or under natural infestation in the field (0%; Table 23). Similar incidence was observed among the *parasorghums*, where most of the accessions showed increased deadheart formation under artificial egg infestation in no-choice greenhouse conditions compared to both artificial fly infestation in the greenhouse and natural fly infestation in the field (Tables 28, 23). The four wild races of *S.bicolor* subsp. *verticilliflorum* of section *sorghum* showed maximum deadhearts (54.8% to 100%). The susceptible check, CSH 1, showed a deadheart incidence of 98.4% on par with field conditions, while the resistant check IS 18551 showed an increased deadheart percentage (93.65%) compared to field conditions (31.4%) and under artificial fly infestation under no-choice conditions in green house (70.2%).

Larval Survival and Adult Emergence

Maximum adult emergence was observed in the wild accessions of section *sorghum* from 45.8% in race *arundinaceum* to 99.5% in race *aethiopicum* (Table 29). In comparison, the susceptible check, CSH 1 showed 79.4% adult emergence and resistant check showed 50.8%. The larval and pupal period ranged from 14 -16 days for CSH 1, and 14 -18 days for IS 18551. The four wild races were comparable to susceptible CSH-1; whereas in *S.halepense*, the larval and pupal duration was slightly extended (18-22 days). In *heterosorghum*, in spite of 31.2% and 50.8% percentage of deadhearts, very few flies emerged (14.6% in TRC-243492 and 6.2% in IS 18958). Similar results were obtained among the *parasorghums* where adult emergence ranged from 4.2% (*S.australiense* IS 18955) to 45.8% (*S.timorense*). In *S.versicolor* (IS 23177), deadhearts increased to 25.9% when infested with eggs artificially under greenhouse conditions, as compared to 6.2% deadhearts when flies were allowed to oviposit on seedlings under no-choice condition, and 0% under field conditions. However, very few flies emerged (26.3%) under greenhouse conditions in spite of 25.9% deadhearts. There was no adult emergence in *S.nitidum* and *S.versicolor* (IS 14262) in spite of 51.8% and 19.4% deadhearts respectively. The *stiposorghums* showed no adult emergence despite the presence of 3-5% deadhearts. Dead, first instar larvae were observed at or near the growing tip of the main shoot when the few deadhearts were dissected to check for larval survival. Larval mortality was also observed in the main stems of deadhearts of the *para-* and *heterosorghums*. Larval and pupal duration ranged from 15-19 days in the *heterosorghums* and from 15-24 days in the *parasorghums*.

Fig. 29 Screening for Resistance to Sorghum Shoot Fly

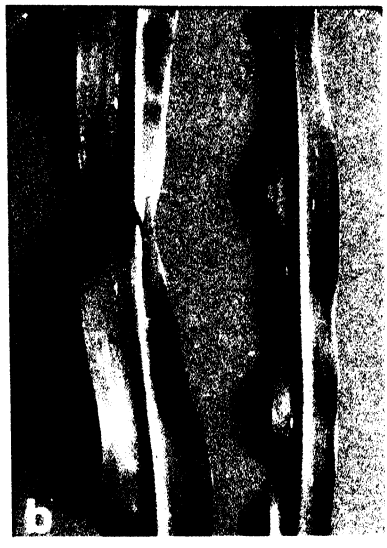
a. Seedling with deadheart symptom with the Sorghum Shoot fly in the inset.

b. Shoot fly eggs on leaf.

c. Interlard fishmeal technique for field screening.

d, e. Resistant (IS 18551) and susceptible checks (CSH – 1) with deadheart symptoms after artificial infestation with eggs under no-choice conditions.


a


b


c


d


e

Table 25 Field Evaluation of Wild Sorghums for Resistance to Sorghum Shoot Fly

Section /Species/ Subsp./Race	Acc. ID	Plants with Eggs (%)			Deadhearts (%)			Average No. of Eggs / Plant		
		1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Chaetosorghum										
S. macrospermum	TRC-241162	3.3	28.0	15.7	3.3	10.1	6.7	0.0	0.4	0.2
Heterosorghum										
S. laxiflorum	TRC-243486	6.7	0.0	3.3	6.7	0.0	3.3	0.1	0.0	0.0
	TRC-243492	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18958	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Parasorghum										
S. australiense	IS 18954	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18955	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18956	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. brevicallosum	TRC-243491	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18957	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. matarankense	TRC-243576	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	RN341	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. nitidum	TRC-243514	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. purpureosericeum	RN285	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18943	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18947	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18951	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18944	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18945	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. timorense	TRC-243437	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TRC-243498	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. versicolor	IS 18926	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 23177	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 14262	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 14275	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18940	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IS 18941	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Stiposorghum										
S. angustum	TRC-243598	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. angustum	TRC-243499	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. ecarinatum	TRC-243574	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. extans	TRC-243601	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. interjectum	TRC-243461	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. intrans	TRC-243571	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. intrans	TRC-243602	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S. stipoideum	TRC-243399	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sorghum										
S.bicolor subsp. *verticilliflorum*										
race *aethiopicum*	IS 27584	98.3	100.0	99.2	88.3	89.5	88.9	1.0	1.9	1.5
	IS 18819	85.0	100.0	92.5	85.0	100.0	92.5	0.9	2.8	1.8
	IS 14564	97.2	100.0	98.6	97.2	100.0	98.6	0.9	1.9	1.4
race *arundinaceum*	IS 18883	79.8	100.0	89.9	74.2	95.6	84.9	0.8	1.6	1.2
	IS 18826	86.7	100.0	93.3	78.3	100.0	89.2	0.9	1.8	1.3
	IS 18830	96.3	100.0	98.2	84.4	100.0	92.2	1.2	2.7	2.0

Contd.

Table 25

Section /Species/ Subsp./Race	Acc. ID	Plants with Eggs (%)			Deadhearts (%)			Average No. of Eggs / Plant		
		1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
race *verticilliflorum*	IS 18865	91.7	100.0	95.8	83.3	100.0	91.7	1.0	2.4	1.7
	IS 14717	90.2	100.0	95.1	85.1	100.0	92.5	0.9	2.8	1.9
	IS 20995	95.4	100.0	97.7	91.7	100.0	95.8	1.0	3.2	2.1
race *virgatum*	IS 18803	97.8	100.0	98.9	97.8	86.7	92.2	1.0	1.6	1.3
	IS 18813	90.4	100.0	95.2	85.0	100.0	92.5	1.0	2.6	1.8
S. halepense	IS 18891	86.1	97.0	91.5	73.9	89.4	81.6	1.1	2.3	1.7
	IS 14299	85.2	100.0	92.6	74.7	88.9	81.8	0.9	1.3	1.1
	IS 14212	80.5	92.3	86.4	53.3	88.1	70.7	0.8	1.5	1.1
	IS 18845	93.3	100.0	96.7	89.4	88.9	89.2	1.1	1.8	1.4
	IS 18849	81.7	100.0	90.8	73.3	81.7	77.5	1.0	1.6	1.3
S. bicolor (SC)	CSH 1	96.7	100.0	98.3	93.3	100.0	96.7	1.0	2.0	1.5
S. bicolor (SC)	ICSV 112	100.0	100.0	100.0	86.7	100.0	93.3	1.1	1.7	1.4
S. bicolor (MRC)	IS 1054	76.9	100.0	88.5	47.6	85.2	66.4	0.8	1.6	1.2
S. bicolor (RC)	IS 18551	45.8	50.6	48.2	32.3	30.6	31.4	0.5	0.5	0.5
S. bicolor (RC)	IS 2146	40.2	51.8	46.0	30.2	32.5	31.4	0.4	0.5	0.5
F Prob Acc. ID		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
F Prob Acc. ID x Year		<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
LSD (5%) Acc. ID				5.81			8.68			0.24
LSD (5%) Acc. ID x Year				8.23			12.28			0.34

Notes: SC=Susceptible check; MRC=Moderately resistant check; RC=Resistant check

Table 26 Field Evaluation of Group Interactions for Resistance to Sorghum Shoot Fly

Group	Plants with eggs (%)			Deadhearts (%)			Average No. of Eggs/Plant		
	1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
MRC	76.90	100.00	88.50	47.60	85.20	66.40	0.80	1.56	1.18
RC	43.00	51.00	47.10	31.30	31.50	31.40	0.44	0.51	0.48
SC	98.30	100.00	99.10	90.00	100.00	95.00	1.04	1.84	1.44
Wild Races	91.70	100.00	95.90	86.40	97.40	91.90	0.96	2.29	1.63
Wild Species	10.70	12.70	11.70	9.20	11.00	10.10	0.12	0.22	0.17
F Prob Group			< .001			< .001			< .001
F Prob Group x Year			0.790			0.270			< .001
LSD (5%) Group			29.920			27.050			0.530
LSD (5%) Group x Yr.			42.31^{NS}			38.25^{NS}			0.750

^{NS}: Not Significant

Notes: SC=Susceptible check; MRC=Moderately resistant check; RC=Resistant check

Table 27 Field Evaluation of Section Interactions for Resistance to Sorghum Shoot Fly

Section	Plants with eggs (%)			Deadhearts (%)			Avg. Eggs/Plant		
	1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Sorghum	85.50	94.80	90.20	76.40	88.40	82.40	0.90	1.90	1.40
Chaetosorghum	3.30	28.00	15.70	3.30	10.10	6.70	0.00	0.40	0.20
Heterosorghum	1.30	0.00	0.70	1.40	0.00	0.70	0.00	0.00	0.00
Parasorghum	0.20	0.20	0.20	0.20	0.20	0.20	0.00	0.00	0.00
Stiposorghum	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F Prob Section			**<0.001**			**<0.001**			**<0.001**
F Prob Section x Year			**<0.001**			**<0.003**			**<0.001**
LSD (5%) Section			**11.480**			**15.310**			**0.400**
LSD (5%) Section x Year			**16.170**			**21.570**			**0.570**

Table 28 Greenhouse Evaluation of Wild Sorghums for non-preference to Sorghum Shoot Fly Oviposition under No-Choice conditions

	Species	Acc. ID	Plants with Eggs (%)	Deadhearts (%)	Average No. of Eggs / Plant
Chaetosorghum					
1	*S. macrospermum*	TRC-241162	76.60	61.50	1.85
Heterosorghum					
1	*S. laxiflorum*	TRC-243492	61.30	7.40	0.85
2		IS 18958	80.50	26.40	0.91
Parasorghum					
1	*S. australiense*	IS 18955	16.60	10.10	0.23
2		IS 18956	15.80	5.80	0.57
1	*S. matarankense*	TRC-243576	0.00	0.00	0.00
1	*S. purpureosericeum*	IS 18944	1.80	0.40	0.03
2		IS 18945	15.20	2.80	0.25
3		RN285	13.00	3.70	0.75
4		IS 18943	73.20	50.30	2.30
5		IS 18947	52.70	24.60	1.38
1	*S. nitidum*	TRC-243514	57.60	9.70	1.31
1	*S. timorense*	TRC-243498	100.00	21.10	2.27
1	*S. versicolor*	IS 23177	51.70	6.20	1.09
2		IS 14262	2.10	0.00	0.02
3		IS 14275	44.20	10.50	0.92
Stiposorghum					
1	*S. angustum*	TRC-243499	9.00	4.00	0.17
1	*S. ecarinatum*	TRC-243574	8.50	3.50	0.09
1	*S. intrans*	TRC-243571	7.10	1.10	0.07
1	*S. extans*	TRC-243601	0.00	0.00	0.00
1	*S. interjectum*	TRC-243461	2.30	1.20	0.02
1	*S. stipoideum*	TRC-243399	0.00	0.00	0.00
Sorghum					
1	*S. bicolor* (SC)	CSH 1	100.00	97.10	2.62
1	*S.bicolor* (RC)	IS 18551	71.30	70.20	1.99
	F Prob		**< 0.001**	**< 0.001**	**< 0.001**
	LSD (5%)		**30.38**	**19.62**	**1.32**

Notes: SC=Susceptible check; RC=Resistant check

Fig. 30 Response of *para-* and *stiposorghums* after artificial infestation with Shoot Fly

a. Immune *S. interjectum* (*stiposorghum*) without deadhearts.

b. Highly resistant *S. purpureosericeum* (*parasorghum*) with one deadheart.

Inset: Seedling with deadheart and dense hair on leaf surface and sheath.


a


S. purpureosericeum
b

Table 29 Shoot Fly emergence in Wild Sorghums after infestation with eggs under No-Choice conditions in Greenhouse

	Species/Subsp./Race	Acc. ID	Deadhearts (%)	Adult emergence (%)	Days to adult emergence
Heterosorghum					
1	*S. laxiflorum*	TRC-243492	31.20	14.62	15 - 19
2		IS 18958	50.80	6.20	15 - 19
Parasorghum					
1	*S. australiense*	IS 18955	28.80	4.20	17.00
2		IS 18956	30.40	6.10	15 - 19
1	*S. matarankense*	TRC-243576	0.00	0.00	-
1	*S. nitidum*	TRC-243514	51.80	0.00	-
1	*S. purpureosericeum*	RN 285	88.90	32.30	13 - 16
2		IS 18943	36.90	44.80	13 - 16
3		IS 18944	0.00	0.00	-
4		IS 18945	12.70	0.00	-
5		IS 18947	61.40	43.50	15 - 19
1	*S. timorense*	TRC-243498	42.40	45.80	15 - 19
2	*S. versicolor*	IS 23177	25.90	26.30	15 - 24
3		IS 14262	19.40	0.00	
1		IS 14275	37.40	31.90	15 - 24
Stiposorghum					
1	*S. angustum*	TRC-243499	4.80	0.00	-
1	*S. ecarinatum*	TRC-243574	5.40	0.00	-
1	*S. extans*	TRC-243601	0.00	0.00	-
1	*S. interjectum*	TRC-243461	4.10	0.00	-
1	*S. intrans*	TRC-243571	3.10	0.00	-
1	*S. stipoideum*	TRC-243399	0.00	0.00	-
Sorghum					
1	*S. halepense*	IS 14212	95.10	68.90	18 - 22
	S.bicolor subsp. *verticilliflorum*				
1	race *aethiopicum*	IS 14564	98.40	99.50	14 - 16
1	race *arundinaceum*	IS 18826	54.80	45.80	14 - 16
1	race *verticilliflorum*	IS 18865	98.40	78.50	14 - 16
1	race *virgatum*	IS 18808	100.00	89.00	14 - 16
1	*S. bicolor* (RC)	IS 18551	93.65	50.80	14 - 18
1	*S. bicolor* (SC)	CSH 1	98.40	79.40	14 - 16
	F Prob		**< 0.001**	**< 0.001**	
	LSD (5%)		**14.87**	**18.97**	

Notes: SC=Susceptible check; RC=Resistant check

Evaluation for Resistance to Spotted Stem Borer

Field Screening

There were significant differences among wild relatives of sorghum in their response to artificial infestation with first instar larvae of *Chilo partellus* in the field, 20 days after seedling emergence (Table 30). Leaf damage score (LDS) varied between 1 and 6.8 over the two years as compared to 6.8 in the susceptible check ICSV 1 and 4.8 in the resistant check IS 2205. Percentage of plants damaged ranged from 0 to 100% in both years whereas stem borer deadhearts ranged from 0 to 100% in 1998 and 0 to 97.3% in 1999. In comparison, the susceptible check had 95.9% damaged plants and 93.6% deadhearts, while the resistant check showed 79% damaged plants and 40.8% deadhearts across the two years.

S.macrospermum of *chaetosorghum* showed a mean LDS of 3.7 with 72.9% plant damage and 59.1% deadhearts over the two years. Accessions belonging to section *sorghum* showed a high LDS ranging from 3.3 to 6.8 accompanied by high deadheart formation ranging from 44.8% to 98.1%. Accessions of sections *hetero- para-* and *stiposorghum* showed very low LDS (<1) and produced no deadhearts due to stem borer damage, except for one accession of *heterosorghum* (TRC-243486) which had 2% deadheart formation. Accession x year interactions were significant for the three variables.

Wild species showed significant group x year effect for all three variables (Table 31). Both the resistant check and the improved resistant varieties (ICSV 700, ICSV 708, and ICSV 743) showed significant variation over years for percentage of plants damaged while the latter group also showed significant interaction with year for deadheart formation. The susceptible check showed non-significant interaction with years for percentage of plants damaged, deadheart formation and LDS.

Differences among taxonomic sections were highly significant for all three variables (Table 32). Section x year interactions were significant for percent plants damaged and deadhearts but not for LDS. Section x year interaction was non-significant for all three variables in *hetero-, para- and stiposorghums.* Section *sorghum* showed significant interaction with years for percent plants damaged, but section x year effects for deadhearts and LDS were non-significant. *Chaetosorghum* showed significant interaction with years for all the variables based on interaction LSD. More leaf feeding but less deadheart formation was observed in the second year compared to the first. Except for *chaetosorghum*, the other sections (*para-, hetero-* and *stiposorghums*) were significantly different from the section *sorghum* for all the variables studied.

