GENETIC ANALYSIS AND MOLECULAR MAPPING OF COMPONENTS OF RESISTANCE TO SHOOT FLY (Atherigona soccata Rond.) IN SORGHUM [Sorghum bicolor (L.) Moench]

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SEPTEMBER, 2002

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Thesis submitted to the University of Agricultural Sciences, Dharwad in partial fulfillment of the requirements for the Degree of

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IN

GENETICS AND PLANT BREEDING

By

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CERTIFICATE

This is to certify that the thesis entitled "GENETIC ANALYSIS AND MOLECULAR MAPPING OF COMPONENTS OF RESISTANCE TO SHOOT FLY (Atherigona s occata Rond.) IN SORGHUM [Sorghum bicolor (L.) Moench]" submitted by Miss.G.M. SAJJANAR for the degree of Doctor of Philosophy in Genetics and Plant breeding, to the University of Agricultural Sciences, Dharwad, is a record of research work done by her during the period of her study in this university under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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Dedicated to Beloved Parents

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Abbreviations

°C	Degree Coloine
°C	Degree Celcius
AFLP	Amplified fragment length polymorphism
	Analysis of variance
APS	Ammonium per sulphate
BAC	Bacterial artificial chromosome
bp	Base pairs
CIM	Composite interval mapping
cm	Centimeter (s)
cM	Centimorgan
CTAB	Cetyl trimethyl ammonium bromide
DAE	Days after emergence
daf	Days to 50% flowering
dH₂O	Distilled water
dhI	Deadhearts I (%)
dhII	Deadhearts II (%)
DHLs	Doubled haploid lines
DNA	Deoxyribonucleic acid
dNTP	Deoxy nitrogenous base 5'triphosphate
drywt	Seedling dry weight
EDTA	Ethylene diamine tetra acetic acid (disodium salt)
g	Grams
GxE	Genotype x environment
GCA	General combining ability
GCV	Genotypic coefficient of variation
gDNA	Genomic DNA
gl	Glossiness (scale 1-5)
h	Hours
h ²	Broad sense heritability
HCl	Hydrochloric acid
JMGRP	Joinmap linkage group assignment module
JMMAP	JoinMap map construction module
JMREC	JoinMap recombination estimation module
JMSLA	JoinMap single locus analysis module
LG	Linkage group
LOD	Log10 of the likelihood odds ratio
M	Molar
MAS	Marker Assisted selction
mg	Milligram (s)
min	Minute (s)
111111	winute (5)

ml	Millilitre (s)
mM	Millimolar
mm	Millimeter (s)
Na_2SO_3	Sodium sulphite
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng	Nanogram
nm	Nanometer
No.	Number (s)
ns	Non-significant
OD	Optical density
oviI	Oviposition I (%)
oviII	Oviposition II (%)
p	Probability
P1	BTx623
P2	IS 18551
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
PCV	Phenotypic coefficient of variation
pН	Hydrogen ion concentration
plht	Plant height (cm)
pslg	Pseudostem length (cm)
QxE	QTL x environment
QTL	Quantitative trait loci
r	Pearson correlation coefficient
r(_s)	Spearman's rank correlation coefficient
R ²	Phenotypic variance explained
RAPD	Random amplified polymorphic DNA
REML	Residual Maximum Likelihood
RFLP	Restriction fragment length polymorphism
RILs	Recombinant inbred lines
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	Revolutions per minute
SCA	specific combining ability
SE	Standard error
sec	Seconds
shtI	Seedling height I (cm)
shtII	Seedling height II (cm)
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats

trlw	Trichome density (lower leaf surface) (no mm ⁻²)
trup	Trichome density (upper leaf surface) (no.mm ⁻²)
UV	Ultraviolet
v	volume
v	Volts
vigI	Seedling vigour I (score 1-5)
vigII	Seedling vigour II (score 1-5)
w	Weight
W	Watts
YAC	Yeast artificial chromosomes
yld	Grain yield per plant (g/pl)
μί	microlitre
μl χ²	Chi-square

Introduction

I. INTRODUCTION

Sorghum (Sorghum bicolor (L.) Moench) is the fifth major cereal crop of world following wheat, rice, maize and barley in terms of production and utilization. Sorghum grain is produced annually as dietary staple for millions of people in semi-arid areas of Asia (mainly India and China) and Africa where drought stress causes frequent failures of other crops. Global cultivation of sorghum is covering an area 42 million hectares with annual production of 58.5 million tonnes. India is a major producer of sorghum with the crop being grown on an area of 10.5 million hectares with production of 9.5 million tonnes (Anonymous, 2001).

The genetic manipulation of the sorghum crop since the 1960's has lead to development of several high-yielding varieties and hybrids. However, realisation of this benefit in the farmers' fields faced with occurrence of biotic and abiotic stresses, is low. Insect pests cause much reduction in yield levels, among which shoot fly is a major one. Shoot fly (*Atherigona soccata* Rond.) is an important pest of sorghum in Asia, Africa and Mediterranean Europe. It was reported as early as the 1970's as one of the serious pests attacking sorghum at the seedling stage (7-30 days after emergence). Its incidence is higher in late-sown crops in the *rainy* and postrainy (*rabi*) seasons in India. The late-sown crops generally suffer greater shoot fly damage because of build up of shoot fly populations on the early-sown crops. The levels of infestation even may go up to 90-100% (Usman, 1972). The losses due to this pest have been estimated to reach as high as 85.9 percent of grain and 44.9 percent of fodder yield (Sukhani and Jotwani, 1980). The annual losses in sorghum production due to shoot fly in India have been estimated at nearly US\$200 million (ICRISAT, 1992).

Adoption of chemical control methods for insect control is not feasible for resourcepoor sorghum-growing farmers of the semi-arid tropics. Therefore, utilization of host plant resistance coupled with timely sowing is the most realistic alternative method for reducing losses caused by the insects. Genetic variability exists in sorghum germplasm for shoot fly tolerance/resistance, but sources of absolute resistance are not available in cultivated sorghum. Although high levels of resistance have been noticed in wild species (Mote, 1984; ICRISAT, 1991), their utilization in breeding programmes is hindered by crossing barriers (personal communication with scientists at ICRISAT, Patancheru). Biophysical plant characters facilitate breeders selection of genotypes resistant to shoot fly in cultivated sorghum. Despite efforts made to manipulate resistance utilizing the existing cultivated sources, the level of resistance achieved so far is low. In addition, neither the efficiency nor the reliability of selection is high when only conventional breeding approaches are used for selection for this trait. The reasons for inefficiency/unreliability of selection include there being several components involved in the resistance and each being controlled by one or more genes (*i.e.*, resistance is a polygenic trait). Further, the action of these genes is influenced by environment. This complicates selection, especially when large number of genotypes are scored in an experiment, hence adversely affecting gain from selection for resistance in an elite genetic background. Marker-assisted selection has the potential to greatly improve the efficiency of selection for such quantitative traits. Since the components of resistance to shoot fly are mostly quantitatively measured, it is important to analyze quantitative trait loci (OTLs) from the point of view of genetics and breeding. For the last two decades sorghum breeders have used the glossiness trait as an indirect selection criteria for shoot fly resistance although genetic control of the trait was not well understood. The ultimate goal of QTL analysis is to develop tools that are useful for marker-assisted selection in practical plant breeding programmes aiming at higher levels of resistance in agronomically elite genetic backgrounds. It can also be helpful in resistance gene pyramiding.

Recently molecular marker technology has made it possible to obtain numerous genetic markers to aid in plant breeding. Studies of QTLs using molecular markers are being conducted in many crop plants. DNA marker-assisted selection can supplement conventional breeding. For this, it is necessary to understand the genetics of component traits, G x E and Q x E interactions, and epistasis. Traditional genetic studies on shoot fly resistance with different sorghum genetic materials have been reported by many workers. However, in all of these information is fragmented. Therefore, a detailed study of the underlying genetic basis of resistance by using appropriate breeding material is the need of hour before formulating more effective breeding strategies. Sufficient numbers of recombinant inbred lines (RILs) were obtained by crossing appropriate parents, viz., BTx623 (susceptible) and IS 18551 (resistant) at ICRISAT, Patancheru. This has opened up new opportunities for a holistic reappraisal of the shoot fly resistance and its component traits. Quantitative genetic analysis of shoot fly resistance requires replicated, multi-environment testing under a wide spectrum of shoot fly

pressure, because of the unpredictability of field environment and possible $G \times E$ interactions. RILs are genetically homozygous and, can be replicated and evaluated in different environments. This allows for measuring the environmental contribution and genotype x environment contributions to total phenotypic variance allowing less-biased estimates of the genotypic variance. Reliable estimates of genetic parameters including heritability, genetic correlations, prediction of genetic gain and $G \times E$ interaction in this breeding material can be useful in applied breeding. Information on heritability and genetic advance of resistance traits helps to identify the characters for which effective selection is possible. Estimates of correlated genetic gain will help in identifying those secondary traits that could be effectively used as a basis for indirect selection for improved resistance.

QTL mapping with RILs will be more efficient than with an F_2 population, because RILs undergo several rounds of meiotic events resulting a two-fold or four fold increase in recombination frequency between two closely linked markers. Higher resolution maps therefore can be constructed with RILs than with single meiosis populations, and more accurate map distances are obtained with RILs than with an F_2 population of similar size (Burr and Burr, 1991).

Genetic maps consisting of molecular markers have been developed in recent years for many crop plants. High-density linkage maps are required for effective marker-assisted selection. In sorghum, linkage maps (individual/integrated) have been developed using a number of RFLP (Subudhi and Nguyen, 2000), AFLP (Boivin *et al.*, 1999) and SSR markers (Bhattaramakki *et al.*, 2000). However, there is a need to fill the remaining gaps with additional markers. Among different types of markers, the SSRs satisfy the best criteria of sufficient polymorphism, repeatability and cost effectiveness required for successful utilization in marker-based selection. In sorghum, a reasonably large number of SSR markers have been developed (often using elite breeding line BTx623 as a source) (Brown *et al.*, 1996; Taramino *et al.*, 1997; Kong *et al.*, 2000; Bhattramakki *et al.*, 2000 and personal communication with scientists at ICRISAT, Patancheru). These are suitable for screening the existing sorghum RIL population to construct a genetic linkage map and to identify QTLs for shoot fly resistance and its component traits. In addition, the molecular marker analysis can be helpful to study the genetic constitution of parents and RILs for shoot fly resistance. The information obtained will be useful to increase our understanding of genetic basis of shoot fly resistance components as well as to examine the various methods of assessing quantitative genetic variation.

A research program was therefore initiated to address the following objectives using the RILs generated at ICRISAT:

Objectives

- 1. Phenotyping RILs for the components of resistance to shoot fly under three different testing environments.
- 2. Study of the genetic architecture of component characters of resistance in relation to varying levels of shoot fly infestation.
- 3. Survey of parental polymorphism at the DNA level using SSR markers.
- 4. Genotyping a subset of the RIL population with a number of SSR markers showing polymorphism.
- Construction of a genetic linkage map and identification of QTLs for resistance to shoot fly and its component traits using the marker genotyped subset of the RIL population.

Review of Literature

II. REVIEW OF LITERATURE

Nearly 150 insect species have been reported as pests on sorghum (Reddy and Davis, 1979; Jotwani *et al.*, 1980) Shoot fly is one of the major pests of sorghum worldwide The adoption of chemical control method is not economically feasible for most sorghum growing farmers. Therefore, utilization of host plant resistance is the most realistic alternative method of reducing the losses caused by sorghum insect pests. No known source of cultivated sorghum accession is reported to confer absolute level of resistance to shoot fly. Further, the level of resistance available is not high and varies across cultivars. The genetic manipulation to increase the resistance is hindered by the complexity of resistance due to its quantitative inheritance, and interaction between host, insect and environment. Resistance of plants to insects is the consequence of heritable plant characters that result in a plant being relatively less damaged than the plant without these characters (Sharma, 1997a). It has been established based on previous studies by several workers that number of component traits are associated with shoot fly resistance.

Recent advances in molecular marker technology have demonstrated in several crops, the feasibility of identification of genomic regions associated with the traits, which are otherwise difficult to manipulate by conventional breeding, using DNA markers linked to such traits. The dissection of quantitative traits into Mendellian factors of inheritance, or so called QTL (Quantitative Trait Loci) provides a powerful tool for identifying genes with minor effects. It also potentially enables transfer of much of the set of genes (linkage blocks) important for resistance to desirable lines. Selection for markers linked to these chromosomal regions has the potential to improve the efficiency of manipulation of quantitatively inherited insect resistance in plant breeding programs.

Below, I review reports on the control of shoot fly, with emphasis on host plant resistance, the sources, mechanisms and inheritance of resistance, and breeding for resistance are reviewed Such an understanding is essential to justify the application of marker technology for genetic analysis of resistance to shoot fly and its components Since the use of molecular marker technology is a new technology, reports on molecular markers, QTL mapping, statistical techniques for mapping QTLs and views on marker-assisted selection are reviewed in general for crops and for sorghum in particular

2.1 Shoot fly resistance and its genetic analysis

2.1.1 Shoot fly control

2.1.1.1 Chemical control

Systemic granular insecticides like phorate, disulfan and carbofuran applied in seed furrows at the time of sowing have given effective control of shoot fly (Vedamoorthy et al., 1965, Usman, 1972 and Sandhu and Young, 1974). However, the dosage required for effective control and consequently the cost involved, is so high that very few sorghum growers have been able to utilize these research findings for the control of shoot fly in their crops (Sukhani and Jotwani, 1982). Soil application and seed treatment with carbofuran have been recommended for late sowings in the *rainy* and postrainy (*rabi*) season sorghums in India (Thimmaiah et al., 1973; Usman, 1973). However, under dryland conditions soil application may not be economical (Patil et al., 1992) since the effectiveness of carbofuran is mainly dependent on soil moisture (Taneja and Henry, 1993). Observations in the last decade have also showed that under high shoot fly pressure even carbofuran seed treatment is ineffective (Chaudhari et al., 1994). The use of insecticides as seed treatment was recommended by Balasubramanian et al (1987), but this recommendation was later withdrawn considering the hazards associated with it (Patıl et al., 1992). Foliar application of endosulfan could not control shoot fly effectively either in the *rainy* (*kharif*) season (Jotwani, 1982) or in the postrainy season (Taneja and Henry, 1993).

2.1.1.2 Biological and cultural control

Very little work has been done to explore the possibility of using parasitoids, predators and pathogens for the control of major pests. Weekly inundation of egg-parasite *Trichogramma chilonis* gave encouraging results in effectively reducing the percentage deadhearts caused by shoot fly (23%) compared to the untreated control (95%). However, being an ectoparasite, its effective periodical release requires constant monitoring for inundative releases during stages of the crop susceptible against shoot fly as well as stemborer (Singh and Rana, 1996).

From previous studies (Jotwani *et al.*, 1970) it has been established that in *kharif* season shoot fly incidence and damage increases with delay in sowing date. Early sowing may help to reduce its menace. However, in some areas and under certain situations early sowing is not feasible Waterlogging results in an increase in deadhearts due to attack by *Atherigona soccata* (Men *et al.*, 1986). Sorghum hybrids do well under irrigation. However, oviposition and deadhearts were higher in treatments with full irrigation than in treatments to which less water was applied during the first 3 weeks after seedling emergence (Nwanze *et al.*, 1996).

2.1.1.3 Host Plant resistance

The use of resistant varieties may offer the best (and perhaps only) economical method of control of certain pests. According to Smith (1989) resistance of plants to insects enables a plant to avoid or inhibit host selection, inhibit oviposition and feeding, reduce insect survival and development, and tolerate or recover from injury from insect populations that would cause greater damage to other plants of the same species under similar environmental conditions Painter (1951) defined resistance in plants to insect attack as the relative amount of heritable qualities possessed by the plant that influenced ultimate degree of damage done by the insect

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2.1.1.3.1 The resistant sources

It was established first by Ponnaiya (1951a) that genetic differences are existed for resistance to shoot fly. Most of the resistant lines were from peninsular India The systematic work on identifying the sources of resistance was initiated in the 1960's (Singh *et al.*, 1978) Subsequently, more than 10,000 varieties from the world collection were systematically screened at different locations (Sharma, 1997b) A number of these varieties consistently showed little damage but none of them was found immune to shoot fly attack (Singh *et al.*, 1968) Young (1972) listed a number of cultivars as promising resistance sources Later several lines were identified in the All India Coordinated Sorghum Improvement Project (AICSP) Identified resistance sources mostly come from *maldandi* (semi-compact head type) or *dagadi* (compact head type) races grown in *rabi* (postrainy) season (Rana *et al.*, 1985)

Several workers have screened sorghum germplasm for resistance to shoot fly (Singh et al., 1968; Dogget et al., 1970; Jotwani 1978, Nimbalkar et al., 1983, Dalvi et al., 1984, Taneja and Leuschner, 1985; Singh and Rana, 1986; Singh et al., 1989, Kishore and Solomon, 1990, Patel and Sukhani, 1990a; Kishore, 1992; Sharma et al., 1992) Wild species of sorghum (Sorghum purpureosericeum and S. versicolor) possess very high levels of resistance to shoot fly (Mote, 1984). Of nearly 14,000 sorghum germplasm sources screened in the field, 42 were found to be less susceptible than others over five seasons, resistance of IS 1054, IS 1071, IS 2394, IS 5484 and IS 18368 was found to be quite stable across locations (Taneja and Leuschner, 1985) Of the 523 lines, 38 were free from Atherigona soccata and the best five entries were IS 5109, IS 15781, IS 15787, IS 15864 and IS 16010 (Rao et al., 1980). In field trials in Haryana, India, in 1977, 288 lines of forage and dual-purpose sorghum were screened for resistance to Atherigona soccata. Of the lines, 120 (41.7%) showed up to 20% deadheart formation, and 30 had less than 10% deadhearts (Khurana and Verma, 1988). Some 205 sorghum genotypes were

evaluated for resistance to shoot fly at Bijapur in *rabi* 1993-94. Only six genotypes were classified as resistant (Balikai *et al.*, 1998). Of the 33 local sorghum varieties evaluated, the most promising ones were PJ-3k, PJ-20k, PJ-4k, PJ-6k, PJ-34k, PJ-19k and PJ-21k (Mote *et al.*, 1981).

Most of the sources resistant to shoot fly originate from postrainy season sorghums grown in India under residual soil moisture conditions (Sharma, 1997b). Cultivars M 35-1 (IS 1054), IS 1057, IS 2123, IS 2146, IS 4664, IS 2205, IS 5604 and IS 18551 have been widely tested and possess moderate levels of resistance to shoot fly. M 35-1, grown widely during the postrainy season in India, is a selection from landraces grown in the postrainy season (Sharma 1997b). Improved varieties CSV 5, CSV 6, CSV 7R, Swati (SPV 504) and CSV 8R have been developed using landraces, and possess moderate levels of resistance to shoot fly control (Singh and Rana, 1986). Some of the improved lines for shoot fly resistance such as ICSV 700, ICSV 705 and ICSV 717 developed at ICRISAT, Patancheru, have better yield potential than the landraces (Agrawal and Abraham, 1985).

Of the 60 accessions screened for resistance to *Atherigona soccata*, IS 2312, IS 2265, IS 3459 and IS 6394 were highly resistant (Deshamane *et al.*, 1980). Of the 74 entries, SPV115 and E333 were most resistant to *Atherigona soccata* (Taley *et al.*, 1980). Among 106 forage and dual-purpose sorghums tested in conditions of heavy infestation, four (IS 2123, IS 5469, IS 5470 and IS 8315) had less than 5% deadheart formation (Singh and Lodhi, 1983). Among the 24 lines tested by Naik and Bhuti (1985), M 35-1 suffered slightly less damage than IS 2312. Out of 67 sorghum lines tested by Jadhav *et al* (1988), the entries IS 688, IS 3952, POD-35 and PVR-10-2 were resistant under moderate incidence of *A. soccata* but susceptible with high incidence. However, IS 1456, IS 7094 and IS 12611 were resistant with both the moderate and high pest incidence. Kushwaha and Kapoor (1995) reported that among twenty sorghum genotypes evaluated, genotype SSV-1333 was the most resistant, followed by SSV-4755, GSSV-153, CSV-15, SSV-1456 and RSSV-3.

It was found that maximum eggs laid per plant and deadhearts occurred in the first week after germination for CSH 1 and SPV 504, while IS 5490 and IS 18551 were undamaged. The cumulative percentages of deadhearts in relation to total plants during the fourth week following germination were: 33.3% in IS 5490, 37.5% in IS 18551, 42.1% in SPV 504 and 91.9% in CSH 1 (Jadhav and Mote, 1986). The average egg population was lowest (0.07-0.13 eggs/plant) in lines IS 5642, IS 5490, IS 5469 and IS 4664. Significantly fewer deadhearts (14.9-18.5 %) were

oserved in the lines IS 5490, IS 4664, IS 5359, IS 5469, IS 5642 and IS 2146 than in the other nes tested (Sharma and Rana, 1983).

Out of 31 high-yielding varieties and hybrids of sorghum, the genotypes CSV 6, SPV 8, SPV 13, SPV 29, SPV 70, CSH 7R, E 303 and IS 5490 showed significantly low susceptibility in both normal and late sowings. In 5 varieties, CSV 3, SPV 10, SPV 14, SPV 16 and SPV 61, the percentage deadhearts in the late sowings decreased significantly over the 3-year period, indicating that selection for resistance is effective (Kishore *et al.*, 1985). Of the forty-one hybrids and 3 controls (CSH 1, CSH 5, CSH 9) assessed, resistance to shoot fly was observed in 2219A x R6830, 2077A x CS3541 x 3691-1-1-2 and 2077A x 285 x PD-2-5 x 285-5 (Lad *et al.*, 1986). The sorghum varieties GM-1, M 35-1 and SPV 86 were crossed with other varieties to increase their resistance to *Atherigona soccata* and the derivatives, RSV-8R and RSV-9R had consistently greater resistance than their parents (Mote and Bapat, 1988).

Among nine selections and their parents, PJ4R x Shenoli 5, ND15 x Improved Saoner 5, M 35-1 x Improved Saoner 5, M 35-1 x PJ4R3 and M 35-1 x PJ4R2 were most resistant to *Atherigona soccata* (Bapat and Mote, 1982a). Advanced generation material (F_3 , F_4 and BCF₃) from crosses involving *Atherigona soccata*-resistant and agronomically superior cultivars was evaluated. F_3 material was the most variable and F_4 the least so for egg count/plant and deadheart percentage. BCF₃ material was relatively more resistant. Five BCF₃ progenies, one F_3 progeny and three F_4 progenies were more resistant than the highly resistant parent IS 5604 while, 11 BCF₃s, 2 F_3 s and 17 F_4 s were more resistant than the commercial variety M 35-1 (Halalli *et al.*, 1983). Of 14 F_2 populations of sorghum tested for resistance to *Atherigona soccata* the crosses involving M 35-1 as one of the parent, viz., M 35-1 x SPV 488, M 35-1 x 19B, M 35-1 x Afzalpur local, M 35-1 x Selection 3 and M 35-1 x IS 2315 were promising on the basis of percentage deadhearts (Balikai and Kullaiswamy, 1999).