Mechanisms of Resistance

Twenty seven accessions of wild sorghums were evaluated in the greenhouse to study the mechanisms of resistance (Figs. 31 and 32).

Non–Preference for Oviposition (Limited-Choice and No-Choice Tests)

Significant differences were observed for non-preference to oviposition by the *C.partellus* females in limited-choice tests in greenhouse among the 27 wild sorghum accessions (Table 33). Overall, the average number of egg masses per plant varied from 0 to 4.13 with average number of eggs per plant ranging from 0 to 317.6. Number of eggs per egg mass varied from 0 to 93.4.

Among the *stiposorghums*, there was no oviposition on *S.extans*. *S.interjectum, S.ecarinatum, S.intrans and S.stipoideum* were least preferred for oviposition relative to both checks. *Stiposorghums* had significantly less number of egg masses ranging from 0.2 to 1.6 compared to resistant check (2.8). Average number of eggs per plant ranged from 7.47 to 62.13 compared to 86.53 in the resistant check (Table 33).

Among the *parasorghums*, there was no oviposition on *S.versicolor* (IS 14262 and IS 14275), and *S.purpureosericeum* (IS 18944). Accessions of *S.timorense*, *S.nitidum*, *S.brevicallosum*, *S.purpureosericeum* (RN 285, IS 18947, IS 18943, IS 18945) and *S.australiense* (IS 18956) were least preferred for oviposition relative to ICSV 1 and had significantly less number of egg masses per plant. In addition, *S.timorense* and *S.purpureosericeum* (RN 285, IS 18945, IS 18947) also had significantly less average number of eggs per plant compared to the resistant check (Table 33).

The two accessions of *S.laxiflorum* belonging to section *heterosorghum* were more preferred for egg laying by the *C. partellus* compared to the susceptible and resistant checks, in terms of egg masses and average number of eggs laid per plant.

In the section *sorghum*, none of the four wild races of subsp. *verticilliflorum* exhibited non-preference for oviposition compared to resistant check (Table 33), and were highly preferred for oviposition over susceptible check by the moths.

Accessions of *para*-and *stiposorghums* that exhibited no oviposition in limited-choice tests and those that showed less ovipositional preference were subjected to no-choice tests. Significant differences for number of egg masses (0.07 - 4.27), average number of eggs per plant (0.53 - 141.93) and number of eggs per egg mass (2.67 - 64.78) were observed (Table 34). The *stiposorghums* [*S.ecarinatum*, *S.intrans*, *S.interjectum*] showed significantly less number of egg masses (0.67 - 2.07) and average number of eggs per plant (37.13 - 86.00) relative to both the susceptible and resistant checks even under no-choice conditions. *S.extans* which showed zero percent oviposition in limited-choice tests, showed 0.33 egg masses per plant, 5.87 eggs per plant and 11.56 eggs

per egg mass compared to the resistant check, which showed 3.4 egg masses per plant, 163.67 average number of eggs per plant and 49.63 eggs per egg mass under no-choice conditions.

Among the *parasorghums*, two accessions of *S.versicolor* [IS 14262 and IS 14275] which exhibited zero oviposition under limited-choice conditions showed slight oviposition under no-choice conditions with 0.07 and 0.13 egg masses per plant, 3.67 and 32 eggs per egg mass and 0.73 and 6.4 average number of eggs per plant, respectively. However, values were significantly lower than the resistant check. Accessions of *S.purpureosericeum* (RN 285, IS 18943, IS 18947) also showed significantly less number of egg masses (0.27 - 0.93) and average number of eggs per plant (14.20 - 51.47) compared to the resistant check under no-choice conditions.

Accession, IS 18944 of *S.purpureosericeum*, which displayed zero oviposition in limited-choice tests was observed to be more susceptible than the resistant check under no choice conditions with 2.6 egg masses per plant, 47 eggs per mass and 120.47 average number of eggs per plant. Similar results were observed for a few other accessions, IS 18945, TRC-243498 and IS 18956. The reverse was observed for accession IS 23177 of *S.versicolor* which showed considerably less oviposition in terms of egg masses (0.07), eggs per mass (2.67) and average number of eggs per plant (0.53) under no choice conditions in contrast to higher values obtained in the limited-choice test for the same variables (1.13, 37.92 and 48.47, respectively) (Table 33).

The distribution of egg masses on the upper and lower surfaces of the leaf is given in Table 35 and Figs. 32-34. There were significant differences among the accessions for number of egg masses per plant, total number of eggs per plant as well as number of eggs per egg mass both on upper and lower surfaces of the leaves. Overall, there were more egg masses and eggs on the upper surface except for *S.timorense* (*parasorghum*) and *S.interjectum* (*stiposorghum*). The resistant and susceptible checks differed significantly from each other for total number of eggs per plant but not for number of egg masses or number of eggs per egg mass.

Antibiosis

Spotted Stem Borer Deadhearts

Under no-choice conditions, when plants were infested with first instar-larvae in greenhouse (10 larvae per plant), seedlings of *stiposorghums* and one *parasorghum* (IS 18944) were not damaged and did not show any deadheart formation (Table 36). Seedlings of *parasorghums* showed very little deadheart formation though there was considerable variation among accessions for leaf damage. While *S.australiense* (IS 18956),

S.matarankense, *S.purpureosericeum* (IS 18943, IS 18944, IS 18945), *S.timorense* and *S.versicolor* showed little leaf damage (LDS about 1), accessions of *S.nitidum* and *S.purpureosericeum* (RN 285 and IS 18947) displayed high leaf feeding (2.7 to 6.0 LDS). However, all these accessions produced very few deadhearts (0 - 14.8%). In the two *heterosorghums*, leaf damage was low (1.3 and 1.7 LDS) but, while TRC-243492 produced only 15.3% deadhearts, IS 18958 showed 82.5%. Compared to the *stipo-*, *para-* and *heterosorghums*, accessions of section *sorghum* were highly susceptible and produced maximum deadhearts in greenhouse, the results being similar to those obtained under field conditions (Table 30). Plants of *S.halepense* (IS 14212) and race *virgatum* of subsp. *verticilliflorum* (IS 18808), were highly damaged (LDS = 6), and produced 98.4% and 98.2% deadhearts respectively. These values were comparable to the susceptible check having an LDS of 7 and showed 98.4% deadheart formation. The resistant check also showed high leaf damage (LDS = 6) and 96.8% plant damage but produced significantly less number of deadhearts (43.4%).

Larval Survival and Adult Emergence

When plants with deadhearts were cut open to check for the presence of larvae 15 days after infestation, no larvae were observed in 11 accessions of *parasorghum*, except in IS 18945 (*S.purpureosericeum*) and IS 18956 (*S.australiense*). Only one larva was recovered in each which stopped feeding by the 24th day and died (Table 36). In *S.laxiflorum* of *heterosorghum*, no larvae were observed in the deadhearts in TRC-243492, but six larvae were recorded from 20 deadhearts in IS 18958 all of which stopped feeding and died within 28 to 30 days. There was no larval survival beyond 30 days and consequently no adult emergence was observed in the *stipo-*, *para-* and *heterosorghums*.

In section *sorghum*, 65% and 55% larvae were obtained from *S.halepense* and race *virgatum* respectively in comparison to 40% and 95% larvae recovered from resistant and susceptible checks. Larval period varied from 37 to 43 days in race *virgatum* and 37 to 45 days in *S.halepense* compared to 30 to 36 days in susceptible check.

Pupal formation ranged from 66% in *S.halepense* to 100% in race *virgatum* and the susceptible check. Pupal period varied from 8 to 12 days in *S.halepense*, 9 to 13 days in race *virgatum*, and 7 to 8 days in ICSV 1 (Table 36). Adult emergence of 27% and 37.5% was recorded in race *virgatum* and *S.halepense* respectively in comparison to 63.2% in the susceptible check.

Table 30 Field Evaluation of Wild Sorghums for Resistance to Spotted Stem Borer

Section /Species/ Subsp./Race	Acc. ID	Plants Damaged (%)			Leaf damage			Deadhearts (%)		
		1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Chaetosorghum										
S. macrospermum	TRC-241162	100.0	45.8	72.9	2.7	4.7	3.7	96.3	21.9	59.1
Heterosorghum										
S. laxiflorum	TRC-243486	14.1	0.0	7.0	1.0	1.0	1.0	3.9	0.0	2.0
	TRC-243492	10.1	3.0	6.5	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18958	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
Parasorghum										
S. australiense	IS 18954	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18955	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18956	0.0	3.9	2.0	1.0	1.0	1.0	0.0	0.0	0.0
S. brevicallosum	TRC-243491	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18957	1.5	0.0	0.7	1.0	1.0	1.0	0.0	0.0	0.0
S. matarankense	TRC-243576	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	RN341	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. nitidum	TRC-243514	1.8	0.0	0.9	1.0	1.0	1.0	0.0	0.0	0.0
S. purpureosericeum	RN285	4.2	10.7	7.4	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18943	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18947	11.3	4.4	7.9	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18951	4.8	0.0	2.4	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18944	16.7	0.0	8.4	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18945	21.7	0.0	10.9	1.0	1.0	1.0	0.0	0.0	0.0
S. timorense	TRC-243437	5.9	7.7	6.8	1.0	1.0	1.0	0.0	0.0	0.0
	TRC-243498	3.9	9.1	6.5	1.0	1.0	1.0	0.0	0.0	0.0
S. versicolor	IS 18926	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 23177	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 14262	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 14275	6.1	0.0	3.0	1.0	1.0	1.0	0.0	0.0	0.0
	IS 18940	0.0	1.9	0.9	1.0	1.0	1.0	0.0	1.9	0.9
	IS 18941	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
Stiposorghum										
S. angustum	TRC-243598	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. angustum	TRC-243499	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. ecarinatum	TRC-243574	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. extans	TRC-243601	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. interjectum	TRC-243461	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. intrans	TRC-243571	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. intrans	TRC-243602	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
S. stipoideum	TRC-243399	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0
Sorghum										
S.bicolor subsp. *verticilliflorum*										
race *aethiopicum*	IS 27584	100.0	73.3	86.7	7.0	5.7	6.3	100.0	90.6	95.3
	IS 18819	100.0	75.4	87.7	5.3	4.3	4.8	70.3	97.3	83.8
	IS 14564	100.0	81.1	90.6	5.3	5.3	5.3	100.0	80.5	90.2
race *aundinaceum*	IS 18883	91.7	52.4	72.1	3.7	3.7	3.7	83.3	81.0	82.2
	IS 18826	100.0	92.4	96.2	5.0	5.7	5.3	76.7	92.4	84.6
	IS 18830	100.0	74.8	87.4	3.0	3.7	3.3	100.0	74.8	87.4
race *verticilliflorum*	IS 18865	100.0	88.3	94.2	3.0	5.0	4.0	86.1	88.3	87.2
	IS 14278	96.3	73.6	85.0	4.7	4.3	4.5	85.2	56.8	71.0
	IS 20995	100.0	93.0	96.5	5.7	4.7	5.2	86.0	91.7	88.8
	IS 14717	100.0	92.1	96.1	2.7	5.0	3.8	78.2	86.8	82.5

Contd.

Table 30

Section /Species/ Subsp./Race	Acc. ID	Plants Damaged (%)			Leaf damage			Deadhearts (%)		
		1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Sorghum (Contd.)										
race *virgatum*	IS 18803	100.0	96.1	98.1	5.0	6.7	5.8	100.0	96.1	98.1
	IS 18808	100.0	89.0	94.5	7.3	6.3	6.8	97.3	72.9	85.1
	IS 18813	100.0	100.0	100.0	4.7	6.3	5.5	98.3	91.3	94.8
	IS 18817	96.7	100.0	98.3	6.3	6.0	6.2	93.0	91.4	92.2
S. halepense	IS 18891	100.0	81.0	90.5	5.0	4.0	4.5	77.8	79.1	78.4
	IS 33712	100.0	100.0	100.0	3.7	3.0	3.3	65.3	76.7	71.0
	IS 14299	100.0	58.5	79.2	3.7	3.7	3.7	76.7	66.1	71.4
	IS 14212	100.0	84.0	92.0	5.3	4.7	5.0	68.8	42.4	55.6
	IS 18845	100.0	72.8	86.4	2.3	4.7	3.5	59.3	70.3	64.8
	IS 18849	94.4	94.4	94.4	3.7	6.0	4.8	53.1	81.2	67.2
S. bicolor (IC)	ICSV 700	100.0	64.4	82.2	7.0	5.3	6.2	66.1	23.4	44.8
S. bicolor (IC)	ICSV 708	100.0	66.4	83.2	6.3	4.3	5.3	67.3	30.3	48.8
S. bicolor (IR)	ICSV 743	100.0	98.6	99.3	6.3	7.3	6.8	83.3	70.3	76.8
S. bicolor (RC)	IS 2205	100.0	58.1	79.0	5.3	4.3	4.8	44.4	37.2	40.8
S. bicolor (SC)	ICSV 1	100.0	91.7	95.9	6.3	7.3	6.8	100.0	87.2	93.6
F Prob Acc. ID				**<.001**			**<.001**			**<.001**
F Prob Acc. IDxYr.				**<.001**			**<.017**			**<.001**
LSD(5%) Acc. ID				**10.56**			**1.04**			**15.21**
LSD(5%) Acc. IDxYr.				**16.65**			**1.51**			**21.99**

Notes: IR = Improved Resistant Cultivar; IC = Improved Cultivar; RC = Resistant Check; SC=Susceptible check

Table 31 Field Evaluation of Group Interaction Effects for Resistance to Stem Borer

Group	Plants damaged (%)			Leaf damage			Deadhearts (%)		
	1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Resistant check	100	76.5	88.2	6.6	5.7	6.1	72.2	41.3	56.8
Susceptible check	100.00	95.90	97.90	6.50	6.30	6.40	97.60	76.60	87.10
Improved resistant	100.00	58.10	79.00	5.30	4.30	4.80	44.40	37.20	40.80
Wild Races	99.00	83.40	91.20	4.70	5.10	4.90	90.40	85.10	87.80
Wild Species	20.00	14.40	17.20	1.50	1.60	1.50	12.50	11.30	11.90
F Prob Group			**<0.001**			**<0.050**			**<0.001**
F Prob Group x Yr.			**<0.001**			**<0.001**			**<0.001**
LSD (5%) Group			**10.56**			**1.05^{NS}**			**15.21**
LSD (5%) Group x Yr.			**16.65**			**1.51**			**21.99**

Notes: ^{NS}: Not Significant

Table 32 Field Evaluation of Sectional Interaction Effects for Resistance to Spotted Stem Borer

Section	Plants damaged (%)			Leaf damage			Deadhearts (%)		
	1998	1999	Pooled	1998	1999	Pooled	1998	1999	Pooled
Sorghum	99.30	82.20	90.80	4.90	5.10	5.00	82.30	74.70	78.50
Chaetosorghum	100.00	45.80	72.90	2.70	4.70	3.70	96.30	21.90	59.10
Heterosorghum	6.00	2.70	4.30	1.00	1.00	1.00	0.80	2.10	1.50
Parasorghum	3.90	1.40	2.70	1.00	1.00	1.00	0.00	0.10	0.10
Stiposorghum	0.00	0.00	0.00	1.00	1.00	1.00	0.00	0.00	0.00
F Prob Section			**<0.001**			**<0.001**			**<.0.001**
F Prob Section x Yr.			**<0.001**			**0.130**			**<0.001**
LSD (5%) Section			**10.56**			**1.05**			**15.21**
LSD (5%) Section x Yr.			**16.65**			**1.51**^{NS}			**21.99**

Notes: ^{NS}: Not Significant

Table 33 Greenhouse Evaluation of Wild Sorghums for non-preference to Oviposition by the Spotted Stem Borer under Limited-Choice conditions

Section / Species / Subspecies / Race	Acc. ID	Egg Masses / Plant	% Relative oviposition		Avg. Eggs / Plant	% Relative oviposition		Eggs / Egg Mass	% Relative oviposition	
			ICSV1	IS2205		ICSV1	IS2205		ICSV1	IS2205
Heterosorghum					**SET 1**					
S. laxiflorum	TRC-243492	3.27	233.33	253.70	132.87	159.11	174.42	39.85	74.64	66.92
	IS 18958	2.93	187.78	229.45	158.93	171.78	205.73	52.91	99.34	88.52
Parasorghum										
S. australiense	IS 18955	3.13	173.89	237.58	123.47	109.00	160.34	38.57	70.71	63.54
S. matarankense	TRC-243576	2.40	149.44	184.62	106.33	137.91	131.30	45.95	86.72	79.25
S. purpureosericeum	RN 285	0.27	28.89	14.65	7.60	9.45	8.35	19.00	37.15	31.18
	IS 18947	0.40	32.22	27.99	7.73	4.99	11.45	19.33	33.75	30.72
S. versicolor	IS 23177	1.13	68.33	87.69	48.47	45.13	61.95	37.92	70.99	62.20
Sorghum										
S.bicolor subsp.*verticilliflorum*										
race *virgatum*	IS 18808	2.87	142.22	239.49	152.00	132.00	204.27	49.92	93.72	83.67
S. bicolor (RC)	IS 2205	1.67	176.11	100.00	95.00	185.96	100.00	59.27	111.29	100.00
S. bicolor (SC)	ICSV-1	2.20	100.00	188.64	122.20	100.00	168.49	53.27	100.00	90.30
	F Prob	**0.134**	**0.472**	**0.469**	**0.136**	**0.271**	**0.423**	**0.081**	**0.053**	**0.027**
	LSD (5%)	**2.436**	**202.57**	**266.11**	**122.074**	**164.34**	**209.46**	**27.777**	**49.738**	**41.619**
Parasorghum					**SET 2**					
S. versicolor	IS 14262	0	0	0	0	0	0	0	0	0
	IS 14275	0	0	0	0	0	0	0	0	0
S. purpureosericeum	IS 18944	0	0	0	0	0	0	0	0	0
	IS 18943	0.53	9.78	15.87	24.4	10.4	30.38	30.5	74.51	120.75
	IS 18945	0.07	1.33	1.59	1.53	0.53	1.57	7.67	13.17	32.92
S. australiense	IS 18956	0.53	10.07	31.75	18.2	7.24	18.03	21.39	42.85	56.65
Stiposorghum										
S. angustum	TRC-243499	1.87	33.53	77.78	62.13	25.98	69.18	37	82.6	91.53
S. interjectum	TRC-243461	0.27	5.33	6.35	10.2	3.51	10.43	12.75	21.91	54.74
Sorghum										
S. halepense	IS 14212	3.27	60.59	103.17	113.53	43.72	126.36	39.61	85.89	113.53
S. bicolor (RC)	IS 2205	2.8	52.2	100	91.13	36.66	100	40.79	91.86	100
S. bicolor (SC)	ICSV 1	5.47	100	239.68	249.33	100	275.43	46.29	100	149.05
	F Prob	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.017**	**0.019**	**0.053**
	LSD (5%)	**1.56**	**30.024**	**80.739**	**56.441**	**18.689**	**56.903**	**30.937**	**70.701**	**103.65**
Parasorghum					**SET 3**					
S. brevicallosum	IS 18957	3.07	65.61	158.40	215.40	99.88	205.39	66.40	152.59	126.35
S. nitidum	TRC-243514	2.20	46.08	121.35	104.60	48.31	104.56	41.95	97.69	81.58
S. timorense	TRC-243498	0.40	8.86	30.90	14.93	8.05	20.78	38.67	91.98	77.66
Stiposorghum										
S. extans	TRC-243601	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S. ecarinatum	TRC-243574	0.20	4.62	15.00	7.47	4.47	11.22	24.00	60.58	48.25
S. stipoideum	TRC-243399	1.60	34.38	86.67	36.60	17.94	38.70	22.23	52.19	43.56
S. intrans	TRC-243571	0.80	16.46	47.18	62.13	27.50	57.98	46.29	101.95	87.14
Sorghum										
S.bicolor subsp.*verticilliflorum*										
race *aethiopicum*	IS 14564	2.40	50.18	139.74	204.40	97.51	208.98	93.40	227.56	198.32
race *arundinaceum*	IS 18826	3.67	79.48	240.51	192.53	97.17	231.15	51.80	122.07	102.57
race *verticilliflorum*	IS 18865	4.13	88.06	253.65	317.60	152.76	341.63	71.68	168.29	143.51
S. bicolor (RC)	IS 2205	1.73	37.28	100.00	86.53	43.38	100.00	51.69	122.34	100.00
S. bicolor (SC)	ICSV 1	4.6	100	304.94	196	100	241.42	42.34	100	83.57
	F Prob	**<0.001**	**<0.001**	**<0.001**	**0.036**	**0.002**	**<0.001**	**0.007**	**0.007**	**0.026**
	LSD (5%)	**1.971**	**37.136**	**116.96**	**188.862**	**71.681**	**114.5**	**38.56**	**92.083**	**89.647**

Notes: SC=Susceptible check; RC=Resistant check

Table 34 Greenhouse Evaluation of Wild Sorghums for non-preference to Oviposition by the Spotted Stem Borer under no-choice conditions

Section / Species	Acc. ID	No. of Egg Masses / Plant	Relative oviposition (%)		Avg. No. of Eggs / Plant	Relative oviposition (%)		No. of Eggs / Egg Mass	Relative oviposition (%)	
			ICSV1	IS2205		ICSV1	IS2205		ICSV1	IS2205
Parasorghum										
S. australiense	IS 18956	4.27	107.24	131.15	22.52	36.18	48.63	94.87	35.61	58.38
S. purpureosericeum	IS 18947	0.27	6.73	7.34	35.50	65.01	79.15	14.20	6.37	8.40
	IS 18943	0.53	12.75	16.37	18.50	36.41	45.93	10.93	4.94	7.30
	RN285	0.93	23.59	29.17	54.03	81.38	112.34	51.47	17.83	31.38
	IS 18945	2.47	61.11	75.79	44.31	72.26	96.62	120.47	46.81	75.20
	IS 18944	2.60	65.01	78.87	47.00	76.39	102.73	120.47	46.81	75.20
S. timorense	TRC-243498	2.53	60.73	72.52	57.70	85.05	119.98	141.93	55.98	92.39
S. versicolor	IS 23177	0.07	1.52	1.59	2.67	6.35	7.52	0.53	0.29	0.36
	IS 14262	0.07	1.59	2.38	3.67	3.89	7.02	0.73	0.18	0.50
	IS 14275	0.13	3.10	3.97	32.00	37.43	63.70	6.40	1.77	4.37
Stiposorghum										
S. ecarinatum	TRC-243574	0.67	17.32	20.44	53.39	79.57	109.52	37.13	13.43	21.23
S. intrans	TRC-243571	2.07	50.72	61.61	41.06	62.08	88.53	86.00	31.90	56.37
S. extans	TRC-243601	0.33	8.25	8.93	11.56	21.62	26.26	5.87	2.78	3.60
S. interjectum	TRC-243461	0.67	16.38	19.74	64.78	96.30	131.83	42.20	16.09	25.99
Sorghum										
S. bicolor (SC)	ICSV 1	4.07	100.00	122.42	71.57	100.00	142.40	287.73	100.00	179.73
S. bicolor (RC)	IS 2205	3.40	83.67	100.00	49.63	72.64	100.00	163.67	62.31	100.00
	F Prob	**< .001**	**< .001**	**< .001**	**< .001**	**0.010**	**<.001**	**< .001**	**< .001**	**< .001**
	LSD (5%)	**0.835**	**22.099**	**28.505**	**31.413**	**53.256**	**67.708**	**58.472**	**24.313**	**43.264**
Notes: SC=Susceptible check; RC=Resistant check										

Table 35 Distribution of Spotted Stem Borer egg masses on the leaf surfaces

Section/Species	Acc. ID	Egg Masses / Plant		Eggs / Egg Mass		Avg. Eggs / Plant	
		Up. Surf.	Lw.	Up. Surf.	Lw.	Up. Surf.	Lw.
Parasorghum							
S. australiense	IS 18956	2.33	1.93	19.34	27.22	44.07	50.80
S.purpureosericeum	IS 18944	1.20	1.40	62.76	24.86	79.93	40.53
	IS 18945	1.33	1.13	36.87	41.13	54.87	53.13
	IS 18943	0.53	0.00	18.50	0.00	10.93	0.00
	IS 18947	0.27	0.00	35.50	0.00	14.20	0.00
	RN285	0.47	0.47	48.42	60.33	22.87	28.60
S.versicolor	IS 23177	0.07	0.00	2.67	0.00	0.53	0.00
	IS 14262	0.07	0.00	3.67	0.00	0.73	0.00
	IS 14275	0.13	0.00	32.00	0.00	6.40	0.00
S. timorense	TRC-243498	0.67	1.87	68.68	55.98	45.27	96.67
Stiposorghum							
S.ecarinatum	TRC-243574	0.60	0.07	53.13	21.00	32.93	4.20
S. intrans	TRC-243571	1.33	0.73	44.57	30.97	53.47	32.53
S. extans	TRC-243601	0.27	0.07	13.33	2.67	5.33	0.53
S. interjectum	TRC-243461	0.13	0.53	40.00	68.83	8.00	34.20
Sorghum							
S. bicolor (SC)	ICSV 1	1.93	2.13	56.54	81.48	102.67	185.07
S. bicolor (RC)	IS 2205	1.87	1.67	51.60	46.58	96.33	67.33
	F Prob	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	LSD (5%)	**0.777**	**0.943**	**32.991**	**32.311**	**33.465**	**60.580**
Notes: SC=Susceptible check; RC=Resistant check							

Fig. 31 Screening techniques for Resistance to Stem Borer and symptoms of infestation

a. Seedling with deadheart symptom with spotted stem borer in the inset.

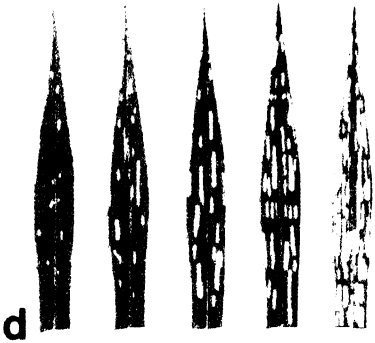
b. Egg masses on leaf.

c. Ragged appearance of sorghum plant after stem borer damage.

d. Leaf damage rating scale.

e. Oviposition cage to study antexenosis under no-choice conditions.

f. Cage technique to study antexenosis under limited-choice conditions.



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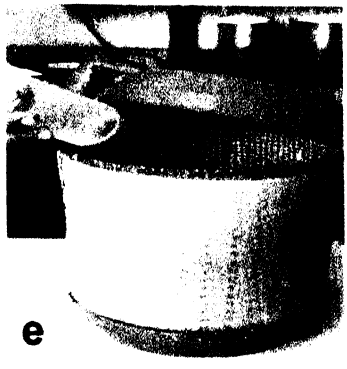
1 3 5 7 9



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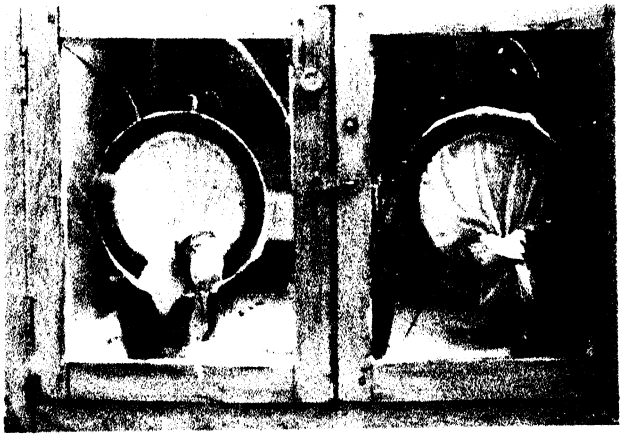