Fourteen advanced breeding lines of *rabi* (postrainy) material and 20 of *kharif* material, and 40 lines from various sources, were tested for resistance to *Atherigona varia soccata*. The first group included lines with less than 40% deadhearts. In the last group, PS18527, PS14533, SPV 491 and RHR5 were highly resistant, with less than 20% deadhearts (Mote *et al.*, 1983). When 45 genotypes of advanced breeding material were examined, SPV 489, SPV 504, SPV 570 and SPV 713 were highly resistant to *Atherigona soccata*, having less than 20% deadhearts, while 22 others were moderately resistant with less than 50% (Nimbalkar *et al.*, 1985). Twentysix advanced sorghum genotypes from ICRISAT were screened against shoot fly (*Atherigona soccata*) and stemborer (*Chilo partellus*) under natural infestation conditions at Hissar during *kharif* 1994 and 1996. Pooled data revealed that deadhearts formed by shoot fly varied from 8.5 to 76.5%. The genotypes IS 18551 and ICSV 93091 recorded less than 10.0% deadhearts due to shoot fly. Deadheart formation due to shoot fly was 15.0-20.0 % in ICSV 700, ICSV 93093, PB15438 and IS 2312. More than 70% deadhearts due to both the pests were recorded in susceptible controls CSH 1, CSH 9 and ICSV 1 (Singh and Grewal, 1997).

IS 18551 and IS 2195 were the best entries with resistance to both shoot fly and stemborer (Nwanze et al., 1991). Six dual purpose sorghums, DS-1, DS-2, DS-3, DS-4, DS-5 and DS-6 were developed from the crosses involving parents with moderate levels of resistance to Atherigona soccata and Chilo partellus, showed resistance to both A. soccata and C. partellus (Kishore, 1994). SPV 1015 (PGS-1), a variety of sorghum with resistance to Atherigona soccata and Chilo partellus, was developed in 1987 by pedigree selection from the cross P601 x P201 (Kishore, 1992). Selection-3 TL, a rainfed rabi sorghum variety for shallow soils, was selected from the local variety Bedar was found to be more resistant to shoot fly [Atherigona soccata] (Gujar et al., 1995). A variety GRS-1 was developed at Gulbarga as an alternative to M 35-1, tolerant of shoot fly (Atherigona soccata) (Patil et al., 1998). Of the improved varieties, PBM2-1, SPV 517 and SPV 297 had the lowest infestation (Shinde et al., 1983a).

Among 29 new male-sterile sorghum lines, together with 8 parental lines and some released evaluated hybrids under heavy attack by *Atherigona varia soccata* the lowest incidence of deadhearts was 40%. The most resistant entries were 365A₃, 367A₁ and 366B₄ (Mote *et al.*, 1983).

2.1.2 Mechanisms of resistance

The major mechanisms of resistance so far known are ovipositional non-preference (oviposition antixenosis) (Soto, 1974), antibiosis (Raina *et al.*, 1981) and tolerance (Dogget *et al.*, 1970). All these three mechanisms suggested by Painter (1951) are known to exist in sorghum for shoot fly resistance. The primary mechanism of resistance to sorghum shoot fly (*Atherigona soccata* Rond.) which has been observed to be non-preference for oviposition and perhaps a low level of antibiosis to the larvae (Young, 1972). Rana *et al* (1981) attributed resistance to a cumulative effect of non-preference, due to some morphological factors and antibiosis.

2.1.2.1 Non-preference for oviposition (Oviposition antixenosis)

The term antixenosis was proposed by Kogan and Ortman (1978) to replace the term nonpreference proposed by Painter (1951). It describes the inability of plant to serve as a host to an insect herbivore. It may be due to morphological or chemical plant characters that affect the insect behaviour adversely resulting in selection of alternative host plant Ovipositional nonpreference by shoot fly in resistant cultivars was first detected by Jain and Bhatnagar (1962) Later several workers consider it as a primary mechanism for shoot fly resistance in sorghum (Blum, 1967; Krishnananda et al., 1970; Jotwani et al., 1971, Pradhan, 1971, Young, 1972; Soto 1974; Narayana, 1975; Sharma et al., 1977; Singh and Narayana, 1978, Singh and Jotwani, 1980a; Singh et al., 1981; Sharma and Rana, 1983, Raina et al., 1984, and Unnithan and Reddy, 1985). The ovipositional non-preference is mainly observed under multi-choice conditions but under no-choice conditions in the field, it has a tendency to be less effective (Soto, 1972) so that the resistant and susceptible varieties are equally damaged (Soto, 1974, Taneja and Leuschner, 1985). Even under greenhouse conditions, in the absence of a preferred host, none of the varieties were highly resistant (Jotwani and Srivastava, 1970) The efficiency of this mechanism is not stable and breaks down under no-choice conditions or under heavy shoot fly pressure in the field (Singh and Jotwani, 1980a; Borikar et al., 1982, Sharma et al., 1997)

In a single-choice test, to study the behavioural resistance it was observed that females exhibited a highly significant non-preference for oviposition on IS 2146, IS 3962 and IS 5613 The first landing by a female was always random, but time spent on these cultivars was very brief and did not result in oviposition. Female flies laid eggs on the non-preferred cultivars only after laying several eggs on alternative CSH 1 plants None of the test cultivars expressed immunity to shoot fly infestation in both choice and no-choice tests (Raina *et al.*, 1984). Thus, non-preference appears to be a relative term since none of the known resistant cultivars were completely non-preferred for egg laying (Sharma and Rana, 1983).

Based on oviposition behaviour, it was reported by Raina (1982) that colour, texture and width of the sorghum leaf were important factors in selection of the oviposition substrate by female flies. Narrowness and erectness of leaves reduced the deadhearts and egg laying as shoot fly had less area for egg laying compared to broad-leaved plants (Mote *et al.*, 1986). Bapat and Mote (1982b) reported leaf colour and hairiness (with trichomes) as non-preference mechanism. The presence of trichomes on the leaf surface was related to a lesser frequency both of oviposition by the shoot fly and subsequent larval damage (Mait *et al.*, 1980). However, trichome numbers appeared to play some role in imparting resistance to oviposition (Raina, 1985).

2.1.2.2 Antibiosis

Antibiosis to shoot fly has been reported by Jotwani and Srivastava (1970), Blum (1972), Soto (1974) and Sharma *et al* (1977). Survival and development were adversely affected when shoot flies were reared on resistant varieties (Jotwani and Srivastav, 1970; Narayana, 1975; Raina *et al.*, 1981; Unnithan and Reddy, 1985) compared with susceptible genotypes (Singh and Narayan, 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). Survival and fecundity were also better on highly susceptible varieties (Singh and Narayana, 1978) but adversely affected on resistant varieties (Taneja and Leuschner, 1985). Survival and longevity of females and fecundity were adversely affected when the larvae were reared on shoot fly resistant genotypes (Raina *et al.*, 1981). Larval and total growth indices were significantly lower in resistant compared with that on susceptible lines (Dhavan *et al.*, 1993).

Based on report that trichomeless cultivars of pearl millet accumulate more dew and stay wet longer (Burton *et al.*, 1977), Raina *et al* (1981) suggested that a similar situation in sorghum would facilitate the movement of freshly hatched larvae to the base of central shoot. On the other hand, trichomed cultivars would tend to dry faster, making the downward journey of the larvae more difficult. The earliest work that referred to antibiosis as a possible mechanism of resistance to shoot fly in sorghum was that of Ponnaiya (1951a, 1951b). He attributed to this an early deposition of irregular shaped silica crystals in the resistant cultivars M.47-3. Blum (1968) confirmed Ponnaiya's observation that plants of resistant cultivars possessed a high density of silica bodies in the abaxial epidermis of the leaf sheaths. He also reported a distinct lignification and thickening of walls of cells enclosing the vascular bundle sheaths within the central whorl of young leaves.

Raina (1985) proposed that three different factors individually or in combination, may contribute to the expression of antibiosis to shoot fly in sorghum: (i) trichomed cultivars hinder the movement of newly hatched larvae to the base of the shoot; (ii) resistant cultivars have greater silica deposits and lignification of cells, which may restrict larval penetration to the base

of the central shoot, and (111) biochemical deficiencies or the presence of chemical factors in resistant cultivars may adversely affect the development and survival of larvae and reduce the fecundity of the resulting adults

Stability parameters for IS 8315 and IS 2123 revealed that the magnitude of oviposition will differ on these two resistant lines under different infestation pressures but there will be relatively less mortality. This is probably an indirect evidence of antibiosis in these two genotypes (Borikar and Chopde, 1982). Some cultivars are preferred for oviposition, however, percent infestation as measured by deadheart production is low mainly due to antibiosis (Mate *et al.*, 1996).

2.1.2.3 Tolerance

Dogget (1972) pointed out that synchronized tillering after the main shoot is killed is potentially a form of recovery resistance, because the tillers in some genotypes express higher levels of resistance than the main shoots. This form of resistance has been referred-as to tiller survival (Blum, 1969a) or recovery resistance (Dogget *et al.*, 1970). Resistant cultivars of sorghum had a very high rate of tiller survival compared with susceptible cultivars and was also suggested that the frequency of tiller survival was related to the rate of tiller growth, so that faster a tiller grew, the greater were its chances of avoiding infestation (Blum, 1972). However, survival of tillers and their development depends upon primary resistance (Sharma *et al.*, 1977). In Africa, it was reported that farmers actually preferred an initial infestation of their sorghum by shoot fly that led to profuse tillering and subsequently a good harvest (Dogget, 1972). However, tolerance can be greatly influenced by the growth conditions of the plant and thus may not always be predictable at various locations, particularly those with irregular patterns of rainfall (Raina, 1985).

2.1.3 Components of resistance

It is established that some characters of sorghum seedlings are associated with shoot fly resistance (Blum, 1968; Maiti and Bidinger, 1979, Raina, 1981, Maiti et al, 1984) Resistant cultivars are usually tall with thin stems having long internodes and short peduncle Also they have narrow glossy and yellowish-green leaves. These leaves possess trichomes on the abaxial surface, which act as physical barriers to penetration of young maggots (Kishore et al., 1985, Mote et al., 1986). Colour of leaves, glossiness of leaves, and presence of trichomes are prominent attributes, which confer resistance to shoot fly in sorghum (Jadhav et al., 1986) In conclusion, entries having greater plant height, narrower leaves, greater trichome density and greater trichome length, yellowish green colour of leaves, glossiness of leaves, and faster initial plant growth rate were found highly resistant to shoot fly while, entries having less plant height, broad leaves, dark green leaf colour, non-glossy leaves, absence of trichomes and slow initial plant growth rate were found highly susceptible to shoot fly. The entries having some of these prominent characters were found moderately resistant to the pest.

2.1.3.1 Glossiness

Sorghum seedlings can be classified as glossy or non-glossy; seedlings with dark green leaves (normal) are non-glossy, and seedlings with light yellow green and shining leaf surfaces are glossy. The glossy trait, a characteristic of most of the winter (*rabi*) sorghum varieties of India (Blum, 1972; Rao *et al.*, 1978) is associated with shoot fly resistance (Blum, 1972; Maiti and Bidinger, 1979; Taneja and Leuschner, 1985; Omori *et al.*, 1988).

The intensity of glossiness of the leaves at seedling stage is positively associated with resistance to shoot fly (Sharma *et al.*, 1997). Glossiness of leaves may possibly affect the quality of light reflected from leaves and influence the orientation of shoot flies towards their host plants. Glossy leaves may also influence the host selection by means of chemicals present in the surface waxes and/or leaves (Sharma, 1993). Expression of glossiness in seedlings is an important trait for identifying shoot fly resistance in sorghum and it is easily identifiable (Agarwal and House, 1982). Agrawal and Abraham (1985) reported that glossiness is highly correlated with shoot fly resistance. Jadhav *et al* (1986) reported negative and highly significant correlation (r = -0.77) between deadhearts and glossiness. Vijaylakshmi (1993) also reported that glossiness was negatively correlated in general with percentage of plants with eggs, number of eggs/100 plants and deadheart percentage in tall as well as dwarf genotypes.

Shoot fly incidence was higher in non-glossy lines than glossy ones in the postrainy season. However, glossiness contributed less to shoot fly resistance during the rainy season. Thus, most of the less susceptible lines are glossy, but all the glossy lines are not necessarily less susceptible to shoot fly (Taneja and Leuschner, 1985). Most of the glossy lines also show the presence of trichomes (Maiti *et al.*, 1984). The association of both the glossy leaf type and trichomes with shoot fly resistance in sorghum has been reported by Maiti and Bidinger (1979). A study of four combinations-glossy leaf and trichomes, glossy leaf only, trichomes only and

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neither - revealed that the mean deadheart percentages were 60.7, 70.9, 83.5 and 91.3, respectively. These results suggested that each of these two traits contributed to the resistance. The glossy leaf characters contributed more than did trichomes, and that the combination of the two traits was more effective than either of the traits alone.

A systemic survey of world germplasm collection indicated a low frequency of accessions with the glossy trait (only 495 of 17536 germplasm accessions screened). A large proportion (84%) of the glossy lines were of Indian origin but some were from elsewhere (Nigeria, Cameroon, Ethiopia, Kenya, Uganda, South Africa and Mexico) (Maiti *et al.*, 1984).

Glossiness is clearly manifested in the seedling stage and gradually disappears as the seedling grows (Maiti *et al.*, 1984). Soil fertility does not affect the glossy expression (in Maiti *et al.*, 1984). The difference between glossiness and non-glossiness can be detected by whether or not spayed water adheres on leaf blades (Tarumoto, 1980).

Glossy lines show variability in seedling morphology, seedling vigour, leaf surface structure, physiological, biochemical and agronomic traits. Glossy sorghums show multiple resistance to shoot fly, stemborer and several other insects and tolerance to abiotic stresses like drought, salinity, high temperature and low nutrient availability. Glossy lines show higher wateruse efficiency and better growth under water stress situation compared to non-glossy ones. Therefore, glossy sorghums may serve as basic resistance sources and a diverse gene pool for improving biotic resistance and abiotic tolerance (Maiti, 1992). Because of the association of the glossy trait with shoot fly resistance and seedling drought resistance, glossiness can be used to identify shoot fly and seedling drought tolerance in preliminary screening of large germplasm and breeding populations (Maiti *et al.*, 1984).

Maiti (1994) reported that at early stage (7 days after emergence), the chlorophyll content was higher in less glossy and non-glossy genotypes compared to highly glossy; with the advancing age, differences in chlorophyll contents became negligible. The epicuticular wax (EW) was at trace levels for all genotypes but at later stages it was higher in the non-glossy line CSH 1 than in the highly glossy line IS 18551. Some of the lines contained low EW content and were tolerant of shoot fly [*Atherigona soccata*], while that contained high EW contents were moderately susceptible.

2.1.3.2. Trichomes

The association between trichomes and pest resistance has been reviewed for numerous plant species by Webster (1975) and Norris and Kogan (1980). Trichomes on sorghum (*Sorghum bicolor* (L.) Moench) leaves are non-glandular hairs that are microscopic in size (approximately 50 µm long) (Gibson and Maiti, 1983). Many workers have established the association of prickle hairs (short pointed trichomes) present on the leaves and leaf sheaths with shoot fly resistance (Blum, 1968; Langham, 1968; ICRISAT, 1978; Maiti *et al.*, 1980; Singh and Rana 1986).

The wild species of sorghum that are nearly immune to shoot fly have a high trichome density on the lower surface of the leaves that contribute to their resistance (Bapat and Mote, 1982b). The importance of trichomes on the under-surface of leaves has been reported by several workers (Blum, 1968; Maiti and Bidinger, 1979; Maiti *et al.*, 1980; Taneja and Leuschner, 1985). Trichomes were clearly a major factor, but not the only factor, involved in resistance (Maiti and Gibson, 1983). Lines possessing both trichomes and the glossy-leaf seedling character were more resistant than lines with only one of these traits (Maiti and Bidinger, 1979).

The role of trichomes in shoot fly resistance and its inheritance have been studied (Maiti and Gibson, 1983; Gibson and Maiti, 1983). Density of trichomes per unit area of leaf lamina surface is genetically controlled, but the presence of trichomes probably is more important for increasing resistance to shoot fly than is density (Maiti and Gibson, 1982). The intensity of trichomes on the adaxial leaf surface was two to six times more than the abaxial leaf surface. Presence of trichomes on the lower surface of leaf and unknown antibiotic factors are likely to create hindrance for egg laying by shoot flies (Biradar *et al.*, 1986).

Maiti and Bidinger (1979) identified 32 lines from 8000 germplasm lines with trichomes on abaxial surface of the leaf blade. These had fewer plants with eggs, fewer plants with deadhearts and lower ratios of plants with deadhearts to plants with eggs than 35 lines without trichomes. Maiti *et al* (1980) observed that the presence of trichomes on the leaf surface resulted in a lower frequency both of oviposition by shoot fly and subsequent larval damage. The resistant cultivars IS 2146, IS 3962 and IS 5613 had high densities of trichomes on the abaxial leaf surface while susceptible hybrid CSH 1 lacked these.

Trichomes have high correlation with oviposition non-preference (genotypic correlation coefficient r = -0.75) (Agrawal and Abraham, 1985). When these correlations were partitioned

into direct and indirect effects through path-coefficient analysis, direct effect of trichomes was low and thus contributed to shoot fly resistance mainly through other traits (Jadhav *et al.*, 1986). Omori *et al* (1983) and Patel and Sukhani (1990b) observed positive correlation between trichome density and resistance to shoot fly. Agarwal and House (1982) found that the level of resistance was greater when both the glossy and trichome traits occurred together. It was reported by Maiti and Gibson (1983) that the correlations between trichome density and percentage of main culms with deadhearts ranged from -0.29 to -0.24 (all nonsignificant at p = 0.10). Karanjkar *et al* (1992) opined that although there are highly significant and negative correlations between the trichome density and shoot fly infestation (deadheart formation) it seems that trichomes do not have any direct role in reducing the deadhearts but help indirectly in reducing oviposition.

Based on unpublished data, it is suggested that trichomes may be less effective during the *rainy* season than during the postrainy season, possibly because of physiological factors or a more severe shoot fly attack during late *rainy* season plantings (Maiti and Gibson, 1983).

2.1.3.3 Seedling vigour

Rapid growth of seedlings may retard the first instar larvae from reaching the growing tip. In contrast, slow growth due to poor seedling vigour, low fertility or environmental stress increases shoot fly damage (Taneja and Leuschner, 1985; Patel and Sukhani, 1990b). Shoot fly resistant lines have rapid initial plant growth (Mote, *et al.*, 1986), greater seedling height and hardness (Singh and Jotwani, 1980c) and have longer stems and internodes and short peduncles (Patel and Sukhani, 1990b). The relationship between vigour of the plant and its escape from shoot fly attack was also reported by Karanjkar *et al* (1992). Earlier studies by Khurana and Verma (1985) and Jadhav *et al* (1986) indicated positive correlation between plant height and shoot fly resistance. Faster growing plants remain in the favorable height (susceptible stage) for a relatively shorter period than the slower growing susceptible plants (Khurana and Verma, 1985). It was concluded that rapid seedling growth and long, thin seedling leaves make plants less susceptible to shoot fly (Singh, 1998). The trichome density and seedling vigour can be used as selection criteria for shoot fly resistance (Karanjkar *et al.*, 1992).

2.1.3.4 Leaf surface wetness

Cultivars with high transpiration rates are preferred for oviposition (Mate et al., 1988) and there are genotypic differences between resistant and susceptible genotypes in surface wetness of the central shoot leaf (Nwanze et al., 1990). Leaf moisture is important for larval movement and deadheart formation (Raina et al., 1981). Leaf blade cuticles of resistant and moderately resistant genotypes are characterized by a smooth amorphous wax layer, and sparse wax crystals. Susceptible genotypes possess a dense meshwork of crystalline epicutucular wax (Nwanze et al., 1992). Leaf surface wetness (LSW) of the central whorl leaf of sorghum seedlings has been associated with susceptibility to *Atherigona soccata* Studies indicated that the presence of (small amounts of) solutes in the surface water may affect larval movement and survival (Sivaramakrishnan et al., 1994).

2.1.4 Correlations

Resistance to sorghum shoot fly is a complex character and depends upon the interplay of a number of component characters, which finally sum up in the expression of shoot fly resistance. It is necessary to study their correlation and causation. It provides the basis for deciding the suitable selection criteria to be considered for the genetic improvement of the crop with respect to the target trait

2.1.4.1 Association of shoot fly resistance with physical factors

2.1.4.1.1 Seedling traits

Two of the componental characters, viz., trichome intensity (abaxial surface), and glossiness intensity, showed negative significant associations with the shoot fly resistance $\{(-0.730 < r < -0.817) \text{ and } (-0.811 < r < -0.935), respectively\}$ (Omori *et al.*, 1983). Similarly, there were negative and highly significant correlations between percentage deadhearts (%), and both trichome density (r = -0.58) and trichome length (r = -0.66) (Jadhav *et al.*, 1986) Although correlation coefficients for the two components with shoot fly resistance were high but they do not play any direct role in building up the total variability in the shoot fly resistance (Omori *et al.*, 1983). In a study by Maiti and Gibson (1983) correlations between trichome density and percentage of main culms with deadhearts ranged from -0.29 to -0.24 (all nonsignificant at p=0.10). It was opined by Omori *et al* (1983) that trichomes do not play any direct role in reducing the deadhearts but help indirectly in reducing oviposition.

According to Omori *et al* (1983) shoot fly egg laying is highly significantly and negatively associated with both trichomes (-0.697 < r < -0.752) and glossiness (-0.747 < r < -0.825) indicating that these traits are deterring ovipositional preference of the shoot fly in

sorghum varieties. The negative correlation between egg laying and glossiness intensity may be caused by the tight positive association of glossiness with trichomes (0.815 < r < 0.833)

A highly significant and positive correlation was observed between the percentage of deadhearts and leaf colour (r = 0.83) (Omori *et al.*, 1983), seedling mass with leaves/plant and leaf width (Singh, 1998). There were negative and highly significant correlations between the percentage of deadhearts and leaf length/breadth ratio (r = -0.58), plant height (r = -0.56) and initial growth rate (r = -0.42) (Jadhav *et al.*, 1986).

2.1.4.1.2 Adult plant characters

Correlation studies between adult plant characters (plant height, days to flower) and shoot fly resistance parameters were carried out with 520 sorghum lines varying in the intensity of leaf surface glossiness at the seedling stage. The results indicated that tall, late-maturing genotypes with high glossiness intensity were the most resistant to *A. soccata* (Maiti *et al.*, 1994). There was a significant negative correlation between the percentage of deadhearts and both grain and fodder yield in the hybrids CSH 5 and CSH 8R (Mote, 1986).

2.1.4.2 Association of shoot fly resistance with biochemical factors

Biochemical constituents of host plants have been reported to affect the growth, survival and reproduction of insects in various ways (Painter, 1951, 1958; Beck, 1965; Schoonhoven, 1968). Antibiosis has been suggested as an additional factor responsible for resistance of sorghum plants to *Atherigona soccata* (Blum, 1972). Antibiosis in this case might be due to some chemical substance present within the plant particularly in the critical penetration zone of the stem, viz., 1 cm above ground (Baghel *et al.*, 1975) Therefore, studies are recommended including chemical analysis and anatomical studies to understand the mechanism of resistance to shoot fly.

According to Painter (1958), low levels of plant resistance to insects such as those involving smaller size and lower fecundity, appear to result from nutritional disturbances. Kalode and Pant (1967) pointed out that an insect susceptible sorghum variety contained higher number of amino acids than an insect resistant variety. In their studies histidine, arginine and aspartic acid were found to be absent in the resistant variety. Pathak (1970) observed lesser aspargine content in rice variety (Mudgo) resistant to brown plant hopper. The female hoppers that fed on this variety had under-developed ovaries with fewer matured eggs. Susceptibility to shoot fly was found to be positively correlated with phosphorous content, and negatively correlated with total phenols content (Khurana and Verma, 1983) and silica content (Bothe and Pokharkar, 1985). In general, biochemical factors such as the presence of irregularly shaped silica bodies in plant tissue, lignification, silica deposition and concentrations of nitrogen, reducing sugars, total sugars, moisture, chlorophyll, lysine, amino acids, phenol and phosphorous have been found to be associated with resistance to shoot fly (Sharma and Nwanze, 1997).

2.1.4.3 Association of shoot fly resistance with physiological factors

The studies on association of shoot fly resistance with physiological factors are meager. Mate *et al* (1996) reported positive and significant correlation between infestation and chlorophyll (r = 0.78), HCN (r = 0.42), nitrogen (r = 0.86) and crude protein (r = 0.87) content of sorghum cultivars.

2.1.5 Inheritance of resistance

Resistance to *Atherigona soccata* is quantitatively inherited (Agrawal and Abraham, 1985) and polygenically controlled (Goud *et al.*, 1983; Halalli *et al.*, 1983). Both additive and non-additive gene actions were involved in the shoot fly resistance (Borikar and Chopde, 1981b; Nimbalkar and Bapat, 1992). However, predominance of additive gene action was reported by several workers (Rao *et al.*, 1974; Balakotaiah *et al.*, 1975; Rana *et al.*, 1975; Sharma *et al.*, 1977; Borikar and Chopde, 1980; Borikar and Chopde, 1981a and b; Rana *et al.*, 1981; Biradar and Borikar, 1985; Patil and Thombre, 1985; Nimbalkar and Bapat, 1987; Singh and Verma, 1988). In contrast, predominance of non-additive gene action was reported by Agrawal and Abraham (1985). Partial dominance for shoot fly resistance was reported by Rao *et al* (1974) and Borikar and Chopde (1980). Sharma *et al* (1977) and Borikar and Chopde (1980) observed continuous variation in different generations and indicated that shoot fly resistance is due to gradual accumulation of genes.

Broad-sense heritability for shoot fly resistance was reported to be around 30% indicating the greater influence of environment (Halalli *et al.*, 1983). In F_1 and F_2 generations the heritability has been estimated as ~50% and 80%, respectively (Sharma *et al.*, 1977), whereas Rana *et al* (1975) reported this to be 25%.

2.1.5.1 Glossiness

Glossiness is simply inherited (Agarwal and House, 1982), being controlled by a single recessive gene (Tarumoto, 1980) and highly heritable. Therefore, it could be used as simple and reliable selection criteria for resistance (Maiti and Gibson, 1983; Omori *et al.*, 1983). Glossiness is highly correlated with shoot fly resistance and path analysis suggests the linkage of glossiness with some unknown inherent antibiotic factors. Intensity of glossiness is quantitatively governed and is controlled by both additive and non-additive genes (Agrawal and Abraham, 1985).

Inheritance of glossiness was studied by Tarumoto (1983) in the F_2 populations of crosses among non-glossy (Gl), glossy (gl), and true glossy (tgl) genotypes. The segregation analysis revealed that the genes controlling the phenotypes of Gl, gl and tgl plants are multiple alleles on the same locus. Similarly, the glossy seedling is reported by Emerson *et al* (1935) in corn (Zea mays L.) in which a series of genes, gl_1 to gl_{10} , each of which causes younger leaves to have glossy surfaces was listed.

2.1.5.2 Trichome density

The inheritance of trichome density has been studied (Gibson and Maiti, 1983; Maiti and Gibson, 1983) and reported that presence of trichomes associated with reduced susceptibility is recessive and controlled by single locus (tr). Heritability for the character was observed to be 75% (Gibson and Maiti, 1983). Trichome density is controlled by both additive and non-additive gene effects (Halalli *et al.*, 1982). Maiti and Gibson (1983) opined that trichomes are clearly major factor, but not the only factor involved in resistance. Jayanthi *et al* (1996) observed season specificity for trichome density reflected in the hybrid groups depending upon the type of parents involved and reported that low density (associated with susceptibility) appeared to be additive.

The mean trichome density on adaxial surface was lower on F_1 s than the average of the parents, indicating the role of partial dominance in respect of trichome density (Biradar *et al.*, 1986). It was observed that R x S and S x R F_1 s exceeded the parental limits. Backcrosses involving 168 (susceptible) as the recurrent parent exhibited higher trichome density on adaxial leaf surface. Complementary type of epistasis coupled with significant heterosis was observed for trichome density on the abaxial leaf surface in crosses SF 863 x 168 and SF 863 x IS 923.

These results indicate the possibility of developing hybrids with higher density of trichomes on their lower leaf surface (Biradar et al., 1986).

2.1.5.3 Seedling height

Seedling height is associated with relative growth rate. The importance of the additive component in the inheritance of seedling height has been suggested (Sharma *et al.*, 1977; Borikar and Chopde, 1982; Halalli *et al.*, 1983) and heritability was moderate for this trait (Halalli *et al.*, 1983). However, some authors (Sharma *et al.*, 1977; Borikar and Chopde, 1981b) reported the nature of gene action for this trait as predominantly non-additive. Rao *et al* (1974) pointed out that the dwarf exotic sorghums are generally susceptible to shoot fly attack and the sources of resistance are generally furnished the tall and late Indian sorghums. Therefore, it was opined that the exotic x Indian crosses are useful in combining resistance with desirable reduced height and earlier maturity.

2.1.5.4 Deadhearts

Both additive and non-additive components of heritable variation were recorded for the trait deadhearts (%) (Borikar and Chopde, 1981b; Halalli *et al.*, 1982; Biradar and Borikar, 1985; Dabholkar *et al.*, 1989; Elbadavi *et al.*, 1997). However, deadhearts (%) is controlled predominantly by additive gene action (Balakotaiah *et al.*, 1975; Sharma *et al.*, 1977; Borikar and Chopde, 1981a; Nimbalkar and Bapat, 1992). In contrast, Kulkarni *et al* (1978) and Dabholkar *et al* (1989) reported non-additive gene action for the trait in their material. In a study by Biradar and Borikar (1985), the dominance (h) and epistatic (i) components were of higher magnitude than the additive component (d).

General combining ability (GCA) variances were higher than specific combining ability (SCA) variances at all three levels of shoot fly infestation (environments) for deadhearts (%) indicating additive gene action for shoot fly resistance (Nimbalkar and Bapat, 1992). Additive gene effects for deadhearts (%) were significant at all three stages (viz., 15, 21 and 28 DAE) and in all three crosses. However, deadhearts occurring between 16 to 21 DAE were governed both by additive and non-additive genetic components. Higher magnitude of non-additive genetic components at a later stage (21 DAE) resulted in significant heterosis for deadhearts (%). Significant negative heterosis for deadhearts (%) was observed in SF 863 x 168 (Biradar *et al.*, 1986).

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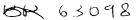
The heritability estimate (%) and genetic advance (% over mean) observed in BCF₃s on the 18 DAE were 36.1% and 15.4%, respectively (Halalli *et al.*, 1983) Borikar and Chopde (1981a) observed heritability estimates for the trait to the extent of 75-77 %, whereas Halalli *et al* (1982) reported low to medium heritability for deadhearts (%) character. However, estimates of narrow sense heritability for deadhearts (%) was high to medium (Nimbalkar and Chopde, 1992).

Two duplicate recessive genes $dh_1dh_1dh_2dh_2$ govern the resistance to deadheart formation Susceptibility is conferred when the plant carries one dominant allele (Sharma and Rana, 1985) However, Borikar and Chopde (1980) reported that at least 3 to 11 genes or gene groups governed for deadhearts (%). The characteristic way in which the seedling mortalities gradually decreased from 65 to 23% in the order of exotics, exotic x exotic, exotic x derivative, derivative x derivative, derivative x Indian, Indian x Indian crosses further confirms that the resistance is due to gradual accumulation of desirable alleles rather than due to one or two major genes (Balakotiah *et al.*, 1975).

2.1.5.5 Oviposition non-preference

The inheritance of ovipositional non-preference has been recorded as additive (Rao *et al.*, 1974; Balakotiah *et al.*, 1975; Biradar *et al.*, 1986) However, both additive and non-additive components of heritable variation are important for the trait egg/plant (Borikar and Chopde 1981b; Halalli *et al.*, 1982; Dabholkar *et al.*, 1989). Under high shoot fly pressure, both additive and non-additive were equally important (Agrawal and Abraham, 1985). The GCA variances for the character eggs/plant in F_1 and F_2 had higher magnitude than the SCA variances indicating the importance of additive gene effects and additive x additive gene interaction (Nimbalkar and Bapat, 1987). Borikar and Chopde (1981b) and Halalli *et al* (1982) also recorded similar results

Although some amount of non-additive gene action is involved, the trait is predominantly under the control of additive gene action for shoot fly resistance (Rao *et al.*, 1974; Balakoaiah *et al.*, 1975; Sharma *et al.*, 1977; Borikar and Chopde, 1980; Borikar and Chopde, 1981a) However, Kulkarni *et al* (1978) reported non-additive gene action for oviposition in their material. The nature of gene action for eggs/plant in two environments was predominantly non-additive, while it was additive in a third environment (Nimbalkar and Bapat, 1992). In the cross SF 863 x 168, significantly non-additive genetic components were associated with negative



heterosis indicating the possibility of exploiting non-preference for oviposition in hybrid development programs (Biradar et al., 1986).

Genetic analysis revealed that one recessive gene (*nponpo*) governs the non-preference to oviposition while, *NpoNpo* or *Nponpo* governs to preference to oviposition (Sharma and Rana, 1985). Borikar and Chopde (1980) reported that one group of dominant genes controls egg/plant 7Narayana (1976) reported high rate of oviposition to be completely dominant over low rate of oviposition.

The heritability estimates for total egg count per plant in F₃ was 50.16% (Halalli *et al.*, 1983), and 80 to 93% (Borikar and Chopde, 1981a). Halalli *et al* (1982) reported low to medium heritability for eggs/plant. However, estimates of narrow-sense heritability for eggs/plant were medium to low (Nimbalkar and Chopde, 1992) or medium (Nimbalkar and Bapat, 1987) The estimates of genetic coefficients of variability (GCV), heritability and genetic advance were high when material was tested under optimum shoot fly pressure (Borikar and Chopde, 1982)

2.1.5.6 Recovery resistance

Both additive and non-additive genetic components were involved in the inheritance of plant recovery (Biradar *et al.*, 1986). However, additive gene action for recovery resistance was reported (Sharma *et al.*, 1977) with fairly high heritability and a positive association of plant recovery with grain yield (Dogget *et al.*, 1970). The predominance of additive gene action for the trait was also reported by several workers (Starks *et al.*, 1970; Borikar and Chopde, 1981a, 1982). According to Borikar and Chopde (1982), tillering was predominantly under non-additive genetic control. The nature of gene action for tillers/100 plants, effective tillers and yield/productive plant was predominantly non-additive (Borikar and Chopde, 1981b). Sharma *et al* (1977) also reported mostly similar results for these traits.

The heritability for plant recovery was observed to be 40 to 70% (Borikar and Chopde 1981a) and significant epistatic effects were reported for this trait (Starks *et al.*, 1970) Heritability estimates were low, probably because of the very high magnitude of the dominance component of genetic variance (Borikar and Chopde, 1982). The heterosis for plant recovery was significant and positive in the cross SF 863 x 168 (Biradar *et al.*, 1986). Blum (1969a) also observed better tiller growth and survival in resistant varieties. However, It was observed that

was observed that there was no correlation between shoot fly resistance and recovery, indicating independent genetic control (Kadam and Mote, 1983).

2.1.6 Breeding for resistance

Predominance of additive genetic variance for deadhearts (%), eggs/plant and plant recovery (%) (Nimbalkar and Bapat, 1992) indicated the exploitation of shoot fly resistance by *inter se* crossing of individuals possessing high values for these traits or by following a biparental mating approach (Borikar and Chopde, 1981b). In backcross breeding the susceptibility decreased with extra dose of the resistant parent in the backcross (168 x IS 923) x IS 923, indicating a dose effect of resistant genes, but increased in the backcross to 168 (Biradar and Borikar, 1985). In a study by Halalli *et al* (1983) three BC₁F₃ progenies, one F₃ progeny and three F₄ progenies were more resistant [in terms of egg count/plant and deadhearts (%)] than the highly resistant parent, suggesting transgressive inheritance of these characters.

Sharma et al (1977) reported that susceptible parents were generally poor combiners, while resistant parents were better combiners for eggs/plant and deadhearts (%) All resistant parents recorded negative GCA effects and all susceptible parents recorded positive GCA effects for deadhearts (%) and eggs per plant (Borikar and Chopde, 1981b), and these results were confirmed by Nimbalkar and Bapat (1987). When eight male sorghum parents resistant to *Atherigona varia soccata* were crossed with three male sterile (MS) parents, the male parent Sel 28 was a good general combiner for yield. The cross 2077A x Sel. 28 showed the highest specific combining ability for resistance and the highest grain yield (Shinde et al., 1983b) Therefore, parental performance is good indication of hybrid behaviour (Rao et al., 1974).

In general, resistant (R) x susceptible (S) crosses exhibited promise for important characters, viz., deadhearts (%), eggs/plant and plant recovery (%) (Borikar and Chopde, 1981b) However, the hybrids involving susceptible parents exhibited much higher damage than that in the crosses between tolerant and resistant parents (Singh and Verma, 1988) Sharma *et al* (1977) reported that the F₁ hybrids from R x R crosses were superior to S x S crosses while, R x S crosses were of the intermediate nature with respect to shoot fly damage

When exotic sorghums and Indian sorghums were planted side by side, the former were highly preferred for oviposition averaging 8 eggs/plant with about 90% of the plant population infested with eggs when compared to 2 eggs per plant with 10% of the plant population infested

with eggs for Indian sorghums (Soto, 1972). Rao et al (1974) also indicated the usefulness of exotic x Indian crosses in combining resistance with agronomic characters. Resistant lines with agronomic worth have been isolated through selection in exotic x Indian crosses (Rana et al., 1975).

In F_1 hybrids shoot fly resistance increased over midparental value under low infestation, but the reverse was true under high shoot fly infestation (Sharma and Rana, 1983). Estimates of genetic coefficients of variability, neritability and genetic advance were better when shoot fly infestation was optimized (Borikar *et al.*, 1982). The study indicated that parameters of inheritance of deadheart (%) and eggs per plant are most influenced by level of shoot fly population. These results indicate that it is essential to consider shoot fly population in resistance breeding studies and selection for resistance may preferably made under conditions of high infestation (Nimbalkar and Bapat, 1992).

2.1.7 Stability of resistance

Studies on G x E interactions are of major importance in developing pest resistant crop varieties. Since the shoot fly population varies with the season and location (Rana *et al.*, 1984), varieties also show variable degrees of damage over different environments. Stability of shoot fly resistance was therefore studied by number of workers to explain the varietal reaction to shoot fly in different environments and to identify the most stable sources of resistance.

Singh *et al* (1978) and Borikar and Chopde (1982) observed unidirectional varietal reaction to changing environments due to non-significance of variety x season interaction. Genotypes, IS 1082, IS 2146 and IS 4664 showed better stability of resistance (Singh *et al.*, 1978). High degree of phenotypic stability and greater resistance to changing levels of shoot fly population were observed in IS 5490 and IS 5604. Genotypes, IS 1071, IS 2394, IS 5484 and IS 18368 were quite stable for shoot fly resistance across the locations (Borikar and Chopde, 1982). The genotypes like IS 2146 and IS 5566 exhibited a high degree of stability for shoot fly resistance (Chundurwar *et al.*, 1992). Rao *et al* (1977) also reported relatively higher stability for shoot fly resistance in IS 5469, IS 5490 and IS 1054. The hybrid IS 5490 x IS 5604 recorded least response to changing levels of shoot fly population (Borikar and Chopde, 1982).

2.2 Molecular marker analysis

2.2.1 Molecular markers

Molecular markers can be used as tools for rapid, detailed and directed genetic manipulation of crop plants. The ability to score genotypes at the molecular level provides a huge increase in the number of available markers for any analysis. The first molecular markers used were isozymes, which are protein variants detected by differences in migration on starch gels in an electric field (Stuber and Goodman, 1983). Indeed these biochemical markers have been particularly useful both in breeding practice (Ainsworth and Gale, 1987) and the further development of marker-aided selection technology (Stuber et al., 1987). Since the late 1960s this class of markers has been extensively applied to a variety of population genetic problems. The limitation with protein markers lies with insufficient protein variation for high-resolution mapping. However, as methods for evaluating variation directly at the DNA level became widely available during the mid 1980s, DNA-based markers replaced isozymes in mapping studies. A significant breakthrough in genetic analysis came when the first genetic map using restriction fragment length polymorphisms (RFLPs) (Botstein et al., 1980) was constructed Since then molecular biology has ushered in a new era with techniques that directly assayed DNA and overcame many of the problems that have previously limited the applied use of biochemical markers

2.2.1.1 DNA Markers

DNA markers are the simply detected differences in genetic information carried by two or more individuals (Paterson *et al.*, 1991). These reveal sites of variation often neutral at the DNA sequence level (Jones *et al.*, 1997). They are phenotypically neutral, polymorphic, abundant and co-dominant in nature (Tanksley, 1993), and therefore extensively used in construction of genetic linkage maps. Several types of DNA markers have been used widely (reviewed by Mohan *et al.*, 1997; Gupta and Varshney, 2000) for linkage mapping, including restriction fragment length polymorphism (RFLPs) (Botstein *et al.*, 1980), random amplified polymorphic DNA (RAPDs) (Williams *et al.*, 1990), simple sequence repeats (SSRs or microsatellites) (Litt and Luty, 1989), amplified fragment length polymorphisms (AFLPs) (Vos *et al.*, 1995) and single nucleotide polymorphisms (SNPs) in combination with DNA chip technology are likely (Wang *et al.*, 1998; Kanazin *et al.*, 2002). Recent reviews of molecular markers useful in mapping plant genomes include Karp *et al* (1997); Malyshev and Kartel (1997); and Mohan *et al* (1997). Each RFLP probe generally scores a single marker locus and marker alleles at these loci are co-dominant, as homozygotes and heterozygotes can be distinguished. The number of detectable RFLPs is impressive (Botstein *et al.*, 1980; Beckmann and Soller, 1983, 1986a, b; Doris-Keller *et al.*, 1987; Soller and Beckmann, 1988), yet RFLPs remain technically complex, laborious and difficult to automate (Reiter, 2001). Ragot and Hoisington (1993) concluded that RAPDs are generally more time/cost effective in small studies where modest number of individuals is to be genotyped, while RFLPs are better for larger studies. AFLP marker data generation involves several steps: restriction digestion, PCR and DNA ligation prior to electrophoretic separation of bands (Vos *et al.*, 1995). Problems with any of these steps can lead to poor gel resolution or unreliable bands that could represent artifacts. However, compared to conventional RFLP techniques, AFLP methods generate many more polymorphic bands per gel track.

AFLP and other highly polymorphic marker systems can be used to fill gaps in RFLPbased genetic linkage maps, following bulk segregant analysis (Michelmore *et al.*, 1991) approaches. The vast majority of polymorphisms that exist in DNA sequence are single base pair differences. Recently much effort has been focussed on the exploitation of SNPs (Nikiforov *et al.*, 1994; Marshall, 1997; Kanazin *et al.*, 2002) and sequence dependent methods for detecting them. A particularly interesting RFLP approach involves the use of tissue specific cDNA clones as the probes (cDNAs are generated from mRNAs of genes being expressed in that tissue). Other categories of markers can also be very useful (reviewed in Rafalski and Tingey, 1993). For example, several studies have used mobile genetic elements such as retroviruses as markers (Rise *et al.*, 1991; Nuzhdin *et al.*, 1993; Keightley and Bulfield, 1993; Ebert *et al.*, 1993; Long *et al.*, 1995).

The *in vitro* amplification of DNA by the polymerase chain reaction PCR (Saiki *et al.*, 1985) has proven to be a revolutionary technique in molecular biology. PCR is rapid, inexpensive and technically simple. More recently researchers have moved to PCR-based methods that all require smaller amounts of starting material and simpler extraction technologies (Young, 2001).

The use of these markers in a breeding programme has the potential to increase efficiency of selection for the traits difficult to manipulate by conventional methods. They offer great scope for improving the efficiency of conventional plant breeding by carrying out selection not directly on the trait of interest but on molecular markers linked to the genes that control the trait. These markers are not environmentally regulated and are therefore unaffected by the conditions in which the plants are grown and are at least theoretically detectable in all stages of plant growth. Therefore, they are used to identify and tag desirable genes even when conditions necessary for reliable phenotyping of the target trait are not present.

2.2.1.1.1 SSR markers

Although known by many names and acronyms, including simple tandem repeats (STRs), microsatellites and simple sequence repeats (SSRs), SSRs have received considerable attention and are probably the current marker system of choice for marker-based genetic analysis and marker-assisted plant breeding (Akkaya *et al.*, 1992; Chin *et al.*, 1996). SSRs are co-dominant, occur in high frequency and appear to be distributed throughout the genomes of most if not all higher plants and animals. They also display high levels of polymorphism even among closely related accessions and are amenable to simple and inexpensive PCR-based assays (see Brown *et al.*, 1996 for review).

The repeat regions are generally composed of di-, tri-, tetra- and sometimes greater length perfectly repeated, nucleotide sequences (Tautz and Ranz, 1984) that exhibit a high degree of polymorphism (Weber and May, 1989). The variability in the number of repeat units is typically the basis of observed polymorphism. The high degree of polymorphism is thought to be the result of increased rates of sequence mutation affecting the number of repeat motifs present at an SSR locus with the observed variation likely due to replication slippage or unequal crossing over (Edwards *et al.*, 1992).

In plant genomes, the overall frequency of microsatellite repeats appears to be generally lower than animal genomes (Morgante and Olivieri, 1993; Wu and Tanksley, 1993), although the incidence of closely spaced repeats has been borne out experimentally (Gupta *et al.*, 1994; Zietkiewicz *et al.*, 1994). In humans AC or TC is a very common repeat unit, but in plants AT is more common followed by AG or TC. In general, plants have about 10 times less SSRs than humans (Mohan *et al.*, 1997).