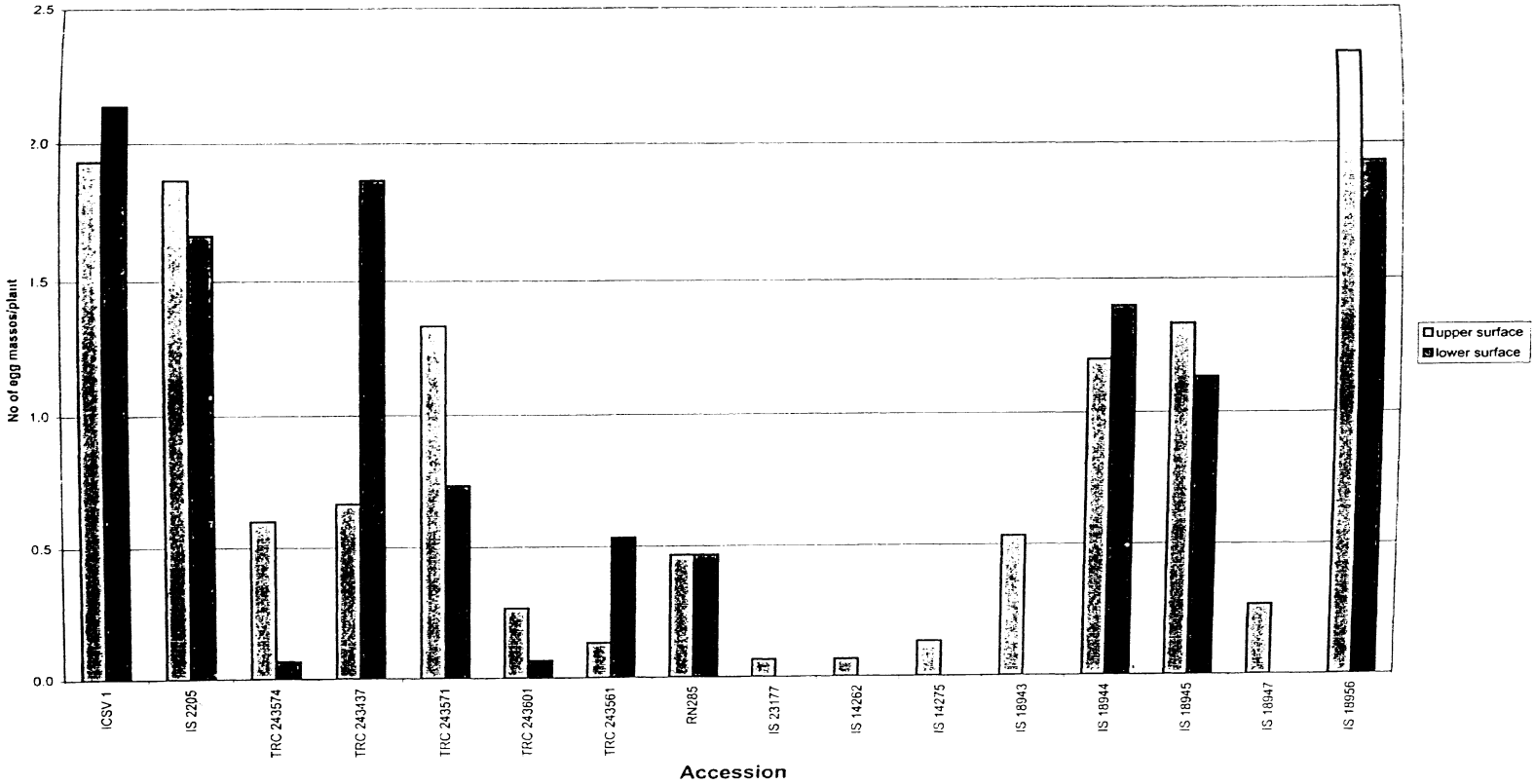


Fig. 32 Distribution of Stem Borer Egg Masses per Plant on the leaf surfaces

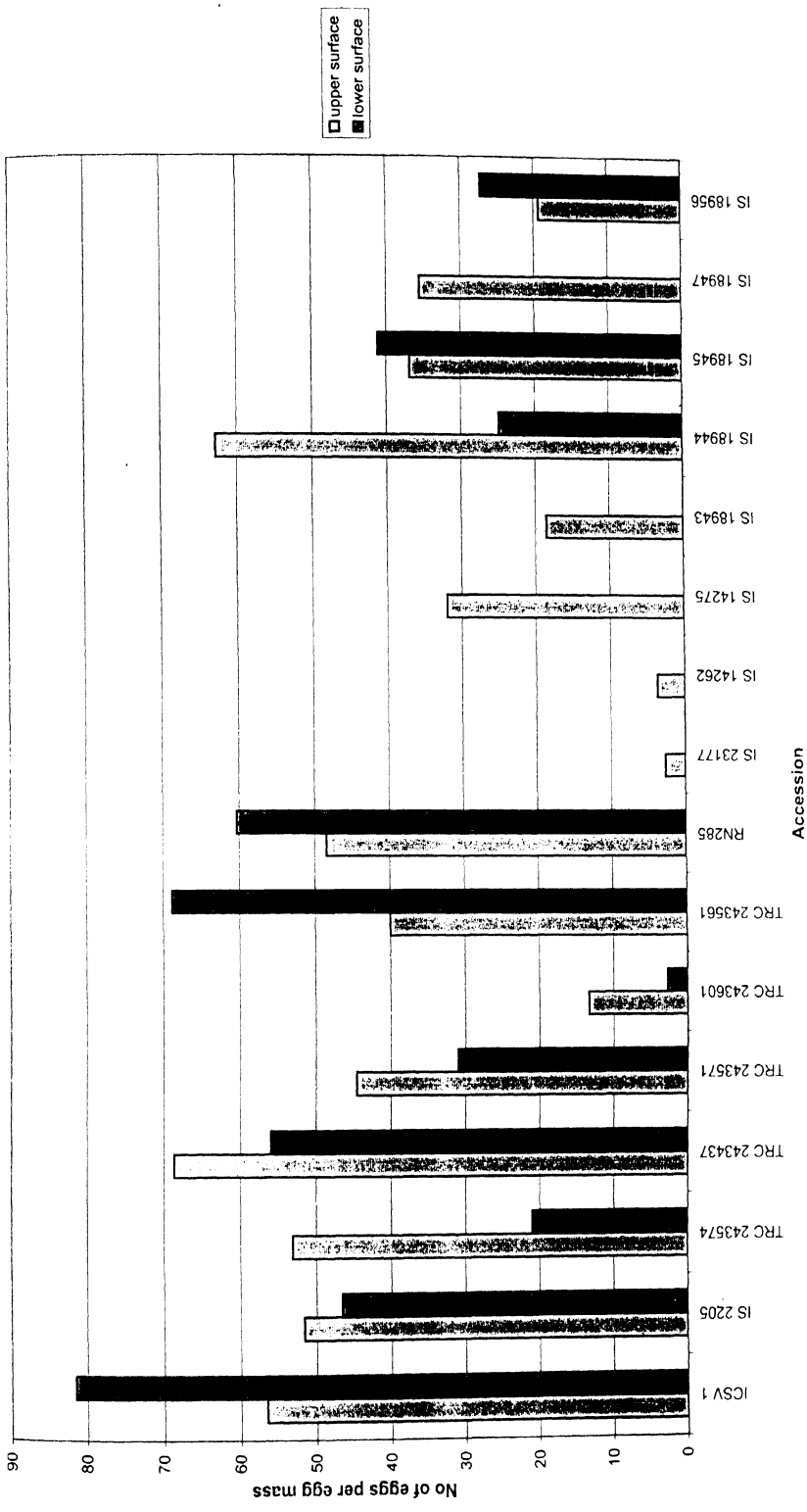


Fig. 33 Distribution of Stem Borer Eggs per Egg Mass on the leaf surfaces

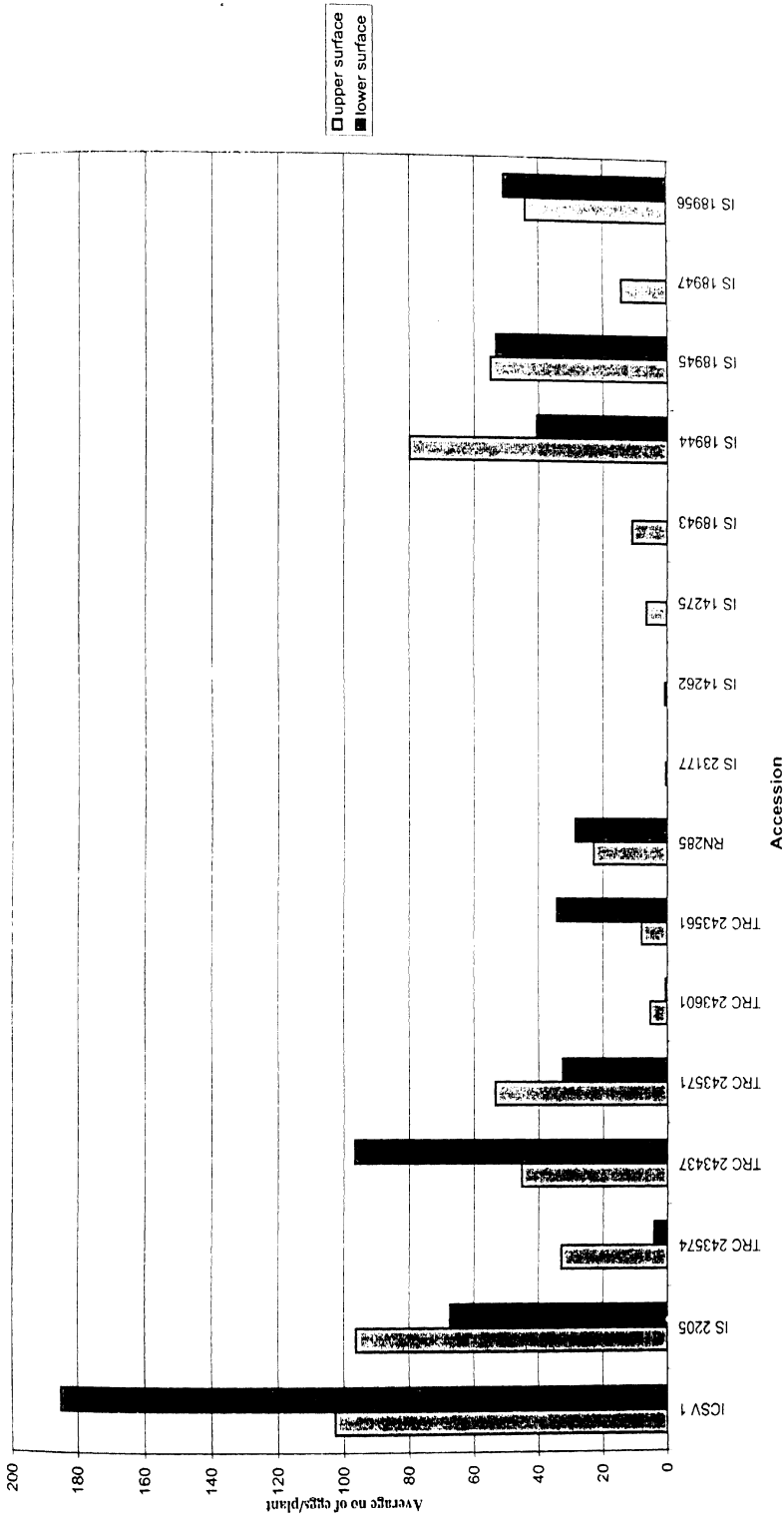


Fig. 34 Distribution of Average Eggs per Plant on the leaf surfaces

Table 36 Adult emergence after artificial infestation with first instar larvae of Spotted Stem Borer under No-Choice conditions in Greenhouse

Section / Species / Subsp. / Race	Acc. ID	Plants damaged (%)	Dead hearts (%)	Leaf damage score	Larvae recovered	Remarks on Larval and Pupal development and emergence of Adults
Heterosorghum						
S. laxiflorum	TRC-243492	29.0	15.3	1.3	0	
	IS 18958	100.0	82.5	1.7	6	Larvae died in 28-30 days
Parasorghum						
S. australiense	IS 18955	22.9	10.5	1.0	0	
	IS 18956	73.1	11.1	1.0	1	Larvae stopped feeding on 24th day
S. matarankense	TRC-243576	33.3	5.2	1.0	0	
S. nitidum	TRC-243514	93.7	0.0	2.7	0	
S.purpureosericeum	RN 285	100.0	11.1	6.0	0	
	IS 18943	37.5	0.0	1.0	0	
	IS 18944	0.0	0.0	1.0	0	
	IS 18945	28.6	12.7	1.3	1	Larva died on 24th day
	IS 18947	60.7	8.2	4.3	0	
S. timorense	TRC-243498	22.1	0.0	1.0	0	
S. versicolor	IS 23177	41.5	0.0	1.0	0	
	IS 14262	7.7	0.0	1.0	0	
	IS 14275	71.0	14.8	1.0	0	
Stiposorghum						
S. angustum	TRC-243499	0.0	0.0	<1	0	
S. ecarinatum	TRC-243574	0.0	0.0	<1	0	
S. extans	TRC-243601	0.0	0.0	<1	0	
S. intrans	TRC-243571	0.0	0.0	<1	0	
S. interjectum	TRC-243461	0.0	0.0	<1	0	
S. stipoideum	TRC-243399	0.0	0.0	<1	0	
Sorghum						
S.bicolor subsp. *verticilliflorum*						
race virgatum	IS 18808	98.2	98.2	6.0	55 % (11)	Larval period: 37-43 days. Pupal period: 9 - 13 days. Adult emergence: 2 normal males; 1 male with malformed wings; 1 moth half emerged from pupa; 7 pupae with no emergence.
S. halepense	IS 14212	98.4	98.4	6.0	65% (13)	Larval period: 37-45 days. Pupal period: 8 - 12 days. Adult emergence: 3 males; 5 pupae with no emergence; 3 escaped; 2 died.
S. bicolor (SC)	ICSV-1	98.4	98.4	7.0	90% (19)	Larval period: 30-36 days. Pupal period: 7 - 8 days. Adult emergence: 6 males; 5 females; 3 egg masses; larvae hatched.
S. bicolor (RC)	IS 2205	96.8	43.4	6.0	40% (8)	Larvae died by 30th day
	F Prob	**< 0.001**	**< 0.001**	**< 0.001**		
	LSD (5%)	**5.9**	**4.4**	**0.4**		

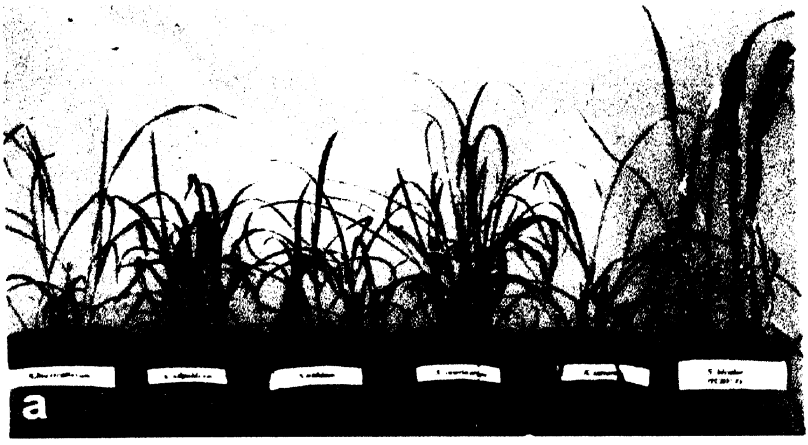
Notes: SC=Susceptible check; RC=Resistant check

Fig. 35 Response of Wild Sorghums to artificial infestation with Stem Borer

a. Resistant *para-* and *stiposorghums* along with susceptible check (ICSV 1).

b. Races *verticilliflorum*, *arundinaceum*, *virgatum* of *S. bicolor* sub species *verticilliflorum* and *S. halepense* along with susceptible check (ICSV 1) exhibiting susceptible response.

c. No deadhearts in *S.nitidium* and *S.ecarinatum* despite egg masses (arrow) in comparison with susceptible *S.bicolor* (centre)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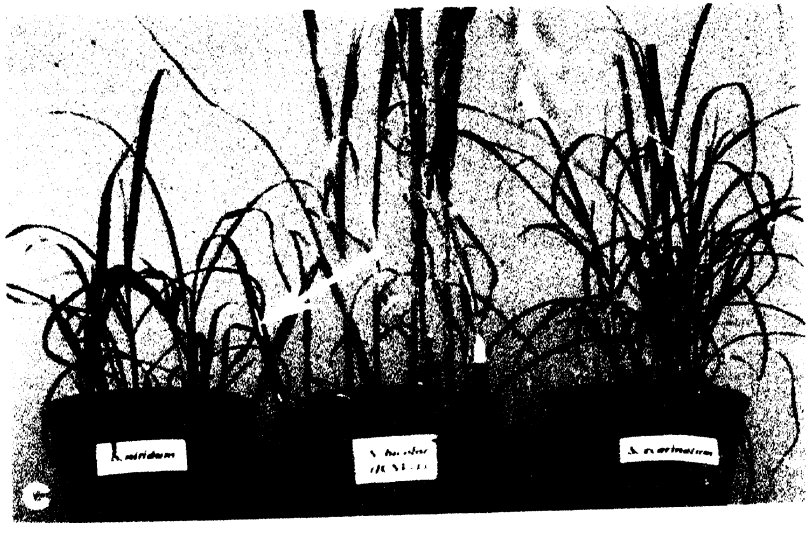
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Discussion

Discussion

Sustained progress in purposeful plant breeding rests on the availability of genetic diversity, which refers to genome differences ranging from a single base pair to rearrangements of entire chromosomes. These variations in genetic make up in interaction with the environment dictate the observable patterns of diversity shown by the multitude of living organisms. This genetic variation within and between species, generated by the processes of mutation, sexual reproduction and selection ensures their capacity for evolutionary change and ecological adaptation. Genetic diversity is also the basic raw material for developing improved genotypes aimed at maintaining and enhancing the productivity, stability and sustainability of agriculture.

Sorghum bicolor, an important cereal crop in the semi arid tropics, has long been considered a genetically diverse species. Cultivated sorghums exhibit a wide diversity in morphological traits including spikelet and panicle type, seed traits, plant height, photo period response and plant architecture. Breeding programmes have been helpful in development of hybrids suitable for diverse agro climatic conditions. However, genetic up gradation is critically limited by the lack of adequate variability especially for pest and disease resistance. Molecular marker studies in the recent past have shown that breeding for improved sorghum varieties has led to a significant reduction in genetic diversity of present day cultivars (Tao *et al.*, 1993; Ahnert *et al.*, 1996; Jordan *et al.*, 1998).

Wild relatives and progenitor species of *Sorghum* represent a potential genetic resource that has not yet been fully explored vis a vis breeding, which could be used to effectively broaden the genetic base and enhance sorghum breeding prospects. In view of this the present investigation was undertaken to study the diversity among wild sorghums at the morphological and molecular levels and also to identify sources of resistance to major biotic stresses such as sorghum downy mildew, sorghum shoot fly and spotted stem borer.

Morphological Diversity

In the present study the 85 accessions could be clearly assigned to one of the five sections into which the genus *Sorghum* is usually subdivided: *sorghum, chaetosorghum, heterosorghum, parasorghum and stiposorghum*. Traits like nodal hairiness, inflorescence characters such as shape of callus at the base of the sessile spikelet and its corresponding mode of articulation at the apex of the peduncle or apex of the rachis internodes,

nature of pedicillate spikelet, panicle branches, raceme joints, awn length and glume size were employed in distinguishing the five sections. These traits have been used to differentiate the sections and species within the genus by several workers (Garber, 1950; de Wet *et al.*, 1970; de Wet, 1978; Lazarides *et al.* 1991). Taxa within section *sorghum* were characterised by absence of nodal hairs, equal glumes of pedicelled spikelets, glabrous nodes and awns which were not prominent; they were also characterised by glabrous leaf blades, an absence of culm and leaf sheath hairiness and an unfringed membrane for the ligule. The four races of subsp. *verticilliflorum* were distinguished based on the nature of the inflorescence and glumes of the pedicillate spikelet. These distinguishing features conform as per the descriptions by de Wet (1978). In the present study, although nodal hairiness served to differentiate the *parasorghums* and *stiposorghums* from the other three sections. The character was variable within accessions of a species. Further, the ring of hairs was often found to be absent from the lower nodes of several specimens, and was absent altogether in *S.extans* of *stiposorghum*, suggesting that this trait is of limited taxonomic value. Such an observation was also made in an earlier study by Lazarides *et al.* (1991). The most reliable diagnostic trait was the callus at the base of the sessile spikelet and its corresponding mode of attachment at the apex of the peduncle or rachis internodes, which in the present study served to consistently distinguish the *para-* and *stiposorghums*. Based on these traits the correct taxonomic identity of the 85 accessions in the wild sorghum collection was established, and assigned to 18 species.

The REML analysis of 85 accessions for the nine quantitative traits showed large genetic differences for all traits. Accession x season interaction was highly significant for all traits. In all traits except for days to 50% flowering, plant height and leaf length, the magnitude of variance component for accessions was larger than that for accession x season interaction. This provides opportunities for genetic improvement in sorghum. Days to flowering was significantly influenced by seasons and varied considerably ranging from 57-157 days in rainy season and from 43-152 days in post-rainy season. The delay in flowering during rainy season could be due to strong photoperiod sensitivity. However, there were a few accessions, which flowered later during the post-rainy than in the rainy season. This was probably because the cumulative temperature requirement was not met with for these accessions though the photoperiod was appropriate (Doggett, 1988). Plant height also varied significantly between the rainy and post-rainy seasons. It ranged from 152 to 305cm in the rainy season and from 80 to 341cm in the post-rainy season. The reduced plant height during post rainy season maybe attributed to the lower temperature and shorter photoperiod, which retards growth resulting in reduced plant height. Conversely, the considerable increase in plant height during the rainy season might have been due to the higher temperatures, longer day-length and

longer growing season which might have encouraged growth. In general, those accessions, which took more time to flower, grew taller in the rainy season thus making them suitable as forage types due to a higher biomass production. The broad sense heritability for leaf width was highest (84%) followed by panicle width (81%), peduncle exertion (79%), basal tillering (74%) and panicle length (72%). This suggests that these traits are less prone to accession x season interactions, and therefore, can be effectively used as selection criteria.