Unique sequences that flank the tandem repeats can be used as highly polymorphic probes or for making PCR primers. There are well-established methods of finding microsatellites by screening phage libraries with oligonucleotide probes. But a quicker, if limited, approach is to examine sequence data banks for their presence (Burr, 2001). SSR-based primers representing tri- tetra- and penta-nucleotide repeats have been used successfully to generate distinct banding patterns that are resolvable on low-resolution agarose gels using ethidium bromide staining (Gupta *et al.*, 1994; and Weising *et al.*, 1995), on high-resolution polyacrylamide gels by silver staining (Buscot *et al.*, 1996), through primer radiolabelling followed by autoradiography (Gupta *et al.*, 1994), or through primer labelling with fluorescent dyes and automated high-resolution visualization of PCR products separated by PAGE-a capillary electrophoresis. As would be predicted, the best product size descrimination is obtained with polyacrylamide-based gel analysis although agarose gel is sufficient for many applications (Vogel and Scolnik, 1997)

In any case SSRs are generally among the most reliable and highly reproducible of molecular markers. Indeed SSRs are now widely recognized as the foundation for many framework linkage maps. SSRs have played a critical role even in merging disparate linkage maps (Bell and Ecker, 1994; Akkaya *et al.*, 1995) since they define specific locations in the genome unambiguously (Young, 2001). These markers can require considerable investment to generate but are then inexpensive to use in mapping and MAS. The large start up costs for this technique should be justifiable for crops where large-scale mapping and MAS are a practical necessity (Hash and Bramel-Cox, 2000).

2.2.1.1.1.1 Sorghum SSR markers

SSR-containing clones isolated from both BAC (bacterial artificial chromosome) and enriched gDNA libraries and database sequences that contain SSRs were the sources for the sorghum SSRs mapped by Bhattramakki *et al* (2000). Targeted isolation of SSR loci using BAC clones as proposed by Cregan *et al* (1999) is likely to be the most efficient method for placing SSR loci in the segments. BTx623 is the reference genotype used for sorghum molecular marker genotyping it was the source of DNA used to construct the enriched libraries and the two sorghum BAC libraries that are currently available (Bhattramakki *et al.*, 2000). PCR primers for the amplification of DNA fragments containing SSRs from sorghum were successfully developed through three different approaches by Brown *et al* (1996) and it was reported that sorghum fragments can be amplified using at least some maize SSR primers (Brown *et al.*, 1996).

Map location of 46 SSR loci (Taramino et al., 1997; Tao et al., 1998; Kong et al., 2000) and 113 novel SSR loci (including four SSR containing gene loci) (Bhattramakki et al., 2000) have been reported to date. SSR markers have been incorporated into an existing RFLP based map of Xu et al (1994) (Kong et al., 1997) and on the map of Peng et al (1999) (Bhattramakki et al., 2000). The number of SSR loci available per sorghum linkage group ranges from 8 to 30. Eight SSR loci that, although monomorphic among the 18 survey accessions, have high degree of homology to known genes (Bhattramakki et al., 2000) have yet to be mapped. The average number of alleles detected per locus at the polymorphic loci was 3.88. (AG/TC)_n and (AC/TG)_n repeats comprise the majority of all SSRs (52%) and 91% of the dinucleotide SSRs at these loci (Bhattramakki et al., 2000). AG/TC repeats also predominated among the SSRs isolated by Brown et al (1996). It was found that as much as 57% of SSR containing triplets rich in G-C base pairs were located in gene coding regions of the total genomic DNA (Wang, Weber, Zhong and Tanksley, 1994).

The estimated average probability that two accessions in a working group will have different alleles at a locus ranges from 0.88 to 0.67 depending upon the working group to which the accessions belong (Kong *et al.*, 2000). In addition, the number of alleles per locus is positively correlated (r = 0.68, which is significant at 1% level) with the number of repeated units at the loci in BTx623, the strain from which the SSRs were originally isolated (Kong *et al.*, 2000). This confirms that most *Sorghum bicolor* SSR loci are sufficiently polymorphic to be useful in marker-assisted selection programs (Kong *et al.*, 2000).

2.2.2 Linkage mapping

Construction of a linkage map is the most fundamental step required for a detailed genetic study and application of the marker-assisted breeding approach in any crop (Tanksley *et al.*, 1989). Comprehensive mapping of quantitative trait loci (QTLs) requires informative markers for all regions of the genome (Paterson *et al.*, 1988; Lander and Botstein, 1989). Likewise, a high density map facilitates marker-assisted selection especially between closely related types (Chittenden *et al.*, 1994), as it provides information on many potentially polymorphic markers in all genomic regions.

Genetic maps show the order of loci along a chromosome and relative distance between them. Such maps are essential for localization of genes affecting both simple and complex traits. Construction of maps is based on the discovery that Mendellian factors or genes controlling inheritance are organized in a linear order on chromosomes. With this fact Sturtevert developed the first chromosome map using segregation data from studies on *Drosophila* (Crow and Dove, 1988). Later chromosome maps in several organisms were developed. The markers on these maps were either genes or morphological features of chromosome. Until recently, construction of chromosome maps proceeded slowly because of lack of polymorphism in genetic markers, time and labour consuming construction of marker stocks, and genetic mapping by indirect observation of recombinant chromosomal segments (Kochert, 1994). Molecular markers have several advantages over these traditional phenotypic markers that were previously available to plant breeders (see Tanksley, 1993). The principle of construction of molecular maps is same as in classical genetic mapping. However, the new consideration in molecular mapping is the fact that a potentially unlimited number of DNA markers can be analysed in a single mapping population (Young, 2001). As predicted by Botstein *et al* (1980) molecular markers have sparked an explosion of genetic maps in humans and wide range of plant and animal species.

Parents selected for mapping experiments should show sufficient polymorphism for both phenotypic characters and molecular markers. This cannot be overemphasized, for in the absence of DNA polymorphism, segregation analysis and linkage mapping are impossible (Young, 2001). Moreover, SSR markers tend to exhibit high levels of polymorphism, even in narrow crosses (Rongwen *et al.*, 1995), providing the possibility of constructing maps in crosses between closely related parents (Young, 2001). In general, any type of marker can be used for construction of linkage maps. However, co-dominant markers (e.g., RFLP and microsatellites associated with unique long-sequence flanking regions) will give more information from F_2 and backcross generations than will markers giving predominantly presence or absence or dominantly inherited polymorphism (Hash and Bramel-Cox, 2000).

Backcross and F_2 populations are suitable for DNA based mapping, but recombinant inbred populations (Burr and Burr, 1991) provide permanent mapping resources because of homozygosity of the lines and can be distributed, replicated and evaluated from experiments in different environments, which is essential for more accurate measurement of any quantitative trait (Burr et al., 1988, Zamir et al., 1993; Goldman et al., 1995; Paran et al., 1995). They are expected to have an increased power of QTL detection because of nearly complete homozygosity at QTL and marker loci (Moreno-Gonzalez, 1993). The process of sibling F_2 plants through at least 5 or more generations of selfing leads to lines that each contains different combinations of linkage blocks from their two original parents. The differing linkage blocks in each individual RIL provides the basis for linkage analysis. The presence of linkage disequilibrium is due to more opportunity for meiotic recombination and thus makes the possibility of distinguishing more closely linked QTLs (Tanksley, 1993). Although lines will become homozygous, some regions of their genomes tend to stay heterozygous longer than expected from theory (Burr and Burr, 1991). However, the rate of recombination is under the genetic control with some modifier genes having a general influence throughout genome and others having fine-scaled influences on specific chromosomal regions (Brooks, 1988). The only limitation in use of RILs is the time required to develop these mapping population. Doubled haploid lines (DHLs) can also be used for linkage mapping with many of the same advantages of RILs (Huen *et al.*, 1991).

Since the resolution of the map and the ability to correctly determine marker order 15 largely dependent on population size, the decision on population size to be used for mapping is critical. When ever possible the larger the population the better (Young, 2001). Based on Monte Carlo simulations, Beavis (1994) concluded that populations smaller than 200 individuals would rarely be successful to find most QTLs and in many cases populations larger than 500 are required. Moreover, if the goal is high resolution mapping in specific genomic regions or mapping QTLs with minor effect, a much larger population is required (Young, 2001).

DNA-based maps can be related to existing cytogenetic maps through the use of aneuploid lines (Helentjaris *et al.*, 1986; Young *et al.*, 1987; Rooney *et al.*, 1994) and substitution lines (Sharp *et al.*, 1989) or in-situ hybridization (ISH) (Zhang *et al.*, 2000) Recently focus has shifted to the relationship between genetic and physical maps (Zhang and Wang, 1997). Eventually the distances between DNA markers need to be described not only by recombination frequency, but by actual physical distance. This kind of information will be abundantly clear in *Arabidopsis* and rice through complete physical mapping and eventual genome sequencing (Yu *et al.*, 2002; Goff *et al.*, 2002; Bennetzen, 2002). Young (2001) opined that even in more complex plant genomes, positional cloning projects based on YAC and BAC libraries are beginning to shed light on relationships of genetic maps to physical maps. Fine structure mapping of the same genome region using both recombinational and physical techniques is the best method to compare different types of maps directly (Young, 2001).

2.2.2.1 Linkage maps in sorghum

Genetic studies of morphological traits in sorghum began early this century and Dogget (1988) summarized genetic linkage of morphological and physiological mutants involving 49 loci. To date over 200 morphological and agronomically important markers have been identified (Berhan *et al.*, 1993), but only nine linkage groups have been established and these consist of only 2-10 loci (Pereira *et al.*, 1994). The biggest linkage group contains ten linked morphological

34

marker loci (Doggett, 1988). Sorghum genome mapping based on DNA markers began in the early 1990s and since then several genetic maps of sorghum have been developed with large number of DNA-based markers including RFLPs, AFLPs and SSRs. These maps will be useful in advanced breeding and genetic studies.

The construction of first sorghum linkage map was developed using heterologous maize probes (Hulbert et al., 1990). Later several RFLP linkage maps of S. bicolor have been constructed (Binelli et al., 1992; Whitkus et al., 1992; Berhan et al., 1993; Chittenden et al.; 1994; Pereira et al., 1994; Ragab et al., 1994; Xu et al., 1994; Dufour et al., 1996, 1997; Peng et al., 1999). Combined, these maps include over 800 markers (Bennetzen et al., 2000). Five major RFLP maps independently developed in this species (Chittenden et al., 1994; Pereira et al., 1994; Ragab et al., 1994; Xu et al., 1994; Boivin et al., 1999) were successfully aligned and form the first report on the unambiguous alignment of all ten sorghum linkage groups (Subudhi and Nguyen, 1999). The integrated maps will accelerate genome mapping and comparative mapping activity in sorghum and other related grass species.

Most of the early sorghum molecular marker-based genetic maps used maize RFLP probes (Binneli et al., 1992; Whitkus et al., 1992; Melake-Berhan et al., 1993; Pereira et al., 1994) in the context of comparative mapping. In 1994, three different groups (Chittenden et al., 1994; Ragab et al., 1994; Xu et al., 1994) developed RFLP maps using mainly sorghum DNA probes. Dufour et al (1997) published a map based on maize, sugarcane and cereal anchor probes. This composite linkage map was further saturated with the addition of more heterologous probes and AFLP markers (Boivin et al., 1999). A few cDNA clones of sorghum and maize relate to photosynthesis and drought stress were mapped on the map of Subudhi and Nguyen (1999). Similarly, the RFLP maps of Xu et al (1994) and Peng et al (1999) have been improved with addition of over 100 SSR markers (Kong et al., 1997; Bhattramakki et al., 2000). Recently more than 500 AFLP markers have been added to the map of Xu et al (1994), which is currently being used to generate a high quality physical map of the sorghum genome (Subudhi and Nguyen, 1999).

The maps were developed from interspecific crosses (Chittenden *et al.*, 1994; Lin *et al.*, 1995), an inter-subspecific cross (Pereira *et al.*, 1994) and rest of the maps from intra-specific crosses. Both F_2 (Hulbert *et al.*, 1990; Binelli *et al.*, 1992; Melake-Berhan *et al.*, 1993; Chittenden *et al.*, 1994; Pereira *et al.*, 1994; Ragab *et al.*, 1994; Xu *et al.*, 1994) and RIL

populations (Dufour et al., 1997; Tao et al., 1998; Boivin et al., 1999; Crasta et al., 1999; Peng et al., 1999) were equally used in map development. Only the maps of Chittenden et al (1994) and Pereira et al (1994) are complete, containing ten linkage groups. The crosses, types of populations, number of linkage groups, number of markers and type of markers used so far in sorghum maps are summarized by Subudhi and Nguyen (1999).

2.2.3 Marker trait associations

Quantitative characters have been a major area of genetic study for over a century because they are a common feature of natural variation in populations of all eukaryotes (Kearsey and Farquhar, 1998). First attempts at studying them stemmed from the work of Galton (1889) on man before the rediscovery of Mendellian inheritance of qualitative characters through the pioneer work of Fisher (1918), Wright (1934), Mather (1949) and Falconer (1960) to the new era. Despite these studies the number of genes and their interactive effects controlling the expression of quantitative traits are poorly understood.

The basic concept of associating genetic markers with quantitative traits was first proposed by Sax (1923). Since then there has been great interest in genetic dissection of quantitative variation. Geneticists have recognized the potential use of linkages between qualitative genes and QTL for studying the nature of quantitative genetic variation (Sax, 1923; Lindstrom, 1926, 1931; Waxelson, 1933; Smith, 1937; Everson and Schaller, 1955; Thoday, 1961). Unfortunately the relatively small number and sometimes deleterious nature of qualitative marker genes was extremely limiting to linkage studies with quantitative genetic variation (Bubeck *et al.*, 1993).

Analysis of biochemical and DNA markers in crosses between parents that differ for a quantitative trait can be used to find RFLPs linked to genes controlling the quantitative traits or QTLs (Gale and Witcombe, 1992). In plants the first attempts of using at performing genome wide analysis of quantitative variation by allozymes (Tanksley *et al.*, 1982; Edwards *et al.*, 1987). Initially RFLPs were used as DNA markers (Beckmann and Soller, 1983; Lander and Botstein, 1989), but these were followed by PCR markers such as RAPDs, microsatellites and AFLPs that were cheaper, safer and provided more markers per unit of DNA (Westman and Kresovich, 1997). These polymorphic markers provided the framework maps around which the bolygenes/QTL could be located (Kearsey and Farquhar, 1998).

Several statistical approaches have been developed for detecting and quantifying the strength of these associations (Soller and Brody, 1976; Edwards *et al.*, 1987; Lander and Botstein, 1989; Knapp, 1989). The ability to detect a QTL with an RFLP marker is a function of the magnitude of QTL's effect on the character, the size of mapping population being studied and the recombination frequency between the marker and the QTL (Tanksley *et al.*, 1989). The realized QTL effect is a function as to how large an effect the QTL has and how tightly it is linked to the marker or flanking markers (Gale and Witcombe, 1992). There are however dangers associated with the establishment of breeding programs based on correlations of marker genotypes with quantitative traits before the identified factors (QTLs) have been tested in several genetic backgrounds and have been evaluated for associated effects on other characters of agronomic or economic importance (Tanksley and Hewitt, 1988).

It is well understood by plant breeders that G x E interactions exist for many quantitative traits, suggesting that general conclusions about QTLs, particularly those with small effects on the basis of single environments and single populations could lead to erroneous decisions. The use of QTL identification by breeders also will be influenced by the consistency of QTL regions across the germplasm (Bubeck *et al.*, 1993). One challenge of plant breeding is to take advantage of favorable direct effects of QTLs, while maximizing favorable environmental interactions and minimizing unfavorable ones (Bubeck *et al.*, 1993).

A greatly abbreviated list of agronomic traits subjected to marker mapping and QTL analysis includes drought tolerance (Martin *et al.*, 1989), seed hardness (Keim *et al.*, 1990), seed size (Fatokun *et al.*, 1992), maturity and plant height (Lin *et al.*, 1995), disease resistance (reviewed by Young, 1996), oil and protein content (Diers *et al.*, 1992), soluble solids (Tanksley and Hewitt, 1988) and yield (Stuber *et al.*, 1987).)

2.2.4 Marker assisted selection (MAS)

The ability to manipulate genes responsible for quantitative traits is a prerequisite for sustained improvement in crop plants. MAS in pedigree, backcross and population improvement are especially useful for the traits that are otherwise difficult or impossible to deal with by conventional means alone (Hash and Bramel-Cox, 2000). There has been an implicit expectation that marker-based QTL analysis will make it easier and faster for breeders to manipulate these traits (Soller and Beckmann 1983; Tanksley, 1983), but this expectation has often not been realized.

The development of linkage maps with abundant markers in a wide range of crop species, was accelerated by development of newer and simpler DNA marker systems like RAPDs (Williams *et al.*, 1990), AFLPs (Vos *et al.*, 1995), and SSRs, also known as microsatellites (Akkaya *et al.*, 1992). Scientists soon began to believe that the promise of MAS originally proposed by Sax (1923) and Thoday (1961) might soon become a reality (Young, 1999). Analysing plants at the seedling stage, screening multiple characters that would normally be epistatic with one another, deterministically minimizing linkage drag and rapidly recovering recurrent parent's genotype were some of the potential advantages of MAS (Tanksley *et al.*, 1989).

In order to tag any gene of interest with a selection fidelity of 99%, Tanksley (1983) showed that it would be necessary to have marker loci spaced at 20-cM intervals throughout the genome. Selection can be exerted for a number of markers simultaneously, which will have the effect of selecting for QTLs with positive effect on the quantitative trait (Paterson *et al.*, 1988). However, one of the major drawbacks is that when the linked marker used for selection is at a distance away from the gene of interest, this permits crossovers to occur between the marker and the gene. This produces a small percentage of false-positives/negatives in the screening process (Mohan *et al.*, 1997). Therefore, in the final analysis, the success of MAS will depend on identifying highly polymorphic marker(s) as close to the gene as possible to ensure its/their utility across many breeding populations (Mohan *et al.*, 1997).

For efficient MAS some additional steps have been suggested by Young (1999): 1) repetition over several years and locations, 2) repetition in larger sibling populations, 3) repetition in genetically unrelated populations, and 4) detailed analysis in marker-generated nearisogenic lines (NILs) that isolate the effects of individual QTLs.

2.2.5 Statistical techniques for QTL analysis

QTL analysis is predicted on looking for associations between the quantitative trait and the marker alleles segregating in the population. It has two essential stages; the mapping of the markers and association of the trait with the markers. Both of these require accurate data and statistical software (Kearsey and Farquhar, 1998). The basic theory underlying marker mapping has been available since the 1920s (see Mather, 1938), but has to be extended to handle hundreds of markers simultaneously. The availability of computer software packages has made this much easier (Young, 2001).

The traditional approach (Soller and Brody, 1976; Tanksley et al., 1982; Edwards et al., 1987) for detecting a QTL in the vicinity of a marker involves studying single genetic markers one at a time. However, if the QTL does not lie at the marker locus, its phenotypic effect diminishes relative to the true effect of the QTL as the distance (recombination frequency) increases between the marker locus and the QTL (Edwards et al., 1987; Lander and Botstein, 1989). To overcome this, Knapp (1989) developed an approach, which utilizes pairs of markers in a sequential manner and estimates the phenotypic effect of the QTL and its significance in the region bracketed by the two markers in each pair. Lander and Botstein (1989) reported development of method for mapping QTLs, interval mapping using LOD scores. Intervals between adjacent pairs of markers along a chromosome are scanned and the likelihood profile of a QTL being at any particular point in each interval is determined; or to be more precise, the log of the ratio of the likelihoods (LOD) of there being one QTL vs no QTL at a particular point (Lander and Botstein, 1989). An alternative approach using multiple regression was developed by Haley and Knott (1992). It often produces very similar results to LOD mapping both in terms of accuracy and precision, but has the advantages of speed and simplicity of programming. Tests of significance and confidence intervals can be obtained by boot strapping approaches (Visscher et al., 1996). However, it is reported by Tanksley and Nelson (1996) that the statistical detector of QTLs is likely to depend not only on the type of population utilized, but also be on the intralocus and interlocus interactions of the segregating QTLs.

For most mapping projects the most widely used genetic mapping software is MAPMAKER (Lander *et al.*, 1987). MAPMAKER is based on the concept of the LOD score, "the log of the odds ratio" (Morton, 1955). The popularity of MAPMAKER is based on the ease with which it performs multipoint analysis of many linked loci (Young, 2001). The computer program JOINMAP is especially suited to relate one's map to those derived from other mapping populations (Stam, 1993).

To apply a linkage map to QTL analysis, MAPMAKER has been modified to carry out QTL analysis using mathematical models and interfaces very much like the original program (Lander and Botstein, 1989). Other programs like QTL Cartographer (Basten *et al.*, 1998) provide very much the same type of analysis. QTL analysis can also performed by using composite interval mapping (CIM) with the PLABQTL software as described by Rami *et al* (1998) or with QTL cartographer. For intended use of linkage information in marker-assisted breeding, a program like Map Manager (Manley and Cudmore, 1998) helps to keep track of marker data in a population of interest While, Hypergene helps to display graphical genotypes (Young and Tanklsey, 1989) The program qGENE seeks to bring all of these important DNA marker tools together into a single package (Nelson, 1997)

2.3 QTL mapping for insect resistance in cereals

Like other quantitative traits, inheritance of resistance to a number of insects in cereals is polygenic (Khush and Brar, 1991) Phenotypic selection for such traits is difficult Selection based on markers could theoretically ease the manipulation of such traits without affecting other important agronomic traits Molecular mapping experiments for quantitative insect resistance in maize, rice and barley have been conducted and the details are presented in Table 1. The mapping population types generally used were $F_{2.3}$, RLs and doubled haploid lines (DHLs). The size of population varies between 71 (RILs) and 475 ($F_{2.3}$) Taking cognizance of the low power of QTL detection for small sample sizes (<300) found in simulation studies (Uz and Melchinger, 1994) several reasonably large sized RIL mapping populations have been developed in sorghum at ICRISAT, Patancheru. These are being screened for resistance to shoot fly and stemborer Significant Q x E interaction was observed for resistance to corn borers in terms of leaf feeding rates (Jampatong *et al.*, 2002, Bohn *et al.*, 1996, Bohn *et al.*, 1997 and Groh *et al.*, 1998). This indicates the influence of environment on the expression of resistance traits

Among the cereals, extensive QTL mapping experiments were done in maize for resistance to different species of corn borers A commonly held view is that maize is exceptionally polymorphic, due to its highly cross-pollinated nature A sufficiently large number of polymorphic RFLP loci can be found for maize in intraspecific crosses in contrast to many other crops where interspecific crosses are used to overcome lack of marker polymorphism within the cultigen In addition, large numbers of RFLPs that have already been mapped in the maize genome are publicly available (Bohn *et al*, 1996) In case of sorghum RFLP linkage maps (Subudhi and Nguyen, 1999) and an integrated SSR and RFLP linkage map (Bhattramakki *et al.*, 2000) are available (details already reviewed in previous section) These markers are of potential use in mapping sorghum genomic regions associated with resistance to shoot fly, stemborer and midge

Correlating genetic map to physical map would be highly valuable to plant geneticists for map based cloning Recently, an attempt has been made to locate molecular markers (umc105a)

Сгор	Pest	Cross	Mapping population	Size of mapping population	No. of QTLs identified	No. of environments evaluated	character (s)	Q x E interaction	Reference
Make	European corn borer (Ostrinia	B73 1 B52	F2.5	300	7	Two locations	Tunnel length	Non- significant	Schon <i>et al.</i> , 1993
	nubilalis Hubner)	B73Ht x Mo47	F23	244	9	Three	Leaf feeding rates	Significant	Jampatong et al., 200
					7	Three	Tunnel length	Significant	
	Sugarcane corn borer (<i>Diatrea</i> s <i>acchralis</i> Fabricius)	CML131 x CML67	F2.3	171	10	2-3	Leaf feeding rates	Significant	Bohn <i>et al.</i> , 1996
		CML131 x CML67	F _{2.3}	171	10	Same environment two years	Leaf feeding rates	Significant	Bohn <i>et al.</i> , 1997
	Southwestern corn borer (<i>Duatrea</i> gran <i>diosella</i> Dyar)	CML131 x CML67	F ₁₃	171	6	Same environment two years	Leaf feeding rates	Significant	Bohn <i>et al.</i> , 1997
	granuloseus Dyni)	CML131 x CML67	RILs	187	9	Three seasons	Leaf feeding rates	Significant	Groh <i>et al.</i> , 1998
					5	One season	Protein concentration	-	
				145	7	One season	Leaf toughness	-	
		KB 1 CML139	F23	475	7	ŀ	Leaf feeding rates	-	
		KB x CML139	RILs	158	8	Two seasons	Leaf feeding rates	Significant	
			RILs	145	2	One season	Leaf toughness	-	

Table 1. QTL mapping for insect resistance in cereals

(contd)

(contd)									
C See	Pat	Crees	Mapping Size of mappin	Size of mapping	Na. of QTLs	No. of Component environments character (s)		Q x E interaction	Reference
Sorghum	Sorghum Green bug (Schizghlidt	GBIK x Redian	RIL	population population RILs 95	identified evaluated 9 -	- raiwated	Resistance and tolerance to	•	Agrama <i>d al.</i> , 2002
	graminum Road.) Shoot fly	BTx623 x IS 18551	RIL	757		Three	biotypes I and K Different components	•	Unpublished, ICRISAT
	(Althergone soccele Rond.)	296B x IS 18551	RILA	•	•	in progress	* *	•	3
		CSV 745 r PB15881-3 RILs	RILS	•	•	in progress	*	•	Ŧ
	pertetius (Swimoe)	ICSV 745 x PB 15520 RILs	RIL		•	in progress	3	•	Ŧ
Rice	Brown Plant hopper Asominori x IR24 (Nilaparwata	Asominori x IR24	RILS	14	2	•	Ovicidal response (grade of water)	•	Yamasaki et al., 2000
•	(subjections)	IR64 I Azucena	DHLs	131	2	•	mortality) Mechanisms of resistance (antibiosis, antitenosis,		Alam and Cohen, 1998
		Lemont x Teqing	RILS	160	٢	•	tolerance) "	·	Xu <i>et al.</i> , 2002
Bartey	Barley Cereal aphids	Harrington 1 TR306	DHLs	150	2		Aphid density		Moharramipour et al., 1997

on the short arm of chromosome 9, csu145a on the long arm) that flank QTL for resistance to sugarcane com borer (SCB) and southwestern com borer (SWCB) (Sadder and Weber, 2002). It was suggested that further DNA sequences have to be identified before attempting to isolate the QTLs.

Material and methods

III. MATERIAL AND METHODS

3.1 Material

The experimental material consisted of a set of 252 recombinant inbred lines (RILs), the two parents and two susceptible checks. The two inbreds, viz., BTx623 and IS 18551 were the parents of RILs. BTx623 is highly susceptible and IS 18551 is resistant to shoot fly. 296 B and CSH-1 were used as susceptible checks. The salient features of parental lines and susceptible checks are presented in Table 2. The mapping population consisting of 252 RILs (F_{5.6}) was used for phenotypic screening along with parents and checks, and 93 RILs (F_{6.7}) forming subset of this mapping population were genotyped with SSR primer pairs.

3.2 Development of mapping population

The set of recombinant inbred lines (RILs) was used as a mapping population. The RILs were produced at ICRISAT, Patancheru, by 6-7 generations of single seed descent method (i.e., each randomly chosen plant/head contributes a single row of offsprings to the next generation). Segregating generations of a cross were rapidly advanced with no intentional selection, each line is being continued by single plant/head in each generation.

A single representative plant in BTx623 was used as female parent and pollinated with a single representative plant from IS 18551. All the F_1 plants were space planted and were selfed. Seeds of single head from each of the selfed F_2 plants were harvested separately and grown in progeny rows in the next generation. Individual plants were chosen randomly within each progeny row in F_3 and were selfed. The process of random selection and selfing individuals continued up to $F_{5.7}$ generations. In all generations of random selection, the nurseries were protected from infestation of shoot fly and other insects. Bulk seed was harvested from random F_5 plants to produce 252 F_6 recombinant inbred lines (RILs). Each F_6 line represents F_2 plant from which it is derived. The method of developing RILs is diagrammatically represented in Figure 1. The details on pedigree of 252 $F_{6.7}$ RILs of cross BTx623 x IS 18551 is given in Appendix I.

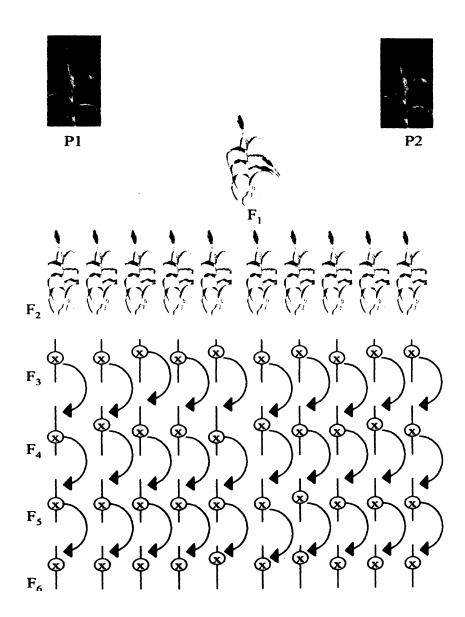


Figure 1. Development of RILs (generation advancement from F_2 onwards by selfing individual plant at random)

Table 2. Salient features of parental lines of RIL mapping population and susceptible checks

Parents and checks	Salient features
BTx623 (Parent)	Derived from cross between IS 40583 (kafir) and
	IS 21807 (caudatum). Grains are white and glumes
	reddish brown. Leaves of seedling are dark green
	(Plate 4) (non-tan), dull, broad and drooping (Plate 1)
	with no trichomes. Highly susceptible to shoot fly.
	High yielding with medium plant height and maturity
	(Plate 3).
IS 18551 (Parent)	Origin from Ethiopia, race durra. Earhead with straw
	colored grain and larger glumes. Leaves of seedling
	are light green, shining (Plate 4), narrow and pointed
	upward (Plate 2) with dense trichomes. Resistant to
	shoot fly. Very tall at maturity (Plate 3).
296 B (Check)	Derived from Aispuri. Semi-compact earhead, white
	grain, tan colored. Leaves of seedling are non-glossy
	with no trichomes. Susceptible to shoot fly.
CSH-1 (Check)	CK60A x IS 84. It has semi-loose earhead with white
	grain. It is non-tan. Leaves of seedling are non-glossy
	with no trichomes. Highly susceptible to shoot fly.



Plate1. The susceptible parent BTx623 bearing dark green, dull, droopy leaves



Plate 2. The resistant parent IS 18551 bearing glossy, erect, narrow, pointed leaves



Plate 3. The two parents of mapping population viz., BTx623 (S) and IS 18551(R) at maturity

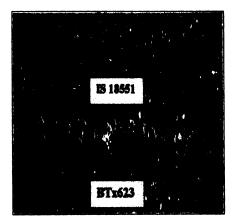


Plate 4. Seedlings showing glossy (Score 1) and non-glossy (Score 5) leaves in resistant (IS18551) and susceptible (BTx6123) parents, respectively

3.3 Evaluation of RILs for phenotypic characters

3.3.1 Locations, seasons and experimental design

A total of 256 entries involving 252 RILs, two parents (BTx623 and IS 18551) and two susceptible checks (296 B and CSH-1) were planted in three environments. The entries were evaluated at ICRISAT, Patancheru (during *kharif* and *rabi*, 2000) and at Dharwar (during early *rabi*, 2000). The experimental material was planted in the month of August 2000 and January 2001 at Patancheru and August 2000 at Dharwar. ICRISAT is located at an altitude of 545 meters above mean sea level at a latitude of 17° 32' N and longitude of 78° 16' E. Dharwar is located at an altitude of 678 meters height above mean sea level at a latitude of 15° 26' N, longitude of 75° 07' E. The three environments are here onwards referred as E1 (*kharif*. Patancheru), E2 (*rabi*, Patancheru) and E3 (early *rabi*, Dharwar).

The genotypes were laid out in an 8 x 32 alpha design (Patterson and Williams, 1976) with four replications. Each replication consisted 32 blocks each with 8 plots. Each entry was planted in 2 row plots of each with 2 m length with ridges 75 cm apart at Patancheru and 45 cm apart at Dharwad. Plots were thinned at 10 DAE (days after seedling emergence) to spacing of 5 cm between plants within rows when the seedlings were at 4-leaf stage.

3.3.2 Resistance screening technique

To attain uniform shoot fly pressure under field conditions the interlard-fish meal technique (Nwanzae, 1997) was followed for screening resistance. Four rows of a susceptible cultivar (CSH-1 or CSH-9) were sown 20 days before sowing the test material (Plate 5). This was done to allow for multiplication of shoot fly for one generation. Ten days after seedling emergence, polythene bags containing moistened fish meal were kept in the test material at uniform intervals covering the entire area to attract the emerging shoot flies from infester rows. Field view of the technique followed is presented in Plate 6. The plant protection measures were avoided until the shoot fly infestation period was complete. However, chemical spray was carried out when the level of shoot fly infestation in the susceptible check CSH-1 was more than 70%.

3.3.3 Observations

Observations on leaf glossiness (1-5 scale), seedling vigour (1-5 scale), trichome density (no./mm²), seedling height (cm), oviposition (%), deadhearts (%), pseudostem length (cm), seedling dry weight (g), days to 50% flowering, plant height (cm) and grain yield (g/pl) were

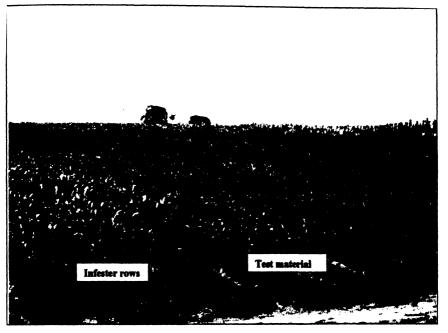


Plate 5. The infestor rows technique to screen resistance to shoot fly under field conditions (infester rows at foreground and test material at the rear end).



Plate 6. The interlard fish meal technique to screen for resistance to shoot fly under field conditions

recorded at all the three environments. The measurements on pseudostem length and seedling dry weight were recorded in two environments E1 and E2.

3.3.3.1 Glossiness

Intensity of glossiness was recorded at 7 DAE on 1 to 5 scale where 1 = high intensity of glossiness and 5 = non-glossy (Plate 3). Leaf glossiness was scored in the morning hours when there was maximum reflection of light.

3.3.3.2 Seedling vigour

Seedling vigour (height, leaf growth and robustness) was scored at 9 DAE and 16 DAE on a 1-5 scale where 1 = high vigour (plants showing maximum height, leaf expansion and robustness) and 5 = low vigour (plants showing minimum growth, less leaf expansion and poor adaptation). The seedlings being recorded at 9 and 16 DAE were designated as seedling vigour 1 and seedling vigour II, respectively.

3.3.3.3 Trichome density

For recording leaf trichome density, the central portion of fifth leaf from the base was taken from three randomly selected seedlings in each entry at 17, 22 and 30 DAE in the environments E1, E3 and E2, respectively. Leaf bits (approximately 0.5 cm²) were placed in 20 ml of acetic acid: alcohol (2:1) in small vials (1.5 cm diameter, 5.75 cm high) overnight. The cleared samples were transferred into 90% lactic acid in small vials and stored for later observations. For microscopic examination, the leaf samples were mounted on a slide in a drop of water and observed under stereomicroscope at a magnification of 10x. The number of trichomes on both lower and upper leaf surfaces was counted in three microscopic fields selected at random and expressed as trichome density (no./mm²). Thus, a total of 3 6 observations per entry mean (3 plants x 3 microscopic fields x 4 replications) were recorded.

3.3.3.4 Seedling height

Seedling height (cm) was measured from base of the plant to tip of top most completely opened leaf on three randomly selected plants in each entry at 20 DAE and 29 DAE. Seedling height recorded at 20 and 29 DAE are being designated as seedling height I and seedling height II, respectively.

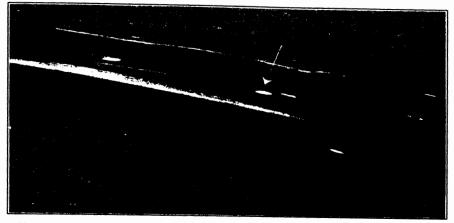


Plate 7. Young leaf of sorghum with eggs laid by shoot fly



Plate 8. Sorghum seedling showing symptom of deadheart

3.3.3.5 Oviposition

Total number of plants with eggs in each entry was recorded twice with an interval of 7 days in all the environments. In the environments E1 and E3 counts were made at 14 and 21 DAE. At environment E2 counts were made at 21 and 28 DAE. A seven-days delay in E2 was planned as shoot fly incidence was also slower in interlards. A typical plant with eggs laid on the lower surface is shown in Plate 7. The observations on oviposition recorded at two stages are referred here onwards as oviposition I and oviposition II. Oviposition counts were expressed in terms of percentage.

Oviposition (%) = Total number of plants

3.3.3.6 Deadhearts

Deadheart counts were recorded twice at an interval of 7 days in all the three environments. In E1, the counts were made at 21 and 28 DAE. In E3 counts were made at 7 days early due to early incidence of shoot fly. However, a seven-days delay in E2 was planned as shoot fly incidence was slower in interlards. A single plant with deadheart symptom due to damage by shoot fly is shown in Plate 8. The observations on deadhearts (%) recorded at two stages of seedling growth are referred here onwards as deadhearts I and deadhearts II. Deadhearts counts were expressed in terms of percentage.

 Number of plants with deadhearts

 Deadhearts (%) =

 x 100

Total number of plants

3.3.3.7 Pseudostem length

The data on pseudostem length (cm) was recorded in E1 and E2 at 29 DAE.

3.3.3.8 Seedling dry weight

Seedling dry weight (g) was recorded on three randomly selected plants at 30 DAE.

3.3.3.9 Days to 50% flowering

Time interval from sowing to anthesis showed at least 50% anthesis was recorded as days to 50% flowering

3.3.3.10 Plant height

Plant height at maturity (cm) was recorded from the soil surface to the tip of plant (including sorghum head)

3.3.3.11 Grain yield

At maturity, matured earhead from all the plants in each entry was harvested and bulked together Average grain yield per plant (g) in each plot was recorded

3.4 Molecular marker analysis

The molecular analysis made in the present study is described below. The details on preparation of solutions and buffers used are presented in Appendix II

3.4.1 Genomic DNA isolation

3.4.1.1 DNA Extraction and purification

DNA was extracted from bulk of about 30-50 individuals from each F_7 line This bulk constituted the genotype of the F_6 plant from which the seeds were obtained for the next generation For DNA extraction, 5 day old etiolated seedlings were used Seeds of each entry were washed with chlorax (10%) to avoid contamination with growth of fungus Around 50 seeds were spread in foldings of paper towels arranged in a plastic tray and sprinkled with water The trays with the seed samples were placed in incubator and the temperature was adjusted to 30 °C Care was taken to avoid drying of paper towels After 5 days the etiolated seedlings (5 g) were taken out from the tray, placed in punched polythene bags and lyophilized in liquid nitrogen These tissues were then stored at -20 °C

For DNA extraction, the following CTAB Maxi-prep method was followed

DNA extraction steps (CTAB maxi-prep)

1 The lyophilized tissue sample was ground into fine powder in liquid nitrogen using autoclaved mortar and pestle

- 2. The ground tissue was transferred to tubes containing about 10 ml of CTAB (2%) solution.
- 3. The tubes containing ground tissue samples were placed in water bath (with gentle shaking) for 2-3 h at 65 °C with periodical shaking at an interval of 20 min Later, the tubes with tissue extract were incubated at room temperature for 15 min
- 4. 10 ml of chloroform and isoamyl alcohol mixture (24 1) was added to the tissue extract and the contents were mixed by shaking gently
- The contents were then transferred to fresh centrifuge tubes and spinned for 20 min at 5000 rpm at room temperature
- 6. The supernatant was transferred to fresh centrifuge tubes About 10 ml of chloroform and isoamyl alcohol mixture (24.1) was added to the supernatant, mixed by inverting and centrifuged at 6000 rpm for 20 min at 2 or 4 °C
- The supernatant was transferred to glass tubes 10 ml of chilled isopropanol was added to each tube, mixed by inverting and incubated at -20 °C for 10 min
- 8. Centrifuged the contents for 20 min with 5000 rpm at 2 or 4 °C The supernatant was discarded.
- 9. The DNA pellet obtained was washed with 70% ethanol and the tubes were inverted on blotter paper to dry the pellet
- 10. RNAse (2 ml) (10mg/ml) was added to each corex tube DNA was redissolved by tapping the pellet suspended in RNase and incubated at room temperature overnight

Purification steps

- 11. 200 ul of sodium chloride (5 M) was added to each corex tube, mixed gently and incubated at 4 °C for 15-20 min
- 16. The contents were centrifuged at 6000 rpm for 20 min and then transferred the supernatant into small corex tubes
- 17. About 2 ml phenol: chloroform (buffered phenol mixed with chloroform in 1 1 proportion) was added to each corex tube and mixed the contents by inverting
- 18. The contents were spinned at 2500 rpm for 10 min and supernatant was transferred into fresh small corex tubes
- 19. Steps 17 and 18 were repeated
- 20. 200 ul sodium acetate (2 5 M, pH 5 2) (about 1/10th volume of aqueous layer) and about 2.5 ml absolute alcohol were added to the supernatant, mixed gently and

incubated at -20 °C for 15-20 min

21 The DNA was spooled in 1.5 ml eppendorf tubes using glass hook, washed with 70% alcohol and dried in vaccum The DNA was dissolved in $T_{10}E_1$ (300 ul) buffer and incubated at 4 ^oC.

3.4.2 Determination of quantity and quality of isolated DNA

It is established that DNA and RNA absorb ultraviolet light so efficiently that optical absorbance can be used as an accurate, rapid and nondestructive measure of their concentration. Therefore, for quantifying the amount of DNA, spectrophotometric readings were taken at a wave-length of 260 nm which allows the calculation of the concentration of nucleic acids in the sample. Double stranded DNA at 50 μ g/ml in aqueous solution has an absorbance (OD) of 1 0 (Maniatis *et al.*, 1982). The procedure used for quantification of DNA is as follows

- 5 μl of DNA sample was added to 995 μl of deionised distilled water, mixed thoroughly and was read the absorption (OD) in a spectrophotometer at 260 nm and 280 nm
- 2. The concentration of DNA in the solution was calculated according to the following formula:

DNA conc $(\mu g/\mu l) = [OD_{260} \times 50 \ \mu g/m l]/5$

The ratio between the readings at 260 and 280 nm (OD_{260}/OD_{280}) was used as an estimate of the purity of the DNA samples Pure preparations of DNA have OD_{260}/OD_{280} values range between 1.7 and 1.8 (Maniatis *et al*, 1982)

The DNA degradation and contamination with other substances were checked by electrophoresis of an aliquat of sample in mini agarose gel (0.7%) It is assumed that large molecular weight DNA appears as a band with sharp strikes, whereas partially degraded material forms a smear of long to small fragments The amount of DNA was also approximated by utilizing UV induced florescence emitted by ethidium bromide molecules intercalated into the DNA. Because, the amount of fluorescence is proportional to the total mass of DNA. The quantity of DNA sample was estimated by comparing the fluorescent yield of the sample with that of a series of standards (e.g., *lambda* DNA). Following procedure was used to accomplish the quality determination

- 1. Ends of perspex tray was sealed with masking tape or clamped (GIBCO BRL) and comb was inserted.
- 2. Agarose gel (0.7%) was prepared by adding 0.42 g agarose to 60 ml of TBE (1X) buffer (EDTA 0.5 M and pH 8)
- 3. The solution was boiled by putting the flask in microwave oven and cooled to 60 °C
- 4. Ethidium bromide (6 µl of conc. 10mg/ml) was added to the gel and mixed gently
- 5. The gel was poured into the tray and air bubbles were removed by using Pasteur pipettes. After the gel was completely set, tape was removed and the gel was placed into the electrophoresis tank.
- 6. Approximately 500 ml of TBE (1X) buffer was poured into the electrophoresis tank enough to cover the gel to a depth of 5 mm.
- 7. Comb was removed carefully.
- 8. About 1/10 volume of loading buffer (6X) with methylene blue dye was added to DNA samples and mixed by gentle tapping and spinning 2-3 sec only in a microfuge. The purpose of adding loading buffer is (i) as it contains glycerol, it makes the samples denser than TBE (1X) and keeps the DNA samples in the well, and (ii) because of its color, it is possible to keep track of the movement of DNA samples in the gel.
- DNA samples were loaded carefully to avoid spillover to adjacent wells. A lane was loaded with DNA size marker (50 ng/µl uncut lambda DNA).
- 10. The lid was put on the gel apparatus and the electrodes were attached by making sure that the negative terminal was at the same end of the apparatus as the wells. Switched on the power supply (80 V).
- 11. The power supply was switched off when dye front was about 2 cm from positive end, and the gel was removed from the gel apparatus.
- 12. The gel was viewed and photographed by using gel documentation system.