Based on the quantitative traits, taxa of section *sorghum* could be clearly differentiated from the members of the other four sections. Within section *sorghum* there were two groups with the cultivated being distinct from the wild races/species except for accessions IS 18805, IS 18820 and IS 18821. This result supports the observations of earlier workers (Liang and Casady, 1966; de Wet and Huckabay, 1967), who also found that cultivated sorghums and their wild progenitors were clearly distinct. This is the first study where wild sorghums of the other four sections have been morphologically compared with section *sorghum*. The clear distinction is indicative of adaptations that are specific to each of the three groups.

Molecular Diversity

Classical methods of estimating genetic diversity and/or relatedness among plants have relied on morphological (phenotypic) traits. The present study revealed wide phenotypic variability. Analysis of the quantitative traits helped to obtain a broad categorisation of the taxa within genus *Sorghum* while also confirming that section *sorghum* was distinct from the other four sections. However, relationships at lower levels of biological organisation were not evident. For instance, within the group comprising wild taxa of section *sorghum*, accessions of the four wild races of subsp. *verticilliflorum* along with those of *S. halepense* and *S. propinquum* formed mixed clusters. Further, though the wholly wild taxa formed a distinct group, accessions of the four component sections did not cluster separately. In order to better understand the extent and distribution of diversity among the wild sorghums a subset of accessions was analysed at the molecular level using (i) four maize mt DNA probes, (ii) four sorghum derived resistance gene candidates, (iii) four AFLP primer combinations and (iv) 10 SSR primer sets.

Diversity in genus *Sorghum* using mt DNA and AFLPs

Evidence from the different sets of molecular data revealed inherent relationships among the 17 *Sorghum* species, which broadly agreed with the accepted system of classification. The strong hybridisation signals obtained with the four mitochondrial DNA (mt) probes in all *Sorghum* species reflect the high homology between maize and sor-

ghum genomes. Different relative intensities observed with some bands of *atp* α and *atp* 6 suggest a variation in the copy number of these genes. Bailey-Serres *et al.* (1986) have previously reported similar results. Mitochondrial patterns were very different across the five sections. Only one pattern generated by *Bam H* I - *cox* I and one band generated by *cox* II with each of the three enzymes were common across 17 *Sorghum* species implying highly conserved naure of *cox* I and *cox* II genes across diverse taxa. Greater polymorphism obtained with *atp* α and *atp* 6 suggest that they are less conserved as compared to *cox* I and *cox* II.

Section *sorghum* was substantiated to be a monophyletic and highly homogenous group quite distinct from the other four sections (*chaeto-, hetero-, para-* and *stiposorghum*). Both mitochondrial data and AFLP profiles revealed high similarity between the diploid species *S. bicolor,* tetraploid *S. halepense* and the four wild races of *S. bicolor* subsp. *verticilliflorum*. These observations are in agreement with earlier studies using isozyme, nuclear, chloroplast and mitochondrial RFLP profiles (Morden *et al.*, 1990; Duvall and Doebley, 1990; Aldrich and Doebley, 1992; Aldrich *et al.*, 1992; Deu *et al.*, 1995).

The mt DNA and AFLP profiles in the present study indicated a closer relationship between the *chaeto-* and *heterosorghum* as compared to the other sections. Sun *et al.* (1994) and Dillon *et al.* (2001) using ITS sequences and Spangler *et al.* (1999) using *ndhF* sequences also showed a close relationship between these two sections.

Based on the mt DNA and AFLP data, the Afro-Asian *parasorghums* were distinctly separate from the Australian *parasorghums*. The only exception was *S. nitidum* from Australia, which clustered with the Afro-Asian *parasorghums* based on mt DNA profiles and with the Australian species based on AFLP profiles. The Australian *parasorghums,* besides having different mitochondrial and AFLP profiles, also differed in appearance from the two Afro-Asian species of *parasorghum* (*S. purpureosericeum* and *S. versicolor*) in having wider leaf blades and much larger open panicles, though they had similar spikelet morphology and possessed the bearded node characteristic of the *parasorghum* species (Ayyangar and Ponnaiya, 1941; Garber, 1950; Lazarides *et al.*, 1991). The separate clustering of the geographically distinct *parasorghums* suggests a polyphyletic origin for this section. Further, close resemblance of the mt DNA and AFLP profiles of the Australian *parasorghums* and the *stiposorghums*, which are confined to the same geographical region indicate their common ancestral origin. This further emphasises that geographical distribution has played a key role in the evolution of these species and focuses on the polyphyletic evolution of the different sections of *Sorghum*.

S.nitidum has the most extensive range of distribution among all the *parasorghum* species and is found in southern China, India, south east Asia, Pacific Islands and northern Australia (Garber, 1950). Considerable variation has been reported in its morphology and ploidy (Celarier, 1958; Gu *et al.*, 1984). Similar mitochondrial profiles between *S. nitidum* from Australia and the Afro-Asian *parasorghums* (*S. purpureosericeum*, *S. versicolor*) in the present study indicate an ancestral association between them. Dillon *et al.* (2001), using ribosomal ITS, also showed that *S. nitidum* from Australia is more closely related to the Afro-Asian *parasorghums*. However, based on AFLP profiles in the present study it was observed to share a close relationship with the Australian *parasorghum* species.

Crossability barriers are known to exist between *S. nitidum* and other *parasorghums*. The close relationship observed between this species from Australia and the other Australian *parasorghum* species suggests that before evolving as separate species, exchange of genetic components might have taken place leaving the mitochondrial component without alteration. These results clearly indicate that the Australian *parasorghums* might have evolved separately as compared to Afro-Asian *parasorghums* confirming the polyphyletic origin for this section. Further studies are essential with collections of *S. nitidum* from its entire distributional range to clarify its relationships with the other species.

Diversity in genus *Sorghum* using SSRs

In *Sorghum*, microsatellite primer sets derived from *S. bicolor* subsp. *bicolor* have been effectively used to evaluate diversity among the cultivated subspecies and races (Brown *et al.*, 1996; Dean *et al.*, 1999; Taramino *et al.*, 1997; Kong *et al.*, 2000; Grenier *et al.*, 2000). However, their application across other species within the genus has not been reported.

In the present investigation, wild sorghums representing 17 species were studied using *S. bicolor* microsatellites to evaluate their utility in assessing species relationships within the genus *Sorghum*. The ten microsatellites amplified alleles in all accessions of the two species within section *sorghum* (*S. halepense* and *S. bicolor* subsp. *verticilliflorum*) and either in *hetero-* and / or *chaetosorghum*. High polymorphism was observed within section *sorghum*. Alleles amplified ranged from two to six across the six accessions tested. However, only two SSRs amplified alleles in the *para* and *stiposorghums*; Sb 6 54 amplified five alleles across five species (*S. nitidum* and *S. versicolor* of *parasorghum* and *S.angustum*, *S. ecarinatum*, and *S. stipoideum* of *stiposorghum*), whereas Sb 1-10 amplified three alleles across four species (*S. purpureosericeum*, *S. australiense*, *S. nitidum* and *S. angustum*).

Similar sized amplification products obtained in these diverse species may reflect inherent relationships (identical by descent). It may also be possible that the co-migrating fragments might simply be a result of mutations, rearrangements and duplications either in the flanking region and/or changes in the repeat itself (just identical in state). Furthermore, the lack of allelic variation obtained among the distantly related taxa compared to the closely related ones in the present study could probably have been caused by changes in repeat sequences (Gupta *et al.*, 1996). Previous studies have shown that microsatellites tend to be more variable in the source species (*S. bicolor*) than in the target species (other 15 species) (Ellegren *et al.*, 1995; Forbes *et al.*, 1995; Hutter *et al.*, 1998).

The absence of amplification across most of the *para-* and *stiposorghums* indicates a lack of homology of the flanking regions containing the selected primer sites (SSR) or the repeat region itself. This confirms *para-* and *stiposorghums* (the target species) are evolutionarily distant from the section *sorghum* (the source species). This has also been confirmed using mt DNA and AFLPs.

S. bicolor microsatellites are valuable tools to fingerprint and evaluate genetic diversity of wild sorghums, but, the problem of the potential non-homology of co-migrating alleles may limit their use to systematic studies of closely related groups. However, assays with a larger number, and different sets of loci coupled with reliable verification of the expected repeats with techniques such as hybridisation with a selective probe and sequence analysis could yield accurate information for phylogenetic and evolutionary studies as well.

Diversity in genus *Sorghum* using Resistance Gene Candidates

Breeding for disease resistance has greatly contributed to improving quality and yield in most crop plants and has led to the identification and isolation of over 30 disease resistance (R) genes from a variety of plant species (Rommens and Kishore, 2000). In the present study, four resistance gene candidates (RGCs - S8-1, S2-2, S27-2 and S30-5) isolated from *S. bicolor* were used to study the diversity in hybridisation patterns across 17 species belonging to five sections of the genus *Sorghum*.

All four RGCs hybridised with members of section *sorghum*, with the highest polymorphism (5-6 alleles) detected for S8-1 and S2-2. Three out of the four RGCs also hybridised to sections *hetero-* and *chaetosorghum* and S8-1 and S2-2 gave weak signals with some members of sections *para-* and *stiposorghum*. Compared to other random molecular markers, the use of RGCs for diversity analysis is an improved approach since functional diversity is being targeted against sequence diversity.

The four RGCs used in the present study are also known to hybridise with maize, rice, sugarcane and pearl millet and also detect allelic polymorphism albeit at a low level (person. commn. Dr S Sivaramakrishnan, ANGRAU). Within the genus *Sorghum*, the four RGCs used in the present study have been shown to hybridise with members of *hetero-* and *chaetosorghum* implying a possible functional and evolutionary relationship with species of these sections.

Wild sorghums of sections *para-* and *stiposorghum* have been shown to be immune to the ICRISAT isolate of the sorghum downy mildew pathogen (Kamala *et al.*, 2002). They are also highly resistant to the sorghum shoot fly and stem borer (this study). However, they are very distantly related to the section *sorghum*. The lack of hybridisation of these RGCs with *para-* and *stiposorghums* suggests that these R genes present in these sections may be different. By isolating more RGCs from *S.bicolor*, it may be possible to locate corresponding alleles, if any, with members of *para-* and *stiposorghums*.

Diversity in *S. bicolor* subsp. *verticilliflorum* of section *sorghum* using AFLPs and SSRs

The analysis of mt DNA, AFLP, SSR and RGC data revealed that section *sorghum* is monophyletic, highly homogenous and quite distinct from other sections. Cultivated types (*S. bicolor* subsp. *bicolor*) within this section have been extensively investigated and assessments of diversity using RFLPs, RAPDs and SSRs have shown varying levels of diversity (Tao *et al.*, 1993; Deu *et al.*, 1994; Cui *et al.*, 1995; Ahnert *et al.*, 1996; Brown *et al.*, 1996; Jordan *et al.*, 1998; Menkir *et al.*, 1997; Dje *et al.*, 1999, 2000; Grenier *et al.*, 2000). Except for a few reports (Morden *et al.*, 1990; Aldrich and Doebley, 1992; Aldrich *et al.* 1992) little information is available on the extent of molecular variation available in wild races of *S.bicolor* subsp. *verticilliflorum*. The present study focused on assessing diversity and analysing population structure within *S. bicolor* subsp. *verticilliflorum* (21 accessions distributed in four races) using AFLPs and SSRs.

The levels of polymorphism between the two techniques differed considerably, ranging from 60% in AFLPs to 100% in SSRs. Similar results were observed by Russel *et al.* (1997) who compared SSRs with AFLPs in barley where polymorphism was 49% in AFLPs and 100% in SSRs. Whenever SSRs have been compared to other systems, they have always revealed the highest level of polymorphism (Wu and Tanksley, 1993; Morgante *et al.*, 1994; Rus-Kortekaas *et al.*, 1994; Saghai Maroof *et al.*, 1994; Maughan *et al.*, 1995; Salimath *et al.*, 1995; Powell *et al.*, 1996).

Average AFLP diversity (H = 0.15) was low for races (H = 0.13) and geographical regions (H = 0.13). Race *verticilliflorum* among the races was most diverse (H = 0.13)

whereas accessions from eastern Africa showed the highest diversity (H = 0.16) as compared to those from central, southern or western African accessions. The AFLP diversity values observed were however similar to the total panmictic heterozygosity (0.15) obtained for the subsp. *verticilliflorum* by Morden *et al.* (1990) using allozymes. Gene diversity is a function of both allelic richness as well as allelic evenness. In the present study, while 146 out of the 240 loci were polymorphic (allelic richness), alleles at as many as 77 loci were present only in one or two accessions. This could probably account for the low diversity values estimated with the AFLPs.

In contrast to AFLP data, high gene diversity was observed with SSRs (H = 0.77). Race *verticilliflorum* was most diverse (H = 0.68) with high allelic richness. This is in agreement with earlier studies that have reported this race to be the most widely distributed and morphologically the most variable (de Wet, 1978; Doggett, 1988). Further, similar gene diversity values for the *verticilliflorums* (H = 0.68) and the *virgatums* (H = 0.67), despite the former having a larger number of alleles (46 vs. 26), is indicative of allelic evenness in the two races. Allelic richness was highest for east Africa, which is in agreement with the fact that this region is believed to be the centre of diversity of sorghum and consequently expected to harbour the highest diversity. The high levels of polymorphism associated with SSR is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Tautz and Renz, 1984; Tautz *et al.*, 1986) rather than only by simple mutations and insertions/ deletions.

Moderate levels of population differentiation were observed for the races (F_{ST} = 0.17 ± 0.03) and geographic regions (F_{ST} = 0.08 ± 0.02) based on AFLP data. Forty percent of the loci were monomorphic and about 53% of the polymorphic alleles were present in only one or two accessions implying that genetic differences between the races and regions may possibly be attributed to the existence of low frequency alleles that are unique to one population or another.

SSR markers revealed lower racial (F_{ST} = 0.03) and regional (F_{ST} = 0.06) differentiation. These values are however, comparable to those reported by Cui *et al.* (1995) with RFLPs. High polymorphism in microsatellites (due to mutations) is reported to drastically deflate F_{ST} expectations (Wright, 1978; Charlesworth, 1998; Nagylaki, 1998; Hedrick, 1999). Therefore even an apparently low F_{ST} for SSRs may indicate important genetic differentiation (Balloux and Lugon-Moulini, 2002). In this study with SSRs an average of 7.6 alleles per locus was obtained with a maximum of ten alleles at a single locus. The effect of this high polymorphism may be a possible cause for low F_{ST} values with SSRs.

The present studies using both AFLP and SSR data suggest that differentiation of the populations within *S. bicolor* subsp. *verticilliflorum* is based on the presence/absence of low frequency alleles (rare alleles). Morden *et al.* (1990) also made similar observations. These results have direct implication for germplasm conservation. While the most common alleles may already be present in most germplasm collections, collection/conservation of unique / rare alleles from Africa needs to be prioritised. These rare alleles may prove to be sources of QTLs for various biotic/abiotic stresses and other agronomic traits as has been noted by Schoen and Brown (1993).

AFLP profiles revealed greater genetic similarity (S_{ij} = 0.41 to 0.74) among all the accessions as compared to SSRs (S_{ij} = 0.00 to 0.60). Distinct races or regions did not cluster together. The MDS also failed to separate the accessions into discrete taxonomic categories, further emphasising that the four wild races of subsp. *verticilliflorum* are closely related to one another with very little taxonomic differentiation between them. The lack of significant differentiation between the races or geographical regions indicates a high level of geneflow and the consequent absence of fixation of different alleles among the races or regions. These findings are in agreement with earlier published reports on the subspecies (Morden *et al.*, 1990; Aldrich *et al.*, 1992).

The analysis of genetic structure of the wild races of *S. bicolor* subsp. *verticilliflorum* based on phenotypic and molecular markers revealed different patterns. The distinct morphological and ecological adaptations were not reflected at the molecular level. The poor correlation (r = 0.12) obtained between morphological and molecular data implied the absence of congruence between the phenotypic and molecular data. This is expected since phenotypic diversity is influenced by both genetic and environmental selection pressures. The differences in diversity estimates between the different molecular markers obtained in the present study may be attributed to the inherent nature of the markers themselves: AFLPs target coding sequences and SSRs target simple sequence repeats.

Evaluation of Wild and Weedy Sorghums for Host Plant Resistance

Cultivation of plant genotypes resistant to pests and diseases has been a principal method of control and several sources of resistance have been identified and utilised in sorghum improvement programmes. Nevertheless, access to diverse germplasm continues to be important since pest/pathogen populations continue to change their virulence patterns necessitating the continual discovery and incorporation of new genes for resistance. The genetic potential of wild species particularly in resistance breeding is well documented for crops such as wheat, rice, maize, barley, potato, tomato, tobacco and sugarcane among others (Hawkes, 1977; Stalker, 1980; Pluknett *et al.*, 1987), and the present study clearly demonstrates the availability of high levels of resistance to sorghum downy mildew, sorghum shoot fly and spotted stem borer among the wild sorghums studied, signifying their potential in sorghum improvement.

Screening for Resistance to Sorghum Downy Mildew

In the present study, most of the cultivated, wild and weedy types of section *sorghum* were highly susceptible to the ICRISAT isolate of the sorghum downy mildew (SDM) pathogen, except for one accession (IS 14383) among the cultivated types, one each among wild races *aethiopicum* and *arundinaceum*, and one of weedy, *S. halepense*, which were found completely free from the disease. One accession of race *virgatum* was identified with only 1.7% disease incidence.

The sorghum line IS 14383, a guinea landrace from Zimbabwe, identified as resistant in this study, is of particular interest since it has recovery resistance, a phenomenon in which plants systemically infected at the seedling stage produce symptomless leaves and shoots and normal panicles. Such an occurrence has been reported earlier in pearl millet and sorghum (Singh and King, 1988; Singh and de Milliano, 1989b). While IS 14383 could be a valuable source of downy mildew resistance, its uniqueness needs to be characterized in terms of secondary metabolites and/or antifungal proteins as well as through molecular markers for enhanced utilisation. There is also a need to test its reaction to other pathotypes in comparison to other resistant sources, particularly QL 3 which is reported to be resistant to 16 different pathotypes (Pawar *et al.*, 1985).

Resistance to SDM has not been observed as a common trait among the cultivated sorghums. Only about 130 accessions were identified as resistant to the ICRISAT culture of the SDM pathogen in a screening of over 16,000 accessions from the world sorghum collection at ICRISAT (Pande *et al.*, 1997). The major sources of resistance (80%) came from Africa, whereas 8% were from the Indian subcontinent, and 12 %

from USA and Australia (primarily breeding material). The resistant accessions among the wild races of section *sorghum* identified in this study are also primarily from eastern Africa (Sudan and Egypt) and India. This is not surprising since the north-east quadrant of Africa is believed to be the centre of domestication and the primary centre of diversity of the crop while India is a secondary centre. Further, since Africa is also probably the primary centre of diversity of the pathogen (Williams, 1984), the region is likely to harbour greater diversity for SDM resistance. IS 18882, the resistant *arundinaceum*, though listed in records as being from USA, also originally must have come from Africa since sorghums were first formally introduced to the Americas only in 1725 (Duncan *et al.*, 1991). This distribution of resistant sources within section *sorghum* appears to validate the generally accepted view that resistance to many diseases/pests is not randomly distributed, but may be found in specific geographical/regional pockets/centers of diversity especially where the crop host and the pathogen have co-evolved (Leppik, 1970; Harlan, 1977). However, it could also be that the predominance of resistance observed among the African accessions, may be just a reflection of the geographical bias in the initial screening sample of 16037 accessions. Ninety five percent of the landraces in the ICRISAT collection are from Africa (78%) and India (17%), which is indicative of the distribution of the largest sorghum growing areas of the world as well as of the history of the crop.

Accessions of 15 species belonging to the *chaeto-*, *hetero-*, *stipo-* and *parasorghums*, constituting the tertiary genepool were all immune except for two accessions of *parasorghum* (*S. purpureosericeum*) that showed about 3% disease incidence in the present investigation. *Chaeto-* and *stiposorghums* are endemic to Australia, the *heterosorghums* are found in Australia as well as the Pacific Islands, while the *parasorghums* are more widespread spanning the three continents of Australia, Asia and Africa. Nevertheless, they all exhibited an immune or near immune reaction to SDM in the present study. In the present study all accessions of *S. versicolor* were resistant but Bonde and Freytag (1979) found that *S. versicolor* from Ethiopia was susceptible to an American isolate of *P. sorghi*. Also, *S. nitidum* from Australia was immune in the present study but Bonman *et al.* (1983) reported that native *S. nitidum* from Thailand was highly susceptible. *S. nitidum* is a highly variable species with an extensive range of distribution and accessional/regional differences could account for the varying reports. Further, these differences in reactions could also be explained by the existence of physiological races or pathotypes with different host ranges within *P. sorghi*. Since the first report (Craig and Fredericksen, 1980), several pathotypes have now been identified (Fernandez and Schaffert, 1983; de Milliano and Veld, 1990; Craig and Odvody, 1992). Those from Africa (Nigeria and Ethiopia) and Asia are more virulent than those from the Americas (Pawar *et al.*, 1985). Alternatively, there may be different species of

Peronosclerospora with different host ranges. The resistance in wild Australian species seems to exemplify this. Though the pathogen was noticed in maize in 1977 (Reddy, 1979), the disease has not been reported in Australia until very recently (Pande *et al.*, 1997). The resistance in the wild species of *Sorghum*, therefore, seems to have developed in the absence of the pathogen signifying allopatric resistance (Harris, 1975). However, although *P.sorghi* is unknown, another downy-mildew-causing species *P.noblei* is known on *S.plumosum*, a *stiposorghum* from temperate New South Wales, Australia (Weston, 1942; Kenneth, 1981). In addition, *P.sacchari*, causing downy mildew of sugarcane is also reported. There are no reports on the susceptibilities of other indigenous Australian sorghums to these pathogens. A comparison of the host ranges of a relatively large number of isolates of *P.sorghi* on the same species/accessions of *parasorghum, heterosorghum, chaetosorghum* and *stiposorghum* coupled with mycological comparisons are required to determine differential susceptibilities of various species of *Sorghum* to downy mildew.

The resistant accessions identified within section *sorghum*, which constitutes the primary and secondary genepool, may be directly used in sorghum breeding to incorporate SDM resistance and produce durable resistance for areas where downy mildew is a serious problem. They may also profitably be used to generate mapping / segregating populations to identify the gene/s or QTLS associated with SDM resistance to enhance marker aided selection in sorghum improvement. However, further studies are required to unequivocally establish a link between sources of resistance and geographic region if any. Even though wild races of *Sorghum* are not priority choices for yield genes in the short term, their potential for improving resistance/tolerance to SDM and other stress environments could prove useful.

The present study also identified many new sources of resistance for SDM from the tertiary genepool, which constitutes the extreme outer limit of the potential genetic resource for crop improvement using conventional breeding methods. Recent advancements in genetic engineering provide for a major expansion of this genepool and offer a distinct possibility of utilizing these species as well.

Screening for Resistance to Sorghum Shoot Fly

Sorghum bicolor is one of the most important hosts of sorghum shoot fly, causing damage at the seedling stage by killing the central shoot (deadheart symptom). The present studies identified several species, accessions of which express host plant immunity to the sorghum shoot fly under both field and greenhouse conditions. Seedling resistance was based on percentage infested seedlings or "deadheart' percentage. In addition, egg counts were used as a measure of ovipositional non-preference. Sorghum

germplasm belonging to *parasorghum* (*S.australiense, S.purpureosericeum, S.brevicallosum, S.timorense, S.versicolor, S.matarankense, S.nitidum*) and *stiposorghum* (*S.angustum, S.ecarinatum, S.extans, S.intrans, S.interjectum, S.stipoideum*) did not suffer any shoot fly damage under multi-choice conditions in the field over two seasons, while *heterosorghum* (*S.laxiflorum*) and *chaetosorghum* (*S.macrospermum*) showed negligible shoot fly damage (Table 25). Twelve of these wild species, screened for the first time, were found to possess very high levels of resistance/immunity in the present study. *S.versicolor* and *S.purpureosericeum* were reported to be immune in an earlier study also (Bapat and Mote 1982; Mote, 1984; ICRISAT, 1988, 1989). The present study further confirmed the high levels of resistance obtained in three accessions of these species (IS 14262, IS 14275, IS 18945) reported earlier (Nwanze *et al.*, 1990a).

Within section *sorghum*, the four wild races belonging to *S.bicolor* subsp. *verticilliflorum* (*aethiopicum, arundinaceum, verticilliflorum* and *virgatum*) were highly susceptible to shoot fly as was *S.halepense*. Earlier studies report these taxa to be common wild hosts of the shoot fly (Nye, 1960; Starks, 1970). Davies and Reddy (1981) reared shoot flies on 21 species of the Poaceae and noticed *S.halepense* was by far the most important alternate host, with *S.verticilliflorum, S.almum, S.virgatum, Echinocloa colonum*, and to a lesser extent, *S.sudanense* also being significant hosts. Delobel and Unnithan (1981) observed that shoot fly populations were higher on wild sorghums than on the local cultivated varieties of *S.bicolor*, suggesting that they acted as a reservoir, especially during the dry season. The high susceptibilities of these wild races and species within section *sorghum* in the present study confirm earlier findings and one may infer that they play a major role as alternate hosts of this insect under natural conditions.

Large differences were observed among the accessions for percentage oviposition and number of eggs per plant in the two years, with oviposition for many of the accessions being greater on susceptible types. Differential environmental conditions as well as varying levels of insect infestation could account for this as reported earlier (Krishnananda *et al.*, 1970; Singh and Narayana, 1978; Singh and Jotwani, 1980a). The resistant checks (IS 18551 and IS 2146) belonging to cultivated species *S.bicolor* were significantly less damaged by shoot fly in terms of egg laying as well as deadhearts as compared to the susceptible check CSH 1 under field conditions (multi choice conditions).These observations are consistent with earlier studies on cultivated sorghums (Taneja and Leuschner, 1985a). Accessions of section *sorghum* showed significant differences across the years for percentage oviposition, eggs per plant and deadhearts formed, but interaction effects were not significant for accessions of *hetero-, stipo-* and *parasorghums* suggesting stability in their response to shoot fly infestation over the two

years. More oviposition was observed on *S.macrospermum* of *chaetosorghum* in the second year but deadheart formation did not increase simultaneously. This suggests that while this accession may be preferred for oviposition at enhanced levels of insect infestation under multi choice conditions, deadheart formation however, is effectively inhibited.

Evaluation of Mechanisms of Resistance to Sorghum Shoot Fly

A knowledge of the mechanisms and the factors contributing to host plant resistance to insects is useful in deciding suitable selection criteria and breeding methods for the genetic improvement of sorghum for resistance to insects (Sharma and Nwanze, 1997). Screening for resistance to insects under greenhouse conditions offers an effective method of identifying insect resistant cultivars since the pattern of occurrence and abundance of insect populations under natural conditions are often sporadic and highly influenced by the environment.

Non-Preference for Oviposition (Antixenosis)

In the present study, more eggs were laid on the resistant cultivar IS 18551 under no-choice conditions in the greenhouse, than under multi choice conditions in the field. Further, under field conditions oviposition in the resistant check was significantly less than the susceptible check but, both resistant and susceptible checks IS 18551 and CSH 1 were equally preferred for egg laying under no-choice conditions. This suggests that ovipositional non preference as a resistance mechanism is effective under multi choice conditions. These results are in conformity with earlier reports (Jotwani and Srivastava, 1970; Soto, 1974; Singh and Narayana, 1978; Taneja and Leuschner, 1985a).

The 22 accessions of the 15 species belonging to *para-*, *hetero-* and *stiposorghums*, which were highly non-preferred under multi-choice conditions in the field, showed varying levels of non-preference for oviposition and deadheart formation under no-choice conditions.

Accessions of *heterosorghum* (*S.laxiflorum*) and *chaetosorghum* (*S.macrospermum*) showed increased levels of egg laying under no-choice conditions compared to field. This again indicates that ovipositional non preference as a resistance mechanism, though effectively operative under multi-choice conditions, is not so effective under no-choice conditions in these two species for which egg laying was similar to that of the resistant check IS 18551.

Among the *para-* and *stiposorghums*, the accessions could be broadly categorised into three groups based on incidence of egg laying under no-choice conditions: (1) where there was no egg laying (absolute non-preference) as in *S.extans*, *S.stipoideum*,

S.matarankense and one accession of *S.versicolor* (accession IS 14262), (2) where there was significant reduction in egg laying as in *S.australiense*, *S.angustum*, *S.ecarinatum*, *S.intrans* and *S.interjectum* and some accessions of *S.versicolor* and *S.purpureosericeum*, and (3) where egg laying was similar to that on the resistant / susceptible check as in *S.timorense*, *S.nitidum* and a few accessions of *S.purpureosericeum* and *S.versicolor*.

The accessions particularly in the first category appear quite promising, as ovipositional non-preference is observed to be absolute, and none of the existing resistant cultivars is known to be completely non preferred for egg laying. It would seem that there is a strong repellent factor that is perceived by the fly at or near the plant surface that completely inhibits oviposition. Relatively reduced oviposition in the other two categories could be due to differential amounts/blends of compounds that inhibit oviposition to varying degrees. Alternatively, the reaction displayed by the wild species in the present study may be due to an absence of an attractant in varying degrees. Some susceptible sorghum genotypes are reported to emit volatile substances that guide the shoot fly females to their hosts for oviposition (Nwanze *et al.*, 1998). It could also be possible that the response observed among the wild species is simply a non-host response with a passive reaction.

An identification of the specific volatiles and their presence or absence can provide a better understanding of what the shoot flies perceive in the environment around the sorghum plant and can also establish whether there are qualitative or quantitative differences in the volatile blends emanating from the leaves of the sorghum plant. Lwande and Bentley (1987) identified (2)-3-Hexene-1-ol acetate as the major volatile trapped from the seedlings of *S.bicolor* (var Serena) to elicit a behavioural response in some adult phytophagous insects. Knowledge of the volatile compounds of sorghum genotypes may be useful in the study of shoot fly-sorghum-plant relationships, especially to determine whether antixenosis might be explored as a complementary control method.

Besides volatile substances, morphological features on the plant/leaf surface may also contribute to absence of or reduced egg laying. In most of the resistant accessions in the present study, medium to high density of hairiness was observed on both the abaxial and the adaxial surfaces of the leaves. In addition, the leaf blades were usually scabrous to puberulent and the ligule was a highly fringed membrane with or without long hair. These features, by being physical irritants may be a cause for reduced oviposition by the shoot fly. Sorghum cultivars with large numbers of trichomes on the leaf have shown reduced oviposition by *A. soccata* (Maiti *et al.*, 1980; Bapat and Mote, 1982), but in other studies deadhearts and density of trichomes were not significantly correlated (Maiti and Gibson, 1983). Also, there are no reports to show that trichomes do actually interfere with fly behaviour. Other factors that have been reported to be

associated with resistance include enhanced seedling vigour (Mote *et al.*, 1986), longer stems, and internodes, and short peduncle (Patel and Sukhani, 1990a), glossiness of leaf surfaces (Maiti and Bidinger, 1979), leaf surface wetness and crystalline epicuticular wax (Nwanze *et al.*, 1992), and differences in silica deposition and lignification (Ponnaiya, 1951 and Blum, 1968).

Phototactic and odour cues from host seedlings are known to have a positive effect on flight orientation and oviposition responses. Information on such stimuli can be used for monitoring shoot fly populations, and may have direct implications in both the breeding for resistance and management of shoot fly. If the adult shoot fly refuses to oviposit or there is a significant suppression in egg laying in the absence of a preferred variety, as in the present study, this type of ovipositional non-preference may be of practical value. In cotton, for example, transfer of an oviposition-suppression factor, identified in *Gossypium barbadense* (L.) into *G.hirsutum* L., resulted in a 25% to 40% reduction in egg laying by the boll weevil, *Anthonomus grandis* Boheman (Maxwell *et al.*, 1969). Similarly, when the cereal leaf beetle *Oulema melanopus* (L) was exposed to pubescent wheat varieties, oviposition was reduced drastically (Schillinger and Gallum, 1968)

Antibiosis

In the present investigation, a significant reduction in the deadheart formation or its complete absence was observed in seedlings of *hetero-, para* and *stiposorghums* in comparison to the resistant check IS 18551, despite the high incidence of egg laying in many of the accessions. In contrast, accessions of section *sorghum* exhibited high incidence of egg laying as well as deadhearts. When seedlings of *hetero-, para-* and *stiposorghums* were manually infested with shoot fly eggs, some genotypes still did not show any deadhearts (*S.extans, S.stipoideum* and *S matarankense* and one accession of *S.purpureosericeum*) whereas others exhibited a slight increase in percentage of deadhearts compared to that obtained after forced oviposition (Table 29). Even so, the deadheart percentage was significantly less compared to the resistant check IS 18551.

Accessions of *stiposorghum* showed a recovery of the few deadhearts and no adult emergence, indicating absence of larval survival. Deadhearts when dissected out revealed dead larvae at or near the growing point of the stem with only traces of feeding. Accessions of *heterosorghum* (*S.laxiflorum*) showed a relatively higher proportion of deadhearts, but, even in these, since no flies emerged, one may infer that though the larvae were initially successful in establishment, they subsequently died. Deadhearts when dissected out, revealed a few live larvae besides the dead ones. Among the *parasorghums*, there was no fly emergence in *S.nitidum, S.purpureosericeum* (IS18945) and *S.versicolor* (IS 14262) despite some deadheart formation, whereas a few flies

emerged in other accessions. Larval mortality was however, noticed in all main stems of the *parasorghums* viz., *S.versicolor, S.purpureosericeum, S.timorense* and *S.nitidum* when deadhearts were cut open for observation.