3.4.3 Testing parental polymorphism using SSR primers

To identify SSR primers showing polymorphism between parents, initial screening of parental lines was carried out before actual genotyping of individuals in RIL mapping population. For this, parental DNA from BTx623 (P1) and IS 18551 (P2) were subjected to PCR amplification by using SSR primer pairs. A total of 96 SSR primer pairs were used to screen the parents of RIL population. The source of these primers is genomic library of BTx623 from which they are isolated and are characterised (Kang *et al.*, 2000). Bhattramakki *et al.*, 2000) The sequence information of both forward and reverse primers is given in Appendix III. From this

screening, SSR primer pairs showing scorable polymorphism between the parents were noted down and used for further screening of the subset of RIL population. The experimental protocol used for testing parental polymorphism is explained in following headings

3.4.3.1 PCR reaction mix and conditions for the amplification of SSR alleles

The standard PCR reagents in total volume of 20 µl were

Genomic DNA template (5 ng/µl)	3 µl
Mg ²⁺ (10 mM)	4 µl
dNTP mix (2 mM)	2 μl
PCR buffer (10X)	2 µl
Deionised distilled water	7 μl
Primer (4 pmoles/µl)	lμl
Taq DNA polymerase (1U/µl)	lμl

According to Caetano-Anolles (1997) the parameters of DNA amplification viz, specificity, efficiency, and fidelity are strongly influenced by the different components of the reaction and by thermal cycling. Therefore, the careful optimization of these parameters will ultimately result in reproducible and efficient amplification. To achieve the optimization, the range of values for components of PCR reaction was varied in the above protocol for different primers used in the present study.

3.4.3.2 PCR cycling

The cycling conditions for SSR primers were set up using touchdown PCR thermocycling (Don et al., 1991). The details of the programme are as follows.

95 °C – 10 min hold	
95 °C - 15 sec (Denaturation)	
* 61 °C - 20 sec (Primer annealing)	* = 1°C drop over cycle for 10 cycles
72 °C - 30 sec (Primer extension)	
95 °C – 10 sec (Denaturation)	
54 °C – 20 sec (Primer annealing)	35 cycles
72 °C - 30 sec (Primer extension)	
72 °C – 2 min hold	
4 °C - soak	

PCR reaction was carried out using a model PTC-100 thermocycler (MJ Research) However, change in annealing temperatures was followed for different primers as per temperature range required for respective primers (mentioned in the Appendix III) to achieve optimization in the amplified products. Before loading, 5 μ l of loading buffer (5X) containing orange dye was added to each reaction sample.

3.4.3.3 Separation of PCR products containing SSRs

For separation and visualization of PCR products both agarose (2%) as well as polyacrylamide gels (6%) were used. Agarose gels were used only for visualization of amplification considering two limitations in their use Firstly, exact sizing of microsatellite alleles cannot be accomplished on agarose. Secondly, it is difficult to distinguish two, three or four base pair differences in DNA fragment length on agarose (Cregan and Quigley, 1998) Therefore, the exact allele sizing of PCR amplified product was performed in polyacrylamide gels.

3.4.3.3.1 Agarose gel electrophoresis

Agarose was casted in 2% gels in TBE buffer (1X) Gels were casted in a horizontal gel frame (GIBCO BRL or Bio-Rad sub cell or OWL separation systems) and products were visualized by incorporating 1 μ l (10 mg/ml) ethidium bromide per 10 ml of gel solution and viewed in a gel documentation system. The procedure of gel electrophoresis was same as described in 3.4.2. An image showing poymorphism for some SSR primer pairs in agarose gel (2%) is presented in Plate 9.

3.4.3.3.2 Polyacrymide gel electrophoresis (PAGE)

For separation and visualization of PCR products, 6% polyacrylamide gels were used The details on gel preparation and visualization of DNA bands are given below

3.4.3.3.2.1 Gel preparation and electrophoresis

Polyacrymide gels allow high resolution of amplified products. For separating amplified products by SSR primers, non-denaturing polyacrymide gels (6%) were used. Before preparation of gel solution, glass plates were cleaned thoroughly with ethanol. Few drops of Repel-Silane-ES were applied to the back plate and rubbed over the surface. This makes it easier to separate the plate from the gel. To other glass plate, a few drops of bind silane were applied and rubbed over entire surface. This prevents gel from dislodging during staining.

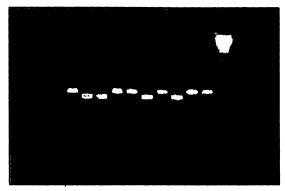


Plate 9. A photographic image showing polymorphism with SSR primers in an agarose gel (2%).

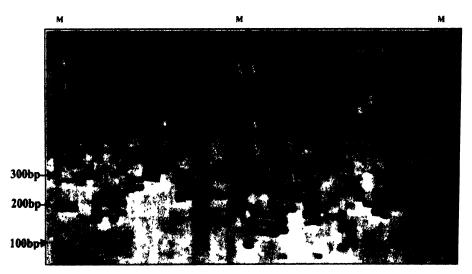


Plate 10. An image of parental polymorphism for SSR primers in polyacrylamide gel w successive pairs of lanes after Lambda marker (M) represent P1 and P2 for 23 primers.

- Caution: i) If plates are not thoroughly washed the bubbles can get trapped while pouring the gel.
 - ii) <u>Silane is carcinogen</u>, so gloves and face mask should be worn when applying to the glass plate.

For a 6% gel (Bio-Rad plates) 75 ml of gel solution was prepared by mixing
 7.5 ml TBE buffer (10X)
 15 ml 29: 1 (w/w) acrylamide/bisacrylamide
 53 ml distilled water

Caution: Acrylamide is a neurotoxin. Always wear gloves, goggles and face mask!

- 2) The contents of gel solution were mixed vigorously. TEMED (90 μ l) was added and mixed by swirling the flask.
- 3) Immediately added 400 µl of 10% (w/v) APS (ammonium per-sulphate) and mixed.
- Acrylamide solution was poured into the syringe which feeds into the glass plates and comb was inserted.
 - NB: Polymerization is catalyzed by the addition of freshly prepared APS. So be quick in pouring solution into the plates.
- 5) After polymerization the gel was set up for running. The comb was removed. Lower tank was filled with TBE (0.5X) approximately 250-300 ml, and back of the plate and upper reservoir with the same (approx 400 ml), ensured that the well was covered. The well was cleaned by aspirating the TBE buffer using a Pasteur pipette to remove small fragments of gel and tiny bubbles. Comb tips were inserted 1 mm deep into the gel. The gel was pre-run to warm it for at least 10 min at 5 V/cm (approx, 400 V, 9 W).
- 6) The samples were made up for loading in 5X loading buffer to give a final concentration of 1X and loaded between 2-5 μl on the gel. Lambda size marker (2 μl with conc. 50 ng/μl) was loaded along with the sample.
- 7) The gel was run at approximately 5 V/cm (400 V, 9 W). The gel was run until the desired resolution was reached. This was determined by the dye front.
- 8) After the run, the plates were carefully pulled apart so that the gel was attached to one plate.

3.4.3.3.2.2 Visualisation of DNA bands

Electrophoresed DNA fragments were detected with silver nitrate staining (Goldman and Merril, 1982). Several protocols for silver staining will be used and most of which require approximately 2 hours. Although commercial kits for silver staining are available from several

manufacturers (e.g., Bio-Rad Laboratories), but the technique was followed with homemade solutions in the present study. Each solution was prepared in separate containers. The same solutions were used twice over a 30 h period except for silver nitrate solution and developer, which were freshly prepared during the staining process.

Following are the steps followed for silver staining

- 1. The gel was rinsed in distilled water for 3-5 min.
- 2. The gel was soaked in 2 litres of 0.1% CTAB (2 g in 2 litres of water) for 20 min.
- 3. The gel was incubated in 0.3% ammonia (26 ml in 2 Litres) for 15 min with shaking.
- 4. Silver nitrate solution was prepared (2 g silver nitrate, 8 ml of 1M NaOH, 6-8 ml 25% ammonia) and titrated with ammonia until the solution became clear and added a further 1 ml of ammonia solution.
- 5. The gel was placed in the silver nitrate solution for 15 min and was gently agitated.
- 6. The gel was then rinsed in water for 1 min.
- The gel was placed in developer (30 g Sodium carbonate, 0.4 ml formaldehyde, 2 litres of water) until the bands became visible.
- 8. The plate was rinsed in water for 1 min to stop staining.
- 9. The gel was placed in fixer (30 ml glycerol in 2 litres of water).
- 10. The gel was kept for air-drying for overnight and was scanned.

NB: To remove the dried gel from plate, the plate was soaked in concentrated sodium hydroxide (NaOH) solution (40 g flakes in 1 litre) for few hours.

The DNA polymorphism among the parents was observed based on length of amplified fragments in terms of number of base pairs by comparing with 100 base pair ladder (50 ng/µl). An image showing parental polymorphism for some SSR primer pairs in polyacrylamide gel visualized by silver staining is presented in Plate 10. Among the different bands observed in 'each lane, the least base pair size of a band was considered for scoring.

3.4.4. Genotyping RILs with SSR primers

3.4.4.1 Monoplex PCR

The subset of mapping population consisting of 93 RILs was screened with the SSR primer pairs showing polymorphism in parents. The same protocol described in 3.4.3 was followed except agarose gel electrophoresis. The PCR products $(2 \ \mu l)$ of SSR primer pairs in RILs were loaded along with parents and ladder (1.5 μl of 100 bp ladder of concentration 50

 ng/μ) using a custom 98 or 100-toothed comb (0.4 cm well center to well center with thickness of 0.4 mm). The DNA bands were visualized by following silver staining. The gels were air dried and scanned. An image of PAGE in which products of single primer were loaded in the gel is presented in Plate 11.

3.4.4.2 Multiplex PCR

Individuals of RIL population were screened with more than one primer simultaneously in a single PAGE. This is based on the concept of differences in size of amplified products with different primers; the information of which was previously known in the parents. While choosing the primers, it was made sure that the amplified products of number of primers do not co-migrate with each other. In this procedure instead of running the PCR reaction with number of primers (pre-amplification multiplex PCR), which otherwise takes lot of time for optimization, the PCR reaction for each primer was set up separately and these monoplex products were pooled together, i.e., post-amplification multiplexing (also called multi-loading). These samples were loaded in the PAGE and bands were visualized with silver staining procedure. The alleles for each primer were scored separately in RILs by comparing with p arental a lleles for respective primer. The example images for two (Xtxp9 and Xtxp312) and three primers (Xtxp32, Xtxp229and Xtxp248) are given in Plates 12 and 13, respectively.

3.4.4.3 Scoring of SSR amplified bands and genotyping

The banding patterns obtained from PCR amplification of various SSR primers in the RILs were scored as follows.

A = Homozygote for the allele from parental strain P_1 (BTx623) at the locus

B = Homozygote for the allele from parental strain P₂ (IS 18551) at the locus

H = Heterozygote carrying the alleles from both P_1 and P_2 parental strains

O = Offtypes showing banding patterns different from the parents

= Missing data for the individual at the locus

After scoring the individual progeny were typed in a Microsoft Excel spread sheet in a format suitable for linkage analysis by JOINMAP (i.e., rows = genotype score at given locus; columns = individual RIL of mapping population).

3.5 Statistical analysis

3.5.1 Phenotypic data analysis

3.5.1.1 Analysis of variance (ANOVA)



Plate 11. An image of PAGE with PCR products of single primer (*Xtxp75*) in 93 RILs of cross BTx623 x IS 18551.

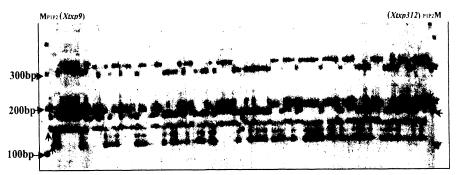


Plate 12. An image of PAGE with PCR products of two primers (*Xtxt9* and *Xtxp312*) in 92 RILs of cross BTx623 x IS 18551.

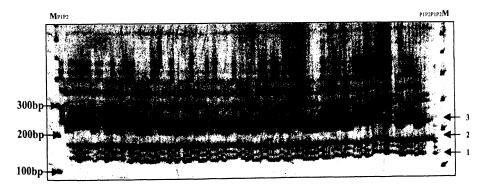


Plate 13. An image of PAGE with PCR products of three primers (1. Xtxp32, 2. Xtxp229 and 3. Xtxp248) in 92 RILs of cross BTx623 x IS 18551.

The analysis of variance for components was performed by residual maximum likelihood algorithm (REML) introduced by Patterson and Thompson (1971). The REML estimates the components of variances by maximizing the likelihood of all contrasts with zero expectation. Variety means were estimated by generalized least squares with weights depending on the estimated variance components according to Patterson (1997). The data was analyzed using GENSTAT package.

3.5.1.2 Phenotypic and genotypic coefficients of variation

The estimates of phenotypic and genotypic coefficient of variation were obtained by using formulae (Singh and Chaudhary, 1999),

 $PCV (\%) = \frac{\sqrt{Vp}}{----} \times 100$

$$GCV(\%) = \frac{\sqrt{Vg}}{2} \times 100$$

where,

PCV = phenotypic coefficient of variation GCV = genotypic coefficient of variation Vp = phenotypic variance Vg = genotypic variance \overline{X} = mean of RILs

3.5.1.3 Phenotypic correlation

Phenotypic correlation coefficients were estimated by using the formula (Singh and Chaudhary, 1996),

$$r_{p} = \frac{Cov_{p}(X, Y)}{\sqrt{Var_{p}(X) Var_{p}(Y)}}$$

where,

 r_p = phenotypic correlation coefficient

 $Cov_p(X, Y) = phenotypic covariance between characters X, Y$

Var_p (X) = phenotypic variance in character X Var_p (Y) = phenotypic variance in character Y

The observed value of correlation coefficient was compared with the tabulated value for (n-2) degrees of freedom for test of significance

3.5.1.4 Estimates of broad sense heritability

Broad sense heritability was estimated in RILs for all resistance components as well as the traits measured at maturity It is the ratio of the total genotypic variance to the phenotypic variance. It was computed as per Falconer (1989) for the data recorded in individual environments, E1, E2 and E3.

 $h^2 = \frac{Vg}{Vp}$

Heritability estimates across the environments in combination of two (E1E2, E2E3 E1E3) and three (E1E2E3) were computed by the formula,

$$h^{2} = \frac{Vg}{Vg + Vge + Ve}$$

$$n_{e} \quad n_{e} x r$$

where,

 h^2 = broad sense heritability Vg = genotypic variance

Vp = phenotypic variance

Ve = environmental variance

 $Vge = G \times E$ interaction variance

ne - number of environments

r = number of replications

3.5.1.5 Superiority of RILs over the parents

The calculation of superiority of RILs over parents for shoot fly resistance and other traits were worked out using following formula,

$$S1 = \frac{RIL - P1}{P1} \times 100$$

RIL - P2 S2 = ----- x 100 P2

where,

S1 = Superiority to P1 (BTx623) S2 = Superiority to P2 (IS 18551)

P1 = Mean of parent 1

P2 = Mean of parent 2

The information obtained by these caculations were used for the estimation of proportion of transgressive segregants (based on means across three environments) lying outside the parental limits for shoot fly resistance components as well as other traits

3.5.1.6 Test of significance of means

To test whether difference between means of parents and RILs is small enough to accept the null hypothesis, i.e., $\overline{X_1} = \overline{X_2}$, t-test was applied and calculations were made using the formula given by Singh and Chaudhary (1996).

$$t = \frac{\overline{X_{1} - \overline{X_{2}}}}{\sqrt{\frac{s_{1}^{2} + \frac{s_{2}^{2}}{n_{2}}}}$$
where, $s_{1}^{2} = \frac{\Sigma (x_{i1} - \overline{X_{1}})^{2}}{n_{1} - 1}$
 $s_{2}^{2} = \frac{\Sigma (x_{i2} - \overline{X_{2}})^{2}}{n_{2} - 1}$

The calculated value of 't' was compared with the tabulated value of 't' for significance at n_1+n_2-2 degrees of freedom.

3.5.1.7 Genetic advance

The estimates of genetic advance and genetic advance as percentage over mean were calculated by the following formula given by Singh and Chaudhary (1999) A 5% selection intensity with i = 2.06 was used.

 $GS = i \sqrt{Vp} \times Vg$ Vpwhere, GS = genetic advance i = standardized selection differential Vp = phenotypic variance of base population Vg = genotypic variance

 $GS(\%mean) = \frac{GS}{\overline{X}} \times 100$

 $\overline{\mathbf{X}}$ = mean of base population

Predicted genetic gain (R) based on highest and lowest measurements for the traits at 5% selection was calculated according to Falconer and Mackay (1996) and expressed as percentage over mean. Thus,

 $R = h^{2}S$ where, $S = \overline{Xs} - \overline{Xp}$ $\overline{Xs} = Mean of the progeny of selected individuals$ $\overline{Xp} = Mean of base population$

Genetic advance was then expressed as a proportion of the RIL mean to compare among the different traits for potential improvement by selection

3.5.1.8 Correlated genetic gain

The extent of correlated response is a function of heritabilities of the primary and correlated characters, as well as the genetic correlation between the characters (Dudley, 1997). The predicted correlated genetic gain were calculated using formula suggested by Falconer (1989).

 $CRy = ih_x h_y r_g \sigma p_y$

where,

CRy = correlated response of character Y when selection is based on character X

i =intensity of selection

 h_x = square-root of the heritability of character X

 h_y = square-root of the heritability of character Y

 r_g = genetic correlation between the two characters X abd Y

 $\sigma p_y =$ phenotypic standard deviation of character Y

The genotypic correlation coefficient was obtained by estimating the variance and covariance components for each character and character pairs using the formula given by Menendez and Hall (1995).

Cov_g(X, Y)

 $\sqrt{\operatorname{Var}_{\mathbf{g}}(\mathbf{X})\operatorname{Var}_{\mathbf{g}}(\mathbf{Y})}$

where,

 r_{g} = genetic correlation

 $Cov_{g}(X, Y) = genetic covariance between characters X, Y$

 $Var_{g}(X) = genetic variance in character X$

 $Var_{g}(Y) = genetic variance in character Y$

3.5.1.9 Spearman's rank correlations

In breeding programs selection involves the ranking of genotypes. To assess the stability of rank order of a set of RILs across the environments, Spearman's rank correlation coefficients (method devised by Spearman, 1904) were estimated by following formula,

$$r(a) = 1 - \frac{6 \Sigma d_1^2}{n (n^2 - 1)}$$

where,

r(a) = Spearman's rank correlation coefficient $d_i =$ difference between the two sets of ranking n = number of observations

3.5.2 Marker data analysis

3.5.2.1 Marker segregation and segregation distortion

Segregation analysis of marker loci was performed by JMSLA module of JOINMAP package. The program makes for each locus a frequency distribution of the genotypes into genotype classes and performs a χ^2 goodness-of-fit test with the expected segregation of 1.1 (1 homozygote of parent P1: 1 homozygote of parent P2) The calculated χ^2 values were compared with table values at 1 degree of freedom for each marker locus.

3.5.2.2 Construction of genetic linkage map

A computer package JOINMAP (Stam and Van Ooijen, 1995, Stam, 1993) was used for construction of linkage map: The JMGRP module of this package was used for grouping the markers (i.e., assignment to the linkage groups) keeping LOD thresh hold value of 4.0 at which best order was obtained. Pair-wise recombination frequency for all the markers belonging to each linkage group was obtained separately by using module JMREC at recombination fraction threshold of 0.499 and LOD threshold of 0.01-0.50. From this pair wise list of recombination estimates together with LOD scores, linkage map was produced by JMMAP module using Kosambi's mapping function (Kosambi, 1944).

Linkage analysis was accomplished using the program Joinmap (Stam, 1993). Linkage distances in terms of centimorgan (cM) values were calculated using Kosambi function The SSR markers used in this study have been mapped in BTx623 x IS 3620C RIL population (Bhattramakki *et al.*, 2000). This linkage map of sorghum was used as reference map for comparison with respect to linkage distances, linkage position and marker order. Markers used for analysis in the present study were therefore assigned to linkage groups based on their known chromosome location and their order was verified by JOINMAP.

3.5.2.3 QTL analysis

Mean of trait data for each individual RIL was used for the QTL analysis of components of resistance. The total number of progeny individuals from the cross BTx623 x IS 18551 used for marker trait associations was 93. In this, the female parent BTx623 (scored as A) was susceptible and male parent IS 18551 (scored as B) was resistant to shoot fly damage QTL mapping was carried out using PLABQTL (Utz and Melchinger, 1995) The output of JOINMAP containing information on linkage groups and linkage distances between the markers on each linkage group were included input file for PLABQTL. After analysis with PLABQTL, the QTLs identified for components of resistance were assigned to the linkage groups based on linkage positions of markers on linkage map developed by Bhattramakki *et al* (2000).

3.5.2.3.1 QTL analysis in single environments

To localize and characterize QTLs controlling components of resistance to shoot fly, the combined phenotypic and molecular data were analyzed with PLABQTL (Utz and Melchinger, 1995). Interval mapping using multiple regression approach with flanking markers (CIM, composite interval mapping) was followed according to the procedure described by Haley and Knott (1992). Since the mapping population used in the present study constitutes RILs, the additive model AA was chosen for analysis in which additive x additive effects were included

The LOD score was calculated from F value in the multiple-regression (Haley and Knott, 1992) as

 $LOD = n \ln (1 + p*F/DFres) * 0.2171$

where,

p = number of parameters fitted

DFres = number of degrees of freedom for residual sum of squares in multiple regression

The percentage of phenotypic variance explained by putative QTL ($R^{2\%}$) was calculated which is based on partial correlation of the putative QTL with the observed variable, adjusted for cofactors (Kendall and Stuart, 1961). In the simultaneous fit, the cofactors are ignored and only the detected QTL and their estimated positions were used for multiple regression to obtain the final estimate of the additive effects and percentage of phenotypic variation that can be explained by the QTL. The adjusted $R^{2\%}$ (adj $R^{2\%}$), the explained final phenotypic variance, was estimated according to Hospital *et al* (1997) The additive effect was

calculated as half the differences between genotypic values of two homozygotes (Falconer, 1989).

Additive effect = (Parent P2 – Parent P1)/2

It was assumed that the second parent IS 18551 (P2) carries the favorable alleles for the different components of resistance and first parent BTx623 (P1) carries favorable alleles for agronomic traits. If the second parent is weaker one, the additive effect was considered as negative.

3.5.2.3.2 QTL analysis across the environments and Q x E interaction

The analysis was done with PLABQTL (following the same procedure described above) to identify QTLs for the traits across two environment combinations and three environments. However, the occurrence of $Q \times E$ interaction was tested for significance by adding digenic epistatic effects to the additive effects in the model. The $Q \times E$ interaction for shoot fly resistance was estimated by a fitted model to the adjusted entry means of each environment as described by Bohn *et al* (1996). A simultaneous fit with detected QTL was performed for each environment. The results were obtained in the form of table showing ANOVA and the estimated effects.

The additive effects were obtained for all detected putative QTL for each environment as well as for means across the environments. The estimates MS (Q x E) were calculated from the difference of the fits of the data from individual environments and means across environments. These values were tested for significance with a sequentially rejective Bonferroni F test (SRBF).

Experimental Results

IV. EXPERIMENTAL RESULTS

The experimental study was carried out to characterize the recombinant inbred lines (RILs) developed by crossing BTx623 (susceptible) and IS 18551 (resistant) genotypes towards understanding the genetic architecture of shoot fly resistance components in sorghum The experiments were conducted under three environments, viz., ICRISAT- Patancheru during *kharif* and *rabi* seasons of 2000 and Dharwad during early *rabi* season 2000. The observations were recorded on different components of resistance to shoot fly and other traits. Genotypic data was collected by screening subset of RIL mapping population with polymorphic SSR primer pairs The results obtained from phenotypic, genotypic, and combined phenotypic and genotypic data analysis are presented herein.