Relatively increased deadheart formation, when seedlings were manually infested with eggs, probably occurred because the eggs were placed directly in the leaf whorl and the larvae did not have to navigate the leaf lamina to reach the whorl. Normally after hatching, the larvae crawl along the leaf lamina to reach the plant whorl and then move downwards through the central shoot till they reach the growing point and after cutting it at the base, feed on the decaying leaf tissues, which results in the formation of the deadheart (Taneja and Leuschner, 1985a).

The above observations indicate the likely presence of a strong compound/blend of compounds that actively inhibit larval survival and development particularly in the main stem of the seedling. Further, there are probably also optical and odour cues on the surface of the leaf among these wild species (by way of trichomes and varying degrees of pubescence) that perhaps disorient the newly hatched larvae and deter them from reaching the whorl. Where a few flies emerged, probably the larvae moved down towards the central shoot, and after partially making a cut, leaving it relatively undamaged, the larvae moved to the side tillers, survived there, and subsequently might have developed into adults. This suggests that tillers had less of an antibiosis effect on the larvae perhaps due to greater toughness/lignification of the main stem tissues compared to the tillers or the presence of larger quantities of antibiotic compounds in the main stem. This would also explain the recovery of the main shoot when the tillers showed complete deadhearts.

Although ovipositional non-preference is observed as the primary mechanism for shoot fly resistance in sorghum, there is evidence from the present study for a high degree of antibiosis, which also contributes to resistance. Others have reported similar results of reduced dead hearts, but low levels of antibiosis on resistant sorghum cultivars (Jotwani and Srivastava, 1970; Blum, 1972; Young, 1973; Soto, 1974; Sharma *et al.*, 1977).

Maximum larval survival and adult emergence was observed among accessions of section *sorghum*, including the four wild races (*aethiopicum, arundinaceum, verticilliflorum* and *virgatum*) and *S.halepense*. These susceptible accessions were comparable to the susceptible check CSH 1 both in terms of larval and pupal periods as well as adult emergence except for *S.halepense* where the developmental period was slightly extended by 2 to 6 days, and the accession of race *arundinaceum*, which showed significantly less adult emergence. Adverse effects of resistant cultivars on the survival, development and fecundity of shoot fly has been noted earlier (Narayana, 1975; Singh and

Narayana, 1978; Singh and Jotwani, 1980b). As in the present study, Raina *et al.* (1981) also observed mortality among the first instar larvae and significantly slow growth of the surviving ones.

Based on the above observations in the present study, it may be inferred that there may be different factors and mechanisms, which individually or in combination may contribute to expression of resistance (ovipositional non-preference and antibiosis) to the shoot fly in sorghum. No single factor has as yet been attributed to cause larval mortality and lowered adult fecundity. Several differences between resistant and susceptible cultivars have been observed for percentage of nitrogen, reducing sugars, total sugars, moisture and chlorophyll content, which in susceptible cultivars are higher than in resistant ones (Singh and Jotwani, 1980c; Patel and Sukhani, 1990b). Also, higher quantities of total amino acids were found in shoot fly resistant cultivars than in susceptible ones (Khurana and Verma, 1982). Woodhead (1982) observed the presence of unusually large amounts of p-hydroxybenzaldehyde in the surface wax of young sorghum plants, but there is no evidence linking this to shoot fly behaviour (Chapman and Woodhead, 1985).

The present study confirms the high susceptibility of section *sorghum* to the sorghum shoot fly. However, extended period of larval and pupal development coupled with lowered adult emergence in some of the wild races/species like race *arundinaceum* and *S.halepense* decreased survival and fecundity of the shoot fly. It is possible that more accessions can be identified with similar reactions that can be exploited for sorghum breeding as they belong to primary/ secondary gene pool. The present investigation has also established that species of *stiposorghum, parasorghum, heterosorghum and chaetosorghum,* all of the tertiary gene pool, are highly resistant if not immune to sorghum shoot fly (*A. soccata*), both in terms of non-preferrence to oviposition and antibiosis.

Antibiosis in combination with ovipositional non-preference would be highly desirable as operating mechanisms for resistance to shoot fly. In view of the immunity/ high resistance observed in these wild sorghums, the exact nature of the resistance conferred by these species needs to be unravelled by further studies and biochemical assays for a better understanding of shoot fly behaviour, particularly in relation to its host species.

Screening for Resistance to Spotted Stem Borer

In the present investigation, 15 wild species of *Sorghum* showed high levels of resistance to the spotted stem borer under conditions of artificial infestation in the field, as well as in the greenhouse, with some accessions showing levels of resistance close to immunity. Seedling resistance was measured as percentage of plants damaged based on leaf injury, and percentage of deadhearts obtained. In addition, the seedlings were graded for leaf damage by giving a score ranging from 0 (no damage) to 9 (very severe damage). Plants damaged by spotted stem borer show typical symptoms of 'window pane' formation which results from the larvae feeding inside the whorl as is evident from the unfolding central leaves showing small or large shot holes on the lamina. Under field conditions, species of *heterosorghum* (*S.laxiflorum*), *parasorghum* (*S.australiense, S.purpureosericeum, S.versicolor, S.matarankense, S.timorense, S.brevicallosum, S.nitidum*) and *stiposorghum* (S.*angustum*, *S.ecarinatum, S.extans, S.intrans, S.interjectum* and *S.stipoideum*) showed negligible damage with a low leaf damage score and no deadhearts, except for one accession of *heterosorghum* which showed 2% dead hearts. In contrast, section *chaetosorghum* (*S.macrospermum*) was observed to be highly susceptible with a high leaf damage score, and high deadheart percentage. These wild species have been evaluated for resistance to stem borer for the first time.

The absence of significant differences in leaf damage score, percentage of plants damaged and percentage of deadhearts over the two years among accessions of *hetero-*, *para* and *stipo sorghums* indicate stability in their resistance response to artificial infestation of borer larvae under field conditions. In contrast, *chaetosorghum* was highly susceptible as evidenced from the large number of deadhearts in the first year. However, in the second season, deadhearts were significantly reduced, as were the number of plants damaged, though leaf feeding/damage was more. This suggests the possible sensitivity of this accession to seasonal variations prevailing during and immediately after artificial infestation. Environmental factors as temperature, humidity and wind velocity might have caused differences in larval establishment and consequently resulting in increased/reduced deadhearts. Similarly, within section *sorghum*, the four wild races of *S.bicolor* subsp. *verticilliflorum* (races *arundinaceum, aethiopicum, verticilliflorum* and *virgatum*) were highly susceptible to stem borer infestation as was *S.halepense*. These wild races/species are commonly affected under natural conditions (Trehan and Butani, 1949; Reddy, 1989) and probably serve as alternate hosts/reservoirs of the insect. Significant differences in accessions for all three parameters over the two years suggest that differential environmental conditions influence the susceptible/resistance response of a particular genotype. ICSV 700, an improved variety bred for

resistance to stem borer, which showed significant differences in % deadhearts in the two years, exemplified the absence of durable resistance among the cultivated sorghums in the present study.

Evaluation of Mechanisms of Resistance to Spotted Stem Borer

Knowledge of principles determining levels of plant resistance to insects enhances the efficient use of various mechanisms in pest management. An understanding of the mechanisms of resistance also allows the effective utilisation of resistant genotypes in stem borer breeding programmes with optimum outlay of time, effort and resources.

Non - Preference for Oviposition (Antixenosis)

In the present study, there was little variation within section *sorghum* in limited-choice tests with the wild races of subsp. *verticilliflorum* as well as weedy *S.halepense* being highly preferred for oviposition both in terms of egg masses and eggs. Further, differences in oviposition between the resistant and susceptible check IS 2205 and ICSV-1 were statistically non-significant. Similarly, the two accessions of *S.laxiflorum* belonging to *heterosorghum* were also highly preferred for oviposition. *Stiposorghums* in general, were least preferred for oviposition and had significantly lower number of egg masses and average eggs per plant. Among the *parasorghums*, there were marked differences in oviposition among the accessions with some being as much or more preferred than the resistant check (*S.australiense, S.matarankense, S.nitidum*), others significantly less preferred (some accessions of *S.purpureosericeum, S.versicolor, S.timorense*) and still others not preferred at all (IS 18944 - *S.purpureosericeum* and IS14262 - *S.versicolor*). Under no-choice conditions, egg laying was observed on all the accessions even on those that were not oviposited at all in the limited-choice tests. However, significant variation was seen in terms of the number of eggs laid among the different accessions. The wide variation observed in number of egg masses and eggs and relative oviposition preference with respect to the resistant check IS 2205, clearly indicates some measure of recognition and preference/non-preference for oviposition by *C. partellus* females on different species/accessions.

Considerable variation was also noticed in the distribution of egg masses and total number of eggs on the leaf surface, but no overall pattern was seen both under limited-choice and no-choice conditions. *Para-* and *stiposorghums* in general were observed to have more egg masses on the lower surface, whereas the opposite was observed for *heterosorghum*. However, two accessions of *S.australiense* (*parasorghum*), *S.ecarinatum* (*stiposorghum*) and race *virgatum* (*sorghum*) showed an almost even distribution of egg masses on the two surfaces. Under no-choice conditions, some of the *para-* and

stiposorghums showed more oviposition on the upper surface. Additionally, *C. partellus* exhibited differential behaviour in the limited- and no-choice tests on the same accessions. Further, some egg laying was also noticed on the walls of the oviposition cage in some of the accessions both in limited and no-choice tests.

Differential egg laying observed in the present study might be due to non-suitability of the given host for oviposition or alternatively, the non-specificity of host surface for oviposition. Roome *et al.* (1977), observed that stem borer moths readily laid eggs on inert material in cages. Nevertheless, if plant leaves are present they are preferred (Chapman and Woodhead, 1985). Physical and chemical characteristics of the plant/ leaf surface, thus, probably influence the moths in their choice of site for egg laying. In most of the accessions of *para-, stipo-, hetero-* and *chaetosorghums* in the present study, a medium- to high-density mat of hairs was observed on both the abaxial and the adaxial surfaces of the leaves. These features, by being physical irritants, may be a cause for reduced oviposition by the stem borer moths. It is known that as a preliminary to oviposition, the insect touches the leaf surface with its antennae and tarsi as well as the ovipositor, which is well endowed with mechanoreceptor hairs (Chadha and Roome, 1980). Thus, these species/accessions which show significantly reduced oviposition relative to the resistant check IS 2205 may be used to investigate chemo receptors and also mechanoreceptors involved in oviposition.

In the present study, under limited-choice conditions, some accessions were relatively unacceptable for oviposition. However, since the leaf/plant surface on these accessions did not completely prevent oviposition when given no-choice, it is still possible that part of the preference shown for the accession over the other surfaces may be mediated by chemical differences in the foliage, which either attract or repel the adults. Moore (1928) demonstrated that volatile chemicals emanating from corn foliage play an important role in the orientation of moths of the European corn borer *Ostrinia nubilalis*.

Significant differences in oviposition on resistant and susceptible genotypes have been established by some workers (Rana and Murty, 1971; Singh and Rana, 1984; Lal and Pant, 1980b; Taneja and Woodhead, 1989; van den Berg and van der Westhuizen, 1997). Differential distribution of egg masses on the upper and lower leaf surfaces has also been observed in several studies and has been attributed generally to trichome density/ hairiness. Ampofo (1985) found that in all maize genotypes and plants at different stages of growth, smooth areas of the lower leaf surface and midrib concavity were preferred by *C. partellus* for oviposition. Durbey and Sarup (1982) also found that moths of *C. partellus* preferred to lay eggs on more or less glabrous lower surface of the leaf. Dabrowski and Nyangiri (1983) reported that maize lines that are hairy, especially on the adaxial surface, had less oviposition. Kumar and Saxena (1985) removed the trichomes on the upper surface thoroughly on one side of the central midrib of the leaf,

leaving the other side intact. Results indicated that the trichomes of the resistant leaf inhibited oviposition, as percentage of eggs laid on the hairless side of the leaf were about five times that on the hairy side in both basal and terminal portions of the leaf. Bates *et al.* (1990) also reported that oviposition by *C. partellus* on maize and sorghum varies greatly in terms of leaf number and leaf blade surface, as well as section and position where oviposition takes place.

The present study demonstrates definitely the presence of ovipositional non-preference by the stem borer. Nevertheless, the choice of site for oviposition does not appear to be guided by its suitability for larval survival, but rather, selected to maximise egg survival. It is known that the larvae after hatching, move to the leaf whorl, feed there and subsequently are able to migrate to neighbouring plants by suspending themselves on silken threads by the 2nd, 3rd or 4th instar stages. This tendency for larval dispersal in early stages could account partially for absence of deadhearts in all *para-* and *stiposorghums*, and some *heterosorghums* in the present study despite considerable egg laying. However, the absence of deadhearts in these wild accessions could also have resulted from the initial failure of the newly hatched larvae to reach the feeding site (plant whorl). Physical and chemical surface characteristics of the culm/leaf probably do not provide the correct cues, thus disorienting the larvae and directing them away from rather than towards the whorl. Further, the ligules, which in these accessions are a highly fringed membrane with or without long hairs, may also act as traps for the climbing larvae preventing their successful establishment. There are some studies which support such an inference (Chapman and Woodhead, 1985).

The present studies are however, inconclusive for ovipositional non-preference in terms of leaf surface, varying both with the accession studied, as well as the evaluation conditions. Further studies are required to determine the value of ovipositional antixenosis in limiting pest damage to indicate its usefulness in breeding programmes.

Antibiosis

The present investigation exhibited wide variation for plant damage, deadheart formation, larval survival, and adult emergence among the accessions tested. These studies were conducted by releasing first-instar larvae directly at the feeding site in the whorl of wild sorghum seedlings under cage conditions in greenhouse. *Stiposorghums* showed traces of leaf feeding and no deadhearts with the consequence that there was no adult emergence. Among the *parasorghums*, despite some amount of plant damage very few deadhearts were formed, and only two larvae were recovered both of which subsequently stopped feeding and died. In comparison, after artificial infestation in the field no deadhearts were found among both *para-* and *stiposorghums*. Among the *heterosorghums* there was considerable plant damage, and up to 82% deadhearts were

obtained but the six larvae that were recovered did not survive to adulthood. Accessions of section *sorghum* showed maximum deadhearts. There was 95% recovery of larvae and pupae from the susceptible cultivar ICSV 1 most of which survived to adulthood. In IS 2205, the resistant check, larval recovery was, however, low and they did not survive. In *S.halepense,* even with high plant damage and deadheart formation, there was 65% larval and pupal recovery, but only 23% of these emerged as adult moths. In race *virgatum* too, there was high plant damage and deadhearts, but, only 36% of the 55% larvae and pupae that were recovered, survived to emerge as moths. Of these, some were abnormal.

The results indicate a high level of antibiosis and/or antixenosis at the feeding site in most of the wild sorghums. In the experiments using artificial infestation, since all plants were artificially infested directly in the whorl, the larvae were not exposed to factors that otherwise could have influenced their movement outside plant. Hence, the absence of deadhearts and low larval recovery in *para-* and *stiposorghums* may be attributed to larval mortality either due to antibiotic effect of chemicals in the plant tissues at the feeding site or the inability to feed due to unsuitability of the plant tissues/anatomical features of the stems. In the few instances where a couple of larvae were recovered, they stopped feeding and died, again suggesting the inability to continue feeding due to tough tissues or the presence of antibiotic compounds that were detrimental to their establishment, development and survival. Others have made similar observations on cultivated sorghum genotypes (Lal and Pant, 1980b; Singh and Verma, 1988; Taneja and Woodhead, 1989). Field experiments by Woodhead *et al.* (1980) showed that damage to the whorls of sorghum by first-instar larvae placed directly into the whorl, was inversely correlated with the amount of HCN produced when the leaves were crushed.

Further, since larval dispersal is easily effected through silken threads by the second instar stage itself, it is likely that the larvae started feeding in the whorl but found the host unsuitable, and therefore migrated to neighbouring plants in search of suitable hosts. This mechanism probably accounted for the absence of deadhearts and consequently also low larval recovery both under field and greenhouse conditions.

Among members of section *sorghum*, larval survival trends were quite different. Maximum survival and adult emergence was noted in susceptible check, ICSV-1, indicating its high suitability as a host. In contrast low larval recovery and their subsequent death in resistant check IS 2205 reflected its unsuitability as a host. In *S.halepense*, three larvae escaped possibly due to non-preference for feeding. Additionally, prolongation of larval period observed in *S.halepense* and race *virgatum* could also be another effect of antibiosis as also the lack of development of all pupae into normal adults in these two accessions. Others have also reported similar results on cultivated sorghum (Dayal, 1989; Taneja and Woodhead, 1989; Saxena, 1990, 1992; Verma *et al.*, 1992).

While these wild races/species within section *sorghum* are highly preferred for oviposition, it is possible that they contain some quantities of antibiotic compounds that is inimical to larval growth and development thus leading to lowered fly populations under natural conditions. Further biochemical studies are needed to identify the compounds that may be responsible for these reactions. It is possible that more accessions may be identified with similar reactions that may profitably be used in sorghum breeding for stem borer resistance.

This study indicated that genotypes though preferred for oviposition might also have high levels of antibiosis to larval feeding and/or exhibit antixenosis to feeding. The degree to which antibiosis is present in a given variety would determine the mechanism of larval survival and the ultimate level of damage incurred. These observations support the view of Ampofo and Nyangiri (1986) that the suitability of plants for feeding neonate larvae is not a major factor determining the choice of oviposition site by *C. partellus* moths.

This investigation has established that high levels of resistance close to immunity are available among the wild sorghums in the tertiary gene pool. Further, various factors, traits and mechanisms appear to contribute to this resistance. Selection of plant material by breeders may be based on this information to increase the levels of and diversify the bases of resistance to *C. partellus* in sorghum.

Allopatric Resistance to Pests and Diseases

In the present study, screening of wild species of *Sorghum* identified several sources with high levels of resistance/immunity to sorghum downy mildew in the primary, secondary and tertiary genepools. Not surprisingly, the resistant sources identified for SDM within the primary and secondary genepools were from Asia and Africa, where both the crop and the pathogen are believed to have originated. It is generally accepted that centres of origin of plants harbour rich sources of resistance to diseases where host and pathogen have coevolved (Leppik, 1970; Harlan, 1977). However, this may not always be true, as is exemplified by the present study, which established that species from Asia and Africa were immune to the SDM pathogen as were the species from Australia, where the pathogen is reported to be a recent introduction. The earliest report of the pathogen in Australia is on maize in 1977 (Reddy, 1979). This type of resistance to diseases and pests, found, in germplasm from areas free of those diseases/pests against which the resistance operates, is considered to be 'allopatric resistance' (Harris, 1975).

These species of the *hetero-, chaeto-, para-* and *stiposorghums* representing the tertiary genepool were also found to be highly resistant/immune to attack by the sorghum shoot fly and the spotted stem borer. Both these insects are unknown in Australia

though present in both Asia and Africa, supporting the idea of allopatric resistance – that a recent co-evolutionary history is not necessary for a plant to possess resistance to a pest. Rather, resistance may be a consequence of a separate biological process important to plant survival, and incidentally also affording resistance to an insect with which it has had no co-evolutionary history. However, it is also possible that infestation by *A. soccata* or *C. partellus* is restricted to section *sorghum* as is reported for *Stenodiplosis sorghicola* Coquillett (Sharma and Franzmann, 2001). Similarly, Harris (1979) studied a wide array of midge specimens collected from sorghum, wild sorghums, wild Poaceae and Cyperaceae from Australia and concluded that species other than *Contarinia sorghicola* have evolved as specific pests of *parasorghums* and *stiposorghums*. *Contarinia plumosi* and *C. roperi* are reported to infest *Sorghum plumosum*, and *C. intrans* infests *S.intrans* and *S.stipoideum*. Other species of gramineae are infested by different species of midges. In the light of this, the response of the *hetero-, chaeto-, para-* and *stiposorghums* to other species of *Atherigona* and *Chilo* need to be investigated to enable a better understanding of host plant – insect relationships.

Many studies have found that both gene order and function are conserved among widely divergent plant taxa (Ahn and Tanksley, 1993, Namuth *et al.*, 1994; Lin *et al.*, 1995). The extent to which this may account for the evolution of allopatric resistance to encounters with new diseases or pests is debatable, but it does seem possible that apparently ' unnecessary' genes for resistance may be maintained in host populations if they carry no fitness cost, or if they are associated with some other 'necessary' character.

Conclusion

An overview of the results shows that *Sorghum* is a very diverse genus at both phenotypic and molecular levels. Section *sorghum* is monophyletic and highly homogeneous quite distant from the other four sections. Divergence of geographically distinct *parasorghums* indicates a polyphyletic origin for this section. The Australian species of *para-* and *stiposorghums* appear to represent a different line of evolution from the other sections, suggesting a polyphyletic origin for the different sections within the genus *Sorghum*.

The accessions identified as resistant to sorghum downy mildew within section *sorghum* may be directly used in sorghum breeding, as they are part of the primary and secondary genepools and can easily hybridise with cultivated sorghums. While confirming that the wild races/species within section *sorghum* are highly preferred for oviposition to shoot fly and stem borer, the present results however suggest the presence of antibiotic compounds that are inimical to larval growth and development. It is possible

that more accessions may be identified with similar reactions that may profitably be used in sorghum breeding.

Sorghum improvement has hitherto relied on exploitation of variability within the primary genepool as gene transfer from one background to another can be readily made. However, the present study demonstrates that wild species of *Sorghum*, several of which have been evaluated for the first time, could be a potentially valuable source of germplasm for sorghum improvement. Accessions of 15 species belonging to sections *stiposorghum, parasorghum, heterosorghum and chaetosorghum*, have been identified as immune / highly resistant to sorghum downy mildew, sorghum shoot fly and the spotted stem borer. These species belong to the tertiary genepool and constitute the extreme outer limit of the potential genetic resource for crop improvement. While their benefit to sorghum improvement through conventional breeding may be limited, recent breakthroughs in cellular and molecular biology have now provided new tools and approaches for utilising the enormous potential that exists within the wild *Sorghum* genepool both as a source of pest and disease resistance and to broaden the genetic base of sorghum breeding.

Summary

Summary

Sorghum [*Sorghum bicolor* (L.) Moench], is an important cereal crop sustaining the livelihood of the resource poor farmers in the semi-arid tropics. Major advancements in crop improvement have resulted in short-statured, photo-insensitive and high-yielding varieties suitable for diverse agro-climatic conditions. Even so, productivity of cultivated sorghum continues to be constrained by various biotic and abiotic stresses (Doggett, 1988).

Wild relatives and progenitor species of *Sorghum* represent a potential genetic resource that has not been fully explored vis-a-vis sorghum breeding. Represented by 24 species, distributed in five taxonomic sections (*sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum*), members of the genus are spread across Asia, Africa and Australia (Doggett 1988; Lazarides 1991). These wild sorghums could be used to effectively broaden the genetic base and provide alternate sources of resistance genes for the long term control of major biotic/abiotic stresses.

With this in view, the present investigation was undertaken to study the diversity among wild sorghums at the morphological and molecular levels and also to identify sources of resistance to sorghum downy mildew, sorghum shoot fly and spotted stem borer.

Eighty-five accessions of wild sorghums, which are distributed in five taxonomic sections, could be clearly identified based on diagnostic morphological traits. Distinct genetic differences were observed for the quantitative traits in both rainy and post-rainy seasons. Leaf width, peduncle exertion, panicle length and number of rachis nodes showed the highest broad sense heritability suggesting that they are less affected by the season and therefore, can be effectively used as selection criteria in breeding programmes. Further, the high phenotypic variability obtained for the quantitative traits facilitated a clear distinction of taxa indicating the existence of group specific adaptations.

Molecular diversity in 22 accessions belonging to the five sections and representing 17 species was assessed using (i) four maize mitochondrial (mt) DNA probes, (ii) four sorghum derived resistance gene candidates, (iii) four AFLP primer combinations and (iv) 10 SSR primer sets. The same AFLP and SSR primer sets were also used to study the intra-specific diversity within 21 accessions representing the four wild races of *S.bicolor* subsp. *verticilliflorum* of section *sorghum*.

The profiles of mt DNA, AFLPs, SSRs and RGCs clearly differentiated the five sections, *sorghum, chaetosorghum, heterosorghum, parasorghum* and *stiposorghum*.

Section *sorghum*, was confirmed to be a highly homogeneous, monophyletic group quite distinctly separated from the other four sections. Within section *sorghum*, the levels of polymorphism within *S.bicolor* subsp. *verticilliflorum* differed considerably, ranging from 61% in AFLPs to 100% in SSRs for the 21 accessions analysed. Overall average AFLP gene diversity (H=0.15) was found to be low in *S.bicolor* subsp. *verticilliflorum*. SSRs, on the other hand, gave a high value of H=0.77. The same trend was also observed among races and geographical regions. Race *verticilliflorum* was most diverse among the races. Accessions from eastern Africa exhibited more diversity as compared to those from southern, central or western Africa. High similarity and less differences observed among the races and geographic regions may be attributable to high gene-flow and the presence of low frequency alleles unique to each population respectively.

Based on both mt DNA and AFLP data *chaetosorghum* and *heterosorghum* showed a closer relationship as compared to other sections.

Both mt DNA and AFLP profiles indicated that the Afro-Asian *parasorghums* were distinct from the Australian *parasorghums* suggesting a polyphyletic origin for this section. *S.nitidum* of *parasorghum* from Australia shared an ancestral relationship (based on mt DNA profile) with the Afro-Asian *parasorghums* and exhibited a close relationship with the Australian *parasorghums* (based on AFLP profiles).

Parasorghums and *stiposorghums* from Australia shared a close relationship based on both mt DNA and AFLP profiles suggesting a separate line of evolution for these sections and further confirming the polyphyletic origin for section *parasorghum*.

Accessions of 17 species, originating from Asia, Australia, Africa, and the USA, were greenhouse tested for resistance against downy mildew. Among the cultivated types tested, a new source of resistance, IS 14383, a landrace guinea sorghum from Zimbabwe, was identified. Thirty-six accessions comprising 15 species from four sections, *parasorghum* (*S.australiense, S.brevicallosum, S.matarankense, S.nitidum, S.timorense, S.versicolor, S.purpureosericeum*), *heterosorghum* (*S.laxiflorum*), *chaetosorghum* (*S.macrospermum*), and *stiposorghum* (*S.angustum*, *S.ecarinatum*, *S.extans*, *S.intrans*, *S.interjectum*, *S.stipoideum*), including all accessions from Australia, exhibited immunity to downy mildew. Among the wild accessions of section *sorghum*, two accessions one each in races *aethiopicum* (IS 18821) and *arundinaceum* (IS 18882) and one weedy accession of *S.halepense* (IS 33712) also exhibited immunity to downy mildew. One accession of race *virgatum* also exhibited resistance to the disease with only 1.7% infection.

Accessions of 17 species originating from Asia, Australia and Africa were evaluated for resistance to the sorghum shoot fly under both field and greenhouse conditions. Germplasm belonging to *parasorghum* (*S.australiense, S.purpureosericeum, S.brevicallosum,*

S.timorense, S.versicolor, S.matarankense, S.nitidum) and *stiposorghum* (*S.angustum, S.ecarinatum, S.extans, S.intrans, S.interjectum, S.stipoideum*) did not suffer any shoot fly damage under multi-choice conditions in the field over two seasons. Sections *heterosorghum* (*S.laxiflorum*) and *chaetosorghum* (*S.macrospermum*) showed negligible shoot fly damage, whereas all accessions of section *sorghum* tested exhibited susceptibility to shoot fly.

Accessions of the 15 species belonging to *chaeto-*, *para-*, *hetero-* and *stiposorghums*, which were highly non-preferred for shoot fly oviposition under multi-choice conditions in the field, showed varying levels of non-preference for oviposition and deadheart formation under no-choice conditions in the greenhouse. Accessions of *heterosorghum* (*S.laxiflorum*) and *chaetosorghum* (*S.macrospermum*) showed increased levels of egg laying under no-choice conditions compared to field. Thus, ovipositional non-preference as a resistance mechanism though effectively operative under multi-choice conditions is not so effective under no-choice conditions in these two species.

When artificially infested with shoot fly eggs in the greenhouse, accessions of *stiposorghum* showed a recovery of the few deadhearts, with no adult emergence, indicating absence of larval survival. Deadhearts when dissected out revealed dead larvae at or near the growing point of the stem with only traces of feeding. Accessions of *heterosorghum* (*S.laxiflorum*) showed a relatively higher proportion of deadhearts, but, even in these, no flies emerged. Among the *parasorghums*, again there was no fly emergence in most of the accessions despite a few deadhearts, and larval mortality was noticed in all main stems when deadhearts were cut open for observation. Members of section *sorghum* were highly susceptibile to the sorghum shoot fly. However, extended period of larval and pupal development coupled with lowered adult emergence in some of the wild races/species (race *arundinaceum* and *S.halepense*) could be profitably exploited for sorghum breeding. Although, ovipositional non-preference is observed as the primary mechanism for shoot fly resistance in sorghum, there is evidence from the present study for a high degree of antibiosis, which also contributes to resistance.

Accessions of 17 species originating from Asia, Australia and Africa were evaluated for resistance against the spotted stem borer under conditions of artificial infestation in the field, as well as in the greenhouse. Under field conditions, species of *heterosorghum* (*S.laxiflorum*), *parasorghum* (*S.australiense, S.purpureosericeum, S.versicolor, S.matarankense, S.timorense, S.brevicallosum, S.nitidum*) and *stiposorghum* (*S.angustum, S.ecarinatum, S.extans, S.intrans, S.interjectum* and *S.stipoideum*) showed negligible damage with a low leaf damage score and no deadhearts except for one accession of *heterosorghum* which showed 2% dead hearts. In contrast, section *chaetosorghum* (*S.macrospermum*) was observed to be highly susceptible with a high leaf damage score, and high deadheart percentage. Within section sorghum all accessions tested exhibited susceptibility to stem borer infestation.

Under no-choice conditions in the greenhouse, egg laying by stem borer was observed on all the accessions even on those that were not oviposited at all in the multi- / limited-choice tests. However, significant variation was seen in terms of the number of eggs laid among the different accessions. The wide variation observed in number of egg masses and eggs and relative oviposition preference with respect to the resistant check IS 2205, clearly indicates some measure of recognition and preference/non-preference for oviposition by *C. partellus* females on different species/accessions. Although, ovipositional non-preference is not the primary mechanism of resistance for the spotted stem borer, the present study demonstrates definitely the presence of some ovipositional non-preference by the stem borer. However, the choice of site for oviposition does not appear to be guided by its suitability for larval survival, but rather, selected to maximise egg survival.

Wide variation was observed for stem borer plant damage, deadheart formation, larval survival, and adult emergence among the accessions tested. The results indicate a high level of antibiosis and/or antixenosis at the feeding site in most of the wild sorghums belonging to *stiposorghums* (traces of leaf feeding; no deadhearts), *parasorghums*, (some plant damage; very few deadhearts; no adults) and *heterosorghums* (considerable plant damage; 82% deadhearts; no adults). Within section *sorghum* accessions showed maximum deadhearts with 95% recovery of larvae and pupae from the susceptible cultivar ICSV 1 most of which survived to adulthood. However, in accessions of *S.halepense* and race *virgatum* larval period was prolonged and all pupae did not develop into normal adult moths.

An overview of the results shows that *Sorghum* is a very diverse genus at both phenotypic and molecular levels. Section sorghum is monophyletic and highly homogenous quite distant from the other four sections. Divergence of geographically distinct *parasorghums* indicates a polyphyletic origin for this section. The Australian species of *para-* and *stiposorghums* appear to represent a different line of evolution from the other sections suggesting a polyphyletic origin for the five sections within the genus *Sorghum*.

The accessions identified as resistant to sorghum downy mildew within section *sorghum* may be directly used in sorghum breeding, as they are part of the primary and secondary genepools and can easily hybridise with cultivated sorghums. While confirming that the wild races/species within section *sorghum* are highly preferred for oviposition to shoot fly and stem borer, the present results however, suggest the presence of antibiotic compounds that are inimical to larval growth and development. It is possible that more accessions may be identified with similar reactions that may profitably be used in sorghum breeding.

Sorghum improvement has hitherto relied on exploitation of variability within the primary genepool as gene transfer from one background to another can be readily made. However, the present study demonstrates that wild sorghum species, several of which have been evaluated for the first time, could be a potentially valuable source of germplasm for sorghum im-

provement. Accessions of 15 species belonging to sections *stiposorghum, parasorghum, heterosorghum and chaetosorghum,* have been identified as immune / highly resistant to sorghum downy mildew, sorghum shoot fly and the spotted stem borer. These species belong to the tertiary genepool and constitute the extreme outer limit of the potential genetic resource for crop improvement. While their benefit to sorghum improvement through conventional breeding may be limited, recent breakthroughs in cellular and molecular biology have now provided new tools and approaches for utilising the enormous potential that exists within the wild *Sorghum* genepool both as a source of pest/disease resistance and to broaden the genetic base of sorghum breeding.

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