4.1 Estimates of phenotypic and genotypic variation

4.1.1 Mean performance of parents and RILs

The mean performances of parents and RILs are presented in Table 3 and Table 4, respectively, for components of resistance to shoot fly at seedling stage and the traits at maturity

The two parents differed phenotypically for all the characters recorded except for seedling dry weight. However, the parental performance under different environments for all the traits except for glossiness also varied With respect to seedling vigour I, the performance of the two parents was on par with each other, while, significant differences between the parents were noticed for seedling vigour II. It was observed that both the parents remained vigorous at later stage of seedling growth in the environment E2

The resistant parental line IS 18551 (P2) recorded high leaf glossiness (score 1 10 - 1 40). high seedling vigour at stage I (score 1.66 - 2.70) and at stage II (score 1.20 - 1 95), high trichome density (no./mm²) on lower (70.82 - 98.18) and upper (1.50 - 3.87) leaf surfaces The parental line (P2) recorded maximum seedling height at stage I (19 47 - 27.34 cm) and at stage II (34.76-45.80 cm). The photographs showing high trichome density (lower and upper leaf surfaces) observed in resistant parent are presented in the Plates 14 - 15 The resistant parent (P2) showed higher pseudostem length (3.60 - 6.55 cm) than P1; it was late flowering (77.70 - 81 24 days), with maximum plant height (201.10 - 285.30 cm) at maturity and low grain yield (9 52 -19.50 g/pl). As expected measurements on oviposition (%) and deadhearts (%) were significantly

	El (kharif	Patancheru)	E2 (rahi	Patancheru)	E3 (carly rab	i Dhamuar)
0						
Character		P2 (IS 18551)	P1 (BTx623)	P2 (IS 18551)	P1 (BTx623)	P2 (IS 18551)
Glossiness	4.90	1.40	4.89	1.16	4.90	1.10
Seedling vigour I	2.50	2.10	1.85	1.66	3.50	2.70
Seedling vigour II	3.10	1.80	2.20	1.95	2.90	1.20
Trichome density (lower) (mm ⁻²)	1.50	76.90	3.87	98.18	2.16	70.82
Trichome density (upper) (mm ⁻²)	0.80	5.20	0.51	2.81	0.99	6.83
Oviposition I (%)	64.31	39.24	23.79	5.20	83.88	75.40
Oviposition II (%)	83.70	67.49	73.12	44.73	93.71	91.43
Deadhearts I (%)	72.13	30.81	45.64	13.08	81.15	53.17
Deadhearts II (%)	82.13	43.18	74.11	20.46	87.03	68.66
Seedling height I (cm)	24.32	27.34	21.94	23.89	15.42	19.47
Seedling height II (cm)	42.95	45.80	43.71	45.25	28 .49	34.76
Seedling dry weight (g)	3.30	3.50	4.42	4.43	-	-
Pseudostem length (cm)	2.70	3.60	3.59	6.55	-	-
Days to 50% flowering	76.12	78.34	71.94	77 .70	79.12	81.24
Plant height (cm)	146.80	285.30	126.20	201.10	132.10	216.50
Grain yield (g/pl)	20.60	9.52	13.00	12.03	25.09	19.50

 Table 3. Means of two parents of cross BTx623 (susceptible) x IS 18551 (resistant) for different components of resistance to shoot fly and other traits in three screening environments

Glossiness (1-5 scale) : 1-high intensity of glossiness, 5-non-glossy

Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour

- data not available

Table 4. Mean and range of phenotypic values in RILs derived from cross BTx623 (susceptible) x IS 18551(resistant) for different components of resistance to shoot fly and other traits in three screening environments

Character	El (khar	rif, Patancheru)	E2 (rab	i, Patancheru)	E3 (early	rabi, Dharwar)
	RĨL	Range	RĨL	Range	RĪL	Range
Glossiness	3.54	1.4-5.0	3.63	1.1-4.9	3.78	1.6-5.0
Seedling vigour I	2.27	1.6-3.3	2.06	1.2-3.9	2.74	1.7-4.0
Seedling vigour II	2.48	1.4-3.6	2.04	1.2-3.8	2.02	1.2-3.0
Trichome density (lower) (mm ⁻²)	39.08	1.5-95.0	50.10	1.6-124.3	40.26	0.26-97.9
Trichome density (upper) (mm ⁻²)	4.88	0.0-19.5	3.70	0.09-25.2	6.17	0.0-24.4
Oviposition I (%)	54.50	41.3-69.8	18.78	6.7-45.7	81.87	70.0-88.4
Oviposition II (%)	74.85	63.7-84.3	68.12	44.6-89.1	92.97	90.0-94.3
Deadhearts I (%)	58.12	34.4-75.2	26.18	5.5-62.0	73.94	53.1-86.4
Deadhearts II (%)	70.26	47.3-87.3	45.19	14.7-80.8	85.42	70.9-92.3
Seedling height I (cm)	24.87	21.1-29.5	23.57	1 9.5-28 .7	16.83	13.5-19.9
Seedling height II (cm)	43.60	39.4-48.0	43.57	35.9-51.0	30.94	26.6-35.6
Seedling dry weight (g)	3.11	2.5-3.8	4.45	4.0-5.0	-	-
Pseudostem length (cm)	2.64	2.1-3.7	4.83	2.6-10.0	-	-
Days to 50% flowering	76.27	67.0-91.8	73.03	65.2-88.2	78.25	72.9-84.2
Plant height (cm)	231.20	136.4-311.1	157.00	101.3-217.4	181.20	104.9-248.1
Grain yield (g/pl)	15.24	5.9-30.4	11.55	4.9-17.0	19.37	10.7-38.0

Glossiness (1-5 scale) : 1-high intensity of glossiness, 5-non-glossy

Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour

- data not available



Plate 14. Trichome density on lower surface of sorghum leaf in resistant parent IS 18551 at 20x



Plate 15. Trichome density on upper surface of sorghum leaf in resistant parent IS 18551 at 20x



Plate 16. RIL (No. 252) with dense trichomes on lower surface of leaf at 20x



Plate 17. RIL with medium dense trichomes on lower surface of leaf at 20x



Plate 18. RIL with sparse trichomes on lower surface of leaf at 20x



Plate 19. RIL (No.252) with dense trichomes on upper surface of leaf at 20x

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lower in P2 than P1 in all the environments. However, the range of phenotypic values for these traits varied significantly in different environments. The phenotypic values for oviposition (%) were lower in environment E2 (5.20 - 44.73%) than in E1 (64.31 - 83.7%) and E3 (75.40 - 91.43%). Similarly, the deadhearts percentage was significantly lower in environment E2 (13.0-25.5) followed by environments E1 (30.80 - 43.20) and E3 (53.2 - 68.7).

The susceptible parental line BTx623 (P1) was non-glossy (score 4.9), with low seedling vigour (score 1.85 - 3.50 and 2.20 - 3.10 at stages I and II, respectively), no trichomes or very few trichomes on both the surfaces (0 - 4.0 no./mm²) and minimum seedling height (15.42 - 24.32 cm at stage I; 28.49 - 42.95 cm at stage II). This parental line recorded lower pseudostem length (2.70 - 3.59 cm) than the resistant parent (P2). It was early in flowering (71.94 - 79.12 days), with lower plant height (126.20 - 146.80 cm) and higher grain yield (13.0 - 25.09 g/pl). It was highly susceptible to shoot fly damage. The parental line was recorded with significantly higher oviposition (%) rates were observed in environment E3 (75.40 - 91.43%) followed by environment E1 (64.31 - 83.7%) than in E2 (23.79 - 73.12%). Similarly, the deadhearts (%) values recorded to be significantly higher under environment E3 (81.0 - 87.0%) followed by E1 (72.1 - 82.1%) and E2 (45.6 - 74.1%).

The mean performance of RILs varied between the environments for the traits studied. The mean values of RILs for the trait glossiness were higher in E3 (score 3.78) and E2 (score 3.63) compared to E1 (score 3.54). In general, the mean values for seedling vigour were higher in the initial stage of plant growth than in the later stage of seedling growth indicating recovery of seedling growth at later stage. However, in environment E2 the mean value for seedling vigour remains same in later stage of seedling growth. The mean values for seedling vigour II were lower in E3 (score 2.02) and E2 (score 2.04) than in E1 (score 2.48). The mean values for trichome density (lower surface) (no./mm²) were higher in environment E2 (50.1) than in environments E3 (40.26) and E1 (39.08). However, for trichome density (upper surface) (no./mm²) higher mean of RILs was observed in environment E3 (6.17) compared to E1 (4.88). The lower RIL mean values were observed for the traits oviposition (%) (18.78 - 68.12%) and deadhearts (%) (26.18 - 45.19%) in the environment E2. However, moderate values for these traits (54.50 - 74.85% and 73.94 - 85.42%) were observed in E1. The mean values for these traits were recorded to be significantly high in E3 (81.87 - 92.97 % and 73.94 - 85.42%). The mean of RILs for seedling dry weight (4.45 g/pl) and pseudostern length (4.83 cm) was higher in environment E2 than in environment E1 (3.11 g/pl and 2.64 cm). With respect to seedling height,

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the average performances of RILs were on par with each other in environments E1 and E2 at seedling stage I (24.87 and 23.57 cm) and II (43.6 and 43.57 cm). However, performance of RILs was lower in environment E3 for seedling height I (16.83 cm) and seedling height II (30 94 cm) compared to E1 and E2.

Regarding traits measured at maturity in RILs, mean days to 50% flowenng (73.0 days), plant height (157.0 cm) and grain yield (11.55 g/pl) were lower in environment E2 compared to means for respective traits in environment E1 (76.27 days, 231.2 cm and 15.24 g/pl) and E3 (78.25 days, 181.2 cm and 19.37 g/pl). The mean performance of RILs for plant height was significantly higher in environment E1 followed by in E3.

4.1.2. Analysis of variance

The analysis of variance for different components showed that variances due to genotypes (RILs) were significant for all the traits studied based on performance in individual environments as well as average over two, and three environments (Table 5).

For the components like glossiness, seedling vigour and trichome density, the variances were higher in E2 and E3 than in E1. However, for other components, viz., oviposition I, oviposition II, deadhearts I, deadhearts II, seedling height I and seedling height II the variances due to genotypes were high in E2 followed by E1 than in E3 In the environments E1 and E2 it was observed that, for shoot fly resistance in terms of deadhearts (%) variances due to genotypes were higher for deadhearts II compared to deadhearts I. However, the reverse was true for this trait in environment E3. For seedling height, the magnitude of genotypic variance was higher in the environment E1 and E2 than in E3. While, the magnitude of genotypic variance was higher for seedling height II compared to seedling height I. For seedling dry weight variances due to genotypes were consistent across E1 and E2. With respect to pseudostem length, significantly higher magnitude of genotypic variances was observed in E2 compared to E1. Among the traits at maturity, plant height and grain yield per plant recorded significantly higher magnitude of genotypic variances in the environment E1 followed by E3 than in E2. But for days to 50% flowering, variances due to genotypes were higher in E1 and E2 than in E3.

Considering the analysis across two environment combinations and three environments, the results showed that variances due to genotypes were significant for all the traits except oviposition II in E2E3 (across E2 and E3) and E1E3 (across E1 and E3). Variances due to G x E

a mon nation nomindor	El (khari)	j	E2 (rabı,	abı,	E3 (early rabi,	y rabi,	EIE2 (across	across	E2E3 (E2E3 (across	El (thrarf E2 (rab., E3 (early rab., E1E2 (across E2E3 (across E1E3 (across	across		EIE2E3 (across
	Patancheru)	SF SF	Patancheru)	SE SE	<u>Dharwar)</u> σ _° ² SJ	<u>ser)</u>	<u>environments</u>) σ ₂ SE	ments) SE	cirvironments) σ _e ² SE	ments) SE	<u>environments</u>) σ ₂ ² SE	SE SE	cuvironments σ _e SE	SE SE
Cliat actes	0.87	800	1 10	010	° 0 97	60 0	0 929	60 0	96 0	60 0	0 88	0.08	0 96	60 0
Seedling vigour I	0 12	0 02	0 30	0 03	0 22	0 03	0 13	0 02	0 14	0 02	0 12	0.02	0 15	0 02
Seedling vigour II	021	0 03	0 29	0 03	0 24	0 03	60 0	0 02	0 03	0 02	0 16	0 02	0 11	0 02
I richome density (lower) (mm ⁻²)	580 80	55 30	988 00	93 20	615 30	59 40	717 00	68 70	726 60	70 40	576 80	54 00	691 80	64 00
Trichome density (upper) (mm ⁻²)	24 58	2 72	23 27	2 38	40 79	4 62	23 45	2 35	29 97	3 14	33 02	3 33	28 73	2 78
Oviposition I (%)	24 58	4 10	44 15	6 11	13 11	2 64	29 63	3 95	11 93	3 09	12 27	2 40	19 07	2 59
Oviposition II (%)	20 27	4 13	48 14	6 77	3 60**	1 74	28 63	4 06	041 ^{ns}	2 86	3 48 ^{ns}	1 93	11 86	2 17
Deadhearts I (%)	39 41	5 27	77 02	8 59	26 06	3 74	45 74	5 50	66 61	4 43	20 24	3 28	30 69	381
Deadhearts II (%)	48 59	6 09	108 85	11 53	14 11	2 64	60 64	7 08	18 65	4 93	17 42	3 13	34 53	4 35
Seedling height I (cm)	3 98	0 63	4 03	0 59	2 16	036	3 22	0 46	1 67	0 33	2 14	0 36	2 53	0 33
Seedling height II (cm)	5 52	1 28	9 46	l 44	4 63	0 89	6 59	0 98	3 20	080	3 84	0 76	4 86	0 70
Seedling dry weight (g)	0 16	0 04	0 13**	0 06	,	•	0 16	0 03	•	,	ı	1	,	•
Pseudostem length (cm)	0 19	0 04	2 71	030	ı	ı	0 57	0 12	•	•	ı	١	١	•
Days to 50% flowering	22 35	2 29	19 80	185	7 78	1 00	16 66	1 77	11 27	1 22	13 06	l 49	13 66	1 40
Plant height (cm)	1446 2	133 40	460 80	44 00	856 00	90 10	742 50	76 90	552 90	57 80	1022 80	100 40	800 60	77 00
Yield (g/pl)	29 80	331	8 02	0 94	27 34	4 78	735	1 50	5 66	2 01	4 96	277	621	1 46
icant Ic vanar rror aılable	•• significant at <i>p</i> =0 05 nce	=0 05				Estimates Figures w Glossines Seedling	Estimates for percentage data are based on angular trans Figures without superscript are significant at $p = 0.01$ Glossiness (1-5 scale) 1-high intensity of glossiness 5- Seedling vigour (1-5 scale) 1-high vigour 5-low vigour	ttage data erscript au er 1-hugh scale) 1-	are based re significa h intensity -high vigo	on angula int at $p =$ of glossin ur 5-low	Estimates for percentage data are based on angular transformation Figures without superscript are significant at $\rho = 0.01$ Glossiness (1-5 scale) 1-high intensity of glossiness 5-non gloss Seedling vigour (1-5 scale) 1-high vigour 5-low vigour	ation jloss,		70

Table 6. G x E interaction variance estimates for components of resistance to shoot fly and other traits in sorghum RIL mapping population derived from cross BTx623 (susceptible) x IS 18551 (resistant) across two and three screening environments

	E1E2	(within	E2E3	(within	E1E3	(across		
Character		n across		cross two		ns and		3 (across
Character		asons)		tions)		tions)		nments)
	σ_{gxe}^2	SE	σ _{gxe} ²	SE	σ _{gxe} ²	SE	σ _{gxe} ²	SE
Glossiness	0.032	0.010	0.025	0.008	0.015**	0.007	0 .024	0.006
Seedling vigour I	0,064	0.013	0.108	0.017	0.037	0.012	0 .070	0.010
Seedling vigour II	0.143	0.021	0.224	0.028	0.045	0.013	0.137	0.015
Trichome density	48 .00	9.20	59.00	10.70	5.90 ^{ns}	5.20	37.70	6.00
(lower) (mm ⁻²)								
Trichome density (upper) (mm ⁻²)	0.71"	0.510	2.300**	0.890	0.000	-	0.94 ^{##}	0.530
Oviposition I (%)	3.99"	2.410	15.990	3.240	5.97**	2.210	8 .500	1.860
Oviposition II (%)	3.48 ^{ns}	2.760	24.110	4.070	7.370	2.490	11.640	2.220
Deadhearts I (%)	9.480	2.640	30.050	4.260	10.130	2.540	16.570	2.240
Deadhearts II (%)	13.750	3.090	39.440	5.140	11.540	2.660	21.650	2.590
Seedling height I (cm)	0.630**	0.310	1.220	0.304	0.802**	0.293	0.876	0.218
Seedling height II (cm)	0.20 ^{ns}	0.720	3.630	0.860	0.98"	0.710	1.680	0.550
Pseudostem length (cm)	0.880	0.119	-	-	-	-	-	-
Days to 50% flowering	4.396	0.596	3.705	0.518	2.920	0,570	3 .640	0.395
Plant height (cm)	180.600	20.300	80.300	16.100	90.200	18.000	117.000	12.800
Grain yield (g/pl)	11.480	1.470	12.130	2.300	23.600	3.540	15.750	1.730

 σ_{gxe}^{2} : variance due to genotype x interaction

Figures without superscripts significant at p = 0.01 level

**significant at p = 0.05

estimates for per centage data are based on tranformed values

ns: non significant

Glossiness (1-5 scale) : 1-high intensity of glossiness, 5-non glossy Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour

El: kharif, Patancheru

E2: rabi, Patancheru

E3: early rabi, Dharwar

SE: standar error

interaction were significant for all the traits across environments except trichome density (upper surface) (Table 6).

4.1.3 Phenotypic and genotypic variability.

The estimates of phenotypic coefficient of variation (PCV%) and genotypic coefficient of variation (GCV%) for shoot fly resistance components and maturity traits in individual and across the environments were obtained and are given in Table 7. In general, estimates of PCV% were higher than GCV%. The ranges of phenotypic values for these traits in different environments are presented in Table 4.

4.1.3.1 Glossiness

Phenotypic scores for the trait in RIL population range widely between 1.4 and 5.0. The range of glossiness was wider and consistent in all the environments. It was observed that both the PCV% and GCV% estimates for the trait were high in each environment. While, the magnitude of variation for the trait was consistent across the environments and there was marginal difference between estimates of PCV% and GCV% observed for the trait.

4.1.3.2 Seedling vigour

The coefficient of variation was higher for seedling vigour II than seedling vigour I. The variability observed was inconsistent across the environments. However, the variability was high in environment E2 and consistent at both the stages.

4.1.3.3 Trichome density on lower leaf surface

The magnitude of estimates of both the PCV% and GCV% was significantly higher for the trait in each environment. It was observed that the estimates of PCV% were marginally higher than GCV% for the trait. The data shows that high magnitude of variability was consistent across the environments. However, the range for phenotypic values (no./mm²) was wider in the nvironment E2 (1.60 - 124.3) followed by E3 (0.26 - 97.90) and E1 (1.5 - 95.0).

The phenotypic variation for trichome density placed RILs between high-density group (similar or more than that in resistant parent), medium density (lower than resistant parent), sparse density and no trichomes groups. Photographs for first three groups are presented in Plates 16 - 18.

in sorghum RIL mapping population derived from cross BTx623 (susceptible) x IS 18551 (resistant) under individual and Table 7. Genotypic and phenotypic coefficients of variation (%) for components of resistance to shoot fly, and other traits

in sorghum RIL mapping population	population						the second s	
across screening environments	nents							
		(incher)	F2 (rahi.	Patancheru)	E3 (carly ra	F3 (rabi. Patancheru) E3 (carly rabi, Dharwar)	environments	SI
	EI (kuari)	EI (Knarl) Falainumu			CCN (%)	PCV (%)	GCV (%) PCV (%)	PCV (%)
	GCV (%)	PCV (%)	GCV (%)	FV (%)				10 90
Character	26.20	26.81	28.49	28.88	25.12	26.48	26.45	0.07
Glossiness	07.67		24 76	26.60	16.33	20.00	15.43	18.02
Seedling vigour I	13.94	19.11	01.47		27 1E	27 13	14.16	18.83
Seedling vigour II	18.05	22.10	25.06 51.00	26.92 63 95	c1.77 06.09	63.43	60.18	61.50
Trichome dunsity	60.79	62.96	ee.10					
	(unner) 101.99	112.73	130.93	140.40	103.71	115.90	109.26	113.84
I ncnome ueusity (upper (mm ⁻²)	2			0000	767	6.36	8.24	10.03
	8.88	11.89	35.04	42.60	10.4	, I , .		5 8.8
(a) i nonisodia	27.2	8 51	9.98	12.29	2.04	4.40	4.20	
Oviposition II (%)	0.0		33 17	36 76	6.71	8.46	10.16	11.95
Deadhearts I (%)	10.31	16.21	20.12		7 T V	6 06	8.47	10.02
Pandhearts II (%)	9.55	11.26	22.55	24.41	t t		90 2	8 47
	7 94	10.56	7.93	10.13	8.40	11.43	8	
Seedling height 1 (City)	20.3	8 21	6.65	8.59	6.78	9.80	5.37	0.12
Seedling height II (cm)	67.6	30.36	1.79	15.91	ı	ı	10.55*	14.99 ⁻
Seedling dry weight (g)	14.40	20.02	33.92	37.64	1	•	19.78*	-60.92
Pseudostem length (cm)		20.02 8 8 8	5.79	5.95	3.52	4.10	4.91	5.20
Days to 50% flowering	0.23	200	12 40	13 87	15.95	17.27	14.64	15.23
Plant height (cm)	16.14	10.44		28.06	27.07	36.98	16.07	25.01
Grain vield (g/pl)	34.28	38.26	40.47	20.02				
				Soular) volues	2			

The estimates for per centage data were based on transfomed (angular) values

* based on measurements in two environments E1 and E2.

PCV: phenotypic coefficient of variation - data not available

Glossiness (1-5 scale) : 1-high intensity of glossiness. 5-non glossy GCV: genotypic coefficient of variation

Seedling vigour (1-5 scale): 1-high vigour. 5-low vigour

4.1.3.4 Trichome density on upper leaf surface

Both PCV% and GCV% estimates were significantly higher in all the environments. High variability was observed for the trait consistently across the environments. In general, the estimates of coefficient of variation were higher in postrainy season across the two locations (E2 and E3) than in *rainy* season (E1). However, the estimates of variation were significantly higher in environment E2 than in E3. The higher variability in postrainy season was also evidenced in a wide range of phenotypic values recorded in E2 (0.09 - 25.2) and E3 (0.0 - 24.4) than in *rainy* season E1 (0.0 - 19.5). As an example, RIL (no. 252) showing high trichome density on upper surface of leaf is presented in Plate 19.

4.1.3.5 Oviposition

As expected the estimates of both PCV% and GCV% for the trait were variable in different environments. Higher estimates were recorded for both oviposition I and oviposition II in environment E2 than in environments E1 and E3. In general, both the PCV% and GCV% estimates were higher for the oviposion I than oviposition II. The PCV% estimates were significantly higher than GCV% estimates at both the stages in each environment.

Similarly, the range of phenotypic values was variable in different environments for both oviposition I and II. For oviposition I (%), the range was wider in environment E2 (6.7 - 45.7%) followed by E1 (41.3 - 69.8%) than in E3 (70.0 - 88.4%). While, the range was narrow for oviposition II (%) in the environment E1 (63.7 - 84.3%) and E3 (90.0 - 94.3%). However, wider range of phenotypic values was observed for oviposition II (%) in E2 (44.6 - 89.1%).

4.1.3.6 Deadhearts

The estimates of both PCV% and GCV% for the trait varied significantly in each environment. Both the estimates were higher at the initial stage of seedling growth (i.e., for percentage deadhearts I). As the growth advanced, the variability for the trait was decreased However, the estimates of variability for damage were higher for deadhearts I (%) in environment E2 (33.12-36.76%) than in E1 (10.31 - 12.57%) and E3 (6.71 - 8.46%) The reduced variability was also evidenced with narrow range of values observed for the trait in E1 and E3. However, the range for phenotypic value was wider in E2 (5.5 - 62.0%) than in E1 (34.4 - 75.2%) and E3 (5.1 - 86.4%).

4.1.3.7 Seedling height

In general, the variability observed for seedling height was low. The variability was comparatively higher for seedling height I than seedling height II. The low variability observed for the trait was consistent across the environments. However, the wider ranges of phenotypic values for seedling height I and seedling height II were recorded in E2 (19.5 - 28.7 cm and 35.9 - 51.0 cm) and E1 (21.1 - 29.5 cm and 39.4 - 48.0 cm) than in E3 (13.5 - 19.9 cm and 26.6-35.6 cm).

4.1.3.8 Seedling dry weight

The estimates of both PCV% and GCV% for seedling dry weight were higher in the environment E1 than in E2. It was observed that the estimates of PCV% were significantly higher than the GCV% values in both E1 and E2. However, the range of phenotypic values for the trait was narrow in both E1 (2.5 - 3.8 g) and E2 (4.0 - 5.0 g).

4.1.3.9 Pseudostem length

The estimates of both PCV% and GCV% for pseudostem length varied between the environments E1 and E2. However, the estimates were higher in environment E2 than in E1. Similarly, significantly higher variability for the trait in environment E2 was evidenced with wider range of phenotypic values (2.6 - 10.0 cm) compared to E1 (2.1 - 3.7 cm).

4.1.3.10 Days to 50% flowering

In general, the magnitude of variability for this trait observed was low in each environment. However, the estimates of both PCV% and GCV% were comparatively higher in environment E1 and E2 than in E3. While, the magnitudes of both the estimates were on par with each other in each environment. Similarly, high variability for the trait was evidenced with wider range of phenotypic values in environments E1 (67.03 - 91.79) and E2 (65.2 - 88.2) compared to E3 (72.9 - 84.2).

4.1.3.11 Plant height

It was observed that the estimates of variability for this trait were higher in environment E1 and E3 than in E2. However, the difference between the estimates of PCV% and GCV% was marginal in each environment. Higher variability observed for the trait was also evidenced with significantly wider range of variability in environments E1 (136.4 - 311.1 cm) and E3 (104.9 - 248.1 cm) than in E2 (101.3 - 217.4 cm).

4.1.3.12 Grain yield per plant

High estimates of both PCV% and GCV% and were observed for the trait. While, the estimates of GCV% were higher in environment E1 followed by E3 than E2. Similarly, the range of phenotypic values for grain yield per plant was wider in the environments E1 (5.9 - 30.4 g) and E3 (10.7 - 38.0 g) than in E2 (4.9 - 17.0 g).

4.1.4 Frequency distributions

The variation observed for shoot fly resistance components and traits at maturity were represented graphically by the frequency distribution of measurements in three environments. The measurements were grouped into equally spaced classes and the frequency of individuals falling in each class was plotted on the vertical scale. The resulting histograms showed smooth curves. In general, frequency distribution for most of the traits under study approximated to normal curve. This can be seen in Figure 2, where the smooth curves drawn through the histograms are normal curves except for the trait trichome density (upper leaf surface). However, the mean and range of values for these characters were varied and so the peaks were seen at different points for each trait in different environments. For example, regarding the trait seedling vigour, shift in peaks was observed at later stage of seedling growth (i.e., seedling vigour II) in each environment.

For the trait glossiness, though the character varied continuously, it showed a kind of bimodal-distribution, which was evidenced clearly in each environment. For the character trichome density (on upper leaf surface), the histograms drawn showed discontinuous distribution, which is more skewed towards no trichome density.

For the sake of convenience, the histograms for resistance traits viz., oviposition I (%), oviposition II (%), deadhearts I (%) and deadhearts II (%) were drawn for two environments, viz., E1 and E2 together and for environment E3 separately. Though range observed was narrow for these traits (Table 7) in environment E3, but shows continuous distribution as expected.

For the traits oviposition I (%) in E2, seedling height I and seedling height II in E3, and pseudostem length, seedling dry weight, oviposition I (%), deadhearts I (%) and deadhearts II (%) were not strictly continuous when histograms for data from different environment were drawn together. Neverthless, one can regard the measurements in such cases as referring to

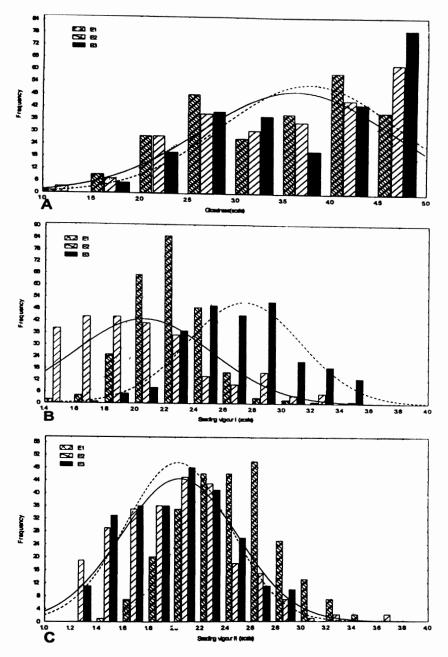


Figure 2 (A-T). Frequency distribution of 252 RILs of cross BTx623 x IS 18551 for components of resistance to shoot fly and other traits in three screening environments, viz., E1 (*kharif*,Patancheru), E2 (*rabi*, Patancheru) and E3 (early *rabi*,Dharwar).

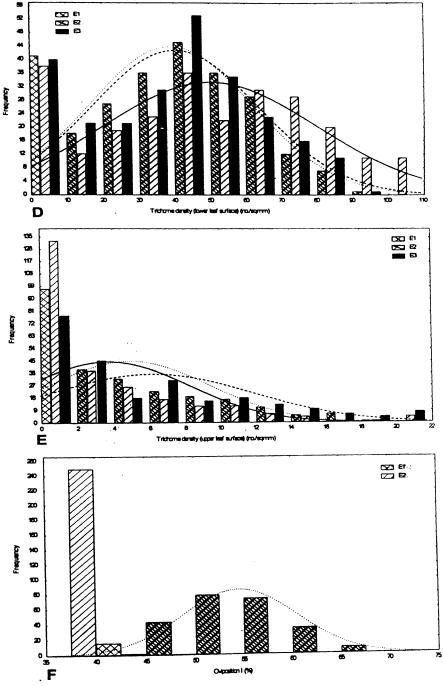


Figure 2. contd.

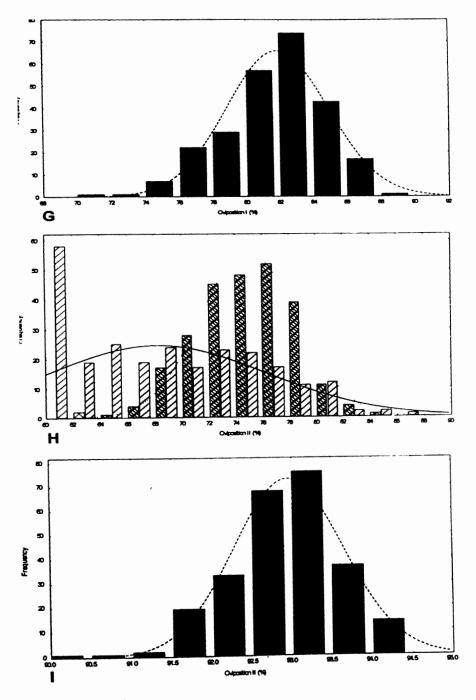


Figure 2. contd.

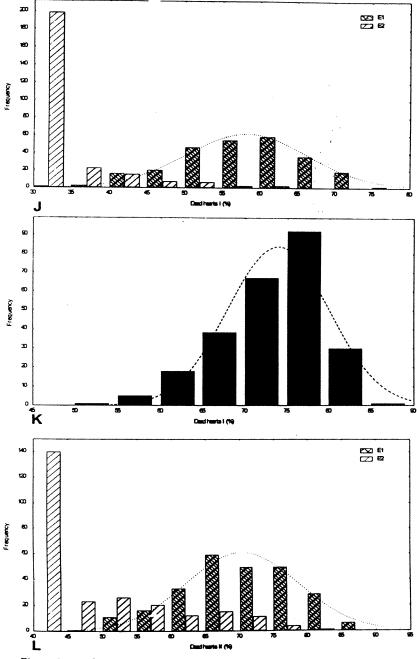
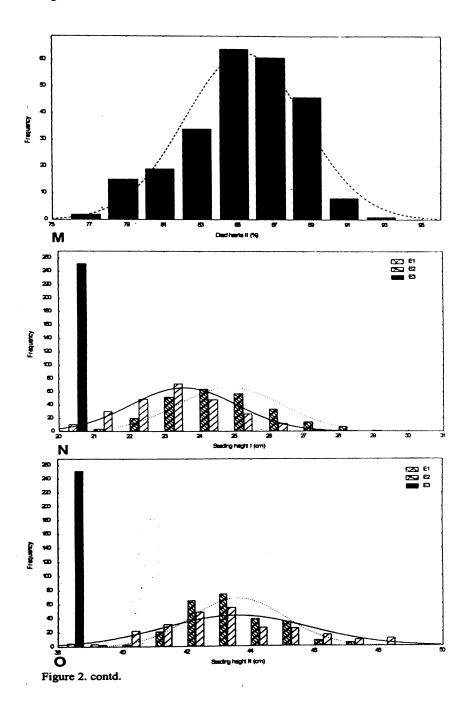


Figure 2. contd.

Figure 2. contd.



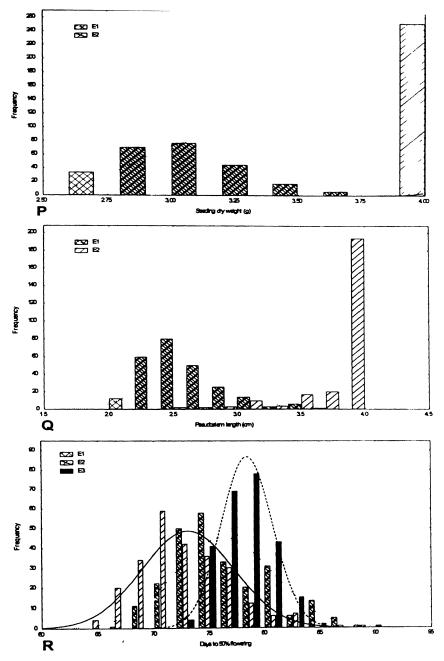


Figure 2. contd.

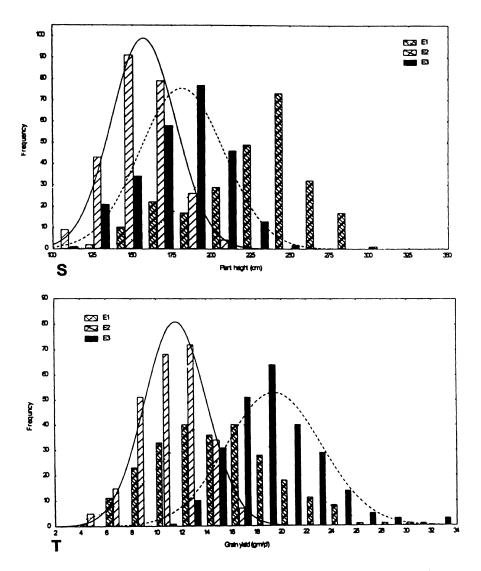


Figure 2G. Frequency distribution of RILs at environment E3 for oviposition 1 (%) Fig ure 2I. Frequency distribution of RILs at environment E3 for oviposition II (%) Figure 2K. Frequency distribution of RILs at environment E3 for Deadhearts I (%) Figure 2M. Frequency distribution of RILs at environment E3 for Deadhearts II (%)

underlying character whose variation is truly continuous. This kind of continuous variation was observed when considered the average performance over the environments (Figure 3).

4.2 Correlations

Correlation coefficients between components of resistance to shoot fly were estimated based on RIL means over the three environments (Table 8). However, considering varying range of phenotypic values observed in different environments for the traits, analysis was also made. using individual environment means (Table 9). This helps to know the varying degree of association in different environments.

4.2.1 Glossiness vs resistance traits and other traits

The intensity of glossiness was found to be negatively associated with resistance traits, viz., oviposition I (r = 0.47), oviposition II (r = 0.32), deadhearts I (r = 0.56) and deadhearts II (r = 0.49) and also days to 50% flowering (r = 0.15) over the three environments. However, significant and positive association was observed for glossiness with seedling vigour II (r = 0.42), seedling height I (r = -0.27), seedling height II (r = -0.23) and plant height (r = -0.14) (Table 8).

Intensity of glossiness was significantly negatively correlated with resistance traits, viz., oviposition I (0.30 < r < 0.47), oviposition II (0.20 < r < 0.43), deadhearts I (0.36 < r < 0.59) and deadhearts II (0.34 < r < 0.50) in different environments (Table 9). In general, the magnitudes of correlation coefficients between glossiness and resistance traits were higher in the environment E3 followed by E1 than in E2. The association of glossiness with seedling vigour I (r = 0.28), seedling vigour II (r = 0.57), seedling height I (r = -0.44) and seedling height II (r = -0.37) was significant and positive in environment E3. Association of glossiness with pseudostem length was significant and positive only in environment E2 (r = -0.16). Regarding association of glossiness with traits at maturity, significant correlations were observed in some environments. The association of the trait was negatively significant with days to 50% flowering (r = 0.15 in E2; r = 0.20 E3). However, its association was significant and positive with plant height (r = -0.13 in E1; R = -0.18 in E3) and grain yield (r = -0.13 in E2).

Trait	18	vigI	vigII	trlw	trup	ovil	ovill	qhI	IIHb	shtI	shtII	daf	plht	yld
1 3 6 .	1													
vigI	0.06	1												
vigII	0.42*	0.68*	-											
trłw	-0.10	0.23*	0.04	1										
trup	-0.10	0.23*	-0.05	0.64*	1									
livo	0.47*	-0.24*	0.22*	-0.38*	-0.40*	-								
oviII	0.32*	-0.04	0.32*	-0.38*	-0.47*	0.65*	-							
Idh	0.56*	-0.15**	0.36*	-0.42*	-0.48*	0.83*	0.74*	1						
IIHb	0.49*	-0.04	0.44*	-0.48	-0.53*	0.77*	0.83*	0.93*	I					
shtl	-0.27*	-0.56*	-0.70*	-0.10	-0.04	0.02	-0.23*	-0.17*	-0.26*	1				
shtII	-0.23*	-0.52*	-0.64*	-0.02	0.04	-0.03	-0.27*	-0.20*	-0.30*	0.76*	1			
daf	0.15**	0.13**	0.24*	-0.14**	0.03	0.09	0.21*	0.18*	0.25*	-0.11	-0.08	-		
plht	-0.14**	-0.30*	-0.28*	-0.19*	-0.12	0.06	0.03	0.03	0.02	0.34*	0.45*	0.51*	Г	
yld	0.04	0.20*	0.20*	0.05	0.14**	0.05	0.02	0.03	0.06	-0.07	-0.10	0.12	0.01	1
* Sign	 Significant at p=0.01 ** Significant at p=0.05 	01												

22 fene	centible: X	N 10001 ST	- Ammorica		wr. (23 (encomplete) x IS 18551 (resistant) unuci unw wr.		were 202 (anscentible) X IS 18551 (resistant) under unw success and and an and and and and and and and	H	IHL	II	2Htl	Shtu
Trait	Env	130	vigI	vigII	triw	trup	IVO	TIMO				
vigl	E	-0.04										
	E2	-0.07										
	E3	0.28*										
viell	EI	0.43*	0.37*									
	E2	-0.02	0.71*									
	E3	0.57*	0.54*									
te luc	El	-0.09	0.20*	-0.10								
	E2	-0.07	•61.0	0.20*								
	E	-0.13**	0.12	-0.06								
	EI	-0.06	0.18*	-0.09	0.63*							
3	E2	-0.11	0.13**	0.03	0.53*							
•	E	-0.10	0.19*	-0.02	0.72*							
	3 2	0.39*	-0.25*	0.37*	-0.27*	-0.27*						
IIVO	5 6	0.30*	-0.19	-0.21*	-0.36*	-0.41						
	1 6	0.47*	-0.11	0.32*	-0.19*	-0.15*						
ļ	3 5	*0C U	0.01	0.42*	-0.33*	-0.38*	0.52*					
ovill	ī	177.D	-0.03	0.04	-0.26*	-0.36*	0.63*					
	E2	-07.0		0 36*	-0.13**	-0.07	0.62*					
	8	0.45*	70.0		# <i>C</i> E UT	-0.34	0.62*	0.66*				
IHÞ	EI	0.47*	-0.14		#00 V	-0 43*	0.84*	0.72*				
	E2	0.36*	-0.11	8.9		+UC UT	*69.0	0.64*				
	E3	0.59*	-0.01	0.51*				0.67*	•06.0			
IIub	EI	0.50*	-0.11	0.50*				•0.79*	0.89*			
	E2	0.34*	0.01	0.10				0.58*	0.80*			
			100	0.15*	*0° (T							

(contd)								l F	14	II4	cht	shtll	drvwt	pslg	đaf	plht
Trait	Env	1 80	vigl	vigII	trlw	đ	IIVO	OVIII								
I I I	E	-0.18*	-0.49*	-0.41*	-0.15**	-0.12	0.16**	-0.09	-0.04	-0.06						
	6	010	-0.47*	-0.63	-0.06	0.03	0.06	-0.19*	-0.13**	-0.26*						
	E3 E3	-0.44*	-0.40*	-0.63*	0.01	0.00	-0.21*	-0.25*	-0.38*	-0.35*						
1141	3 6	200	-0.47*	-0.35*	-0.04	0.02	0.19*	-0.15**	-0.04	-0.08	0.63*					
SILLI	56	010	-034	-0.52*	-0.08	0.01	-0.04	-0.22*	-0.18*	-0.31*	0.58*					
	1 2	41.v	-0.39*	-0.57*	0.10	0.06	-0.18*	-0.21*	-0.28*	-0.31*	0.55*					
1	3 6	000	-0.45*		-0.13**	-0.13**	0.46*	0.04	0.20*	0.19*	0.46*	•09.0				
drywt	36	0.05	800		-0.16**	-0.08	0.19*	-0.05	0.02	-00.0	0.31*	0.54*				
	73	200	-0.41*		-0.04	-0.05	0.25*	-0 .09	0.01	-0.01	0.57*	0.66*	0.84*			
pslg	12 1	10.0-			-0.12	-0.08	-0.07	-0.22*	-0.23*	-0.31*	0.45*	0.67*	0.51*			
	71	01.0-			*96.0-	100-	0.05	0.15**	0.13**	0.17*	-0.01	-0.03	-0.08	-0.06		
daf	E	60.0				0.04	0.18*	0.27*	0.28*	0.34*	-0.21*	-0.15**	-0.13**	-0.40*		
	E2	0.15				0.15**		-0.01	0.04	0.01	-0.08	-0.13**	•	•		
	E	0.20*				010		0.03	0.04	0.03	0.35*	0.39*	0.20*	0.28*	0 33*	
plht	EI	-0.13**							0.09	0.08	0.17*	0.42*	0.10	0.28*	* I† 0	
	E2	-0.07				() () ()			-0.02	-0.02	0 28*	0.21*	٠		0 70*	
	E3	-0.18*	• -0.28*	-0.27		71.0-				20.0	-0 30 *	+CE 0-	-0.23*	-0.34*	-0.22*	-0.47*
vld	EI	0.10	0.22*	• 0.25*	0.23*	0.19*	10.0-	70.0	0.02				**71 V	110	(1 G	-0 03
•	E2	-0.13**	•• 0.09	-0.02	0.06	0.19*	-0.10	-0.11	-0.14**		0.00	0.10		11.0		0.264
	E3	0.04	0.05	0.17*	-0.14**	-0.01	0.16*	0.17*	0.14**	0.13**	-0.02	0.02				

significant at p=0.01
significant at p=0.05
data not available
E1: kharyf. Patancheru
E2: rabi. Patancheru
E3: early rabi. Dharwar

4.2.2 Seedling vigour vs resistance traits and other traits

It was observed that seedling vigour II was significant and negatively associated with oviposition I (r = 0.22), oviposition II (r = 0.32), deadhearts I (r = 0.36) and deadhearts II (r = 0.44), days to 50% flowering (r = 0.24) and grain yield (r = 0.20). Significant and positive association was observed for seedling vigour I and seedling vigour II with seedling height I (-0.57 < r < -0.70), seedling height II (-0.52 < r < -0.64) and plant height (-0.28 < r < -0.30). However, the association of seedling vigour I with trichome density was significant and negative (r = 0.22 and 0.21 on upper and lower leaf surfaces, respectively) (Table 8).

The magnitude and direction of association was variable in different environments and at different stages of seedling growth (Table 9). Seedling vigour I was significantly positively associated with oviposition I (r = -0.25 in E1; r = -0.19 in E2). However, seedling vigour II was significant and negatively associated with oviposition I (r = 0.37 and r = 0.32), oviposition II (r = 0.42 and r = 0.36), deadhearts I (r = 0.45 and r = 0.51) and deadhearts II (r = 0.50 and r = 0.45) in environments E1 and E3. While, the association of seedling vigour II with oviposition I was negative and significant in environment E2 (r = 0.21).

The association of seedling vigour I was significant and negative with trichome density (lower surface) (r = 0.20 in E1; r = 0.19 E2) and trichome density (upper surface) (r = 0.18 in E; r = 0.13 in E2 and r = 0.19 in E3). The association was maintained at later stage of seedling growth, i.e., seedling vigour II only in environment E2 (r = 0.20). As expected, seedling vigour was significantly and positively associated with seedling height at both the stages of seedling growth. However, the magnitude of correlation coefficients were higher for seedling vigour II with seedling height I (r = -0.63 in E2 and E3) and with seedling height II (r = -0.52 in E2; r = -0.57 in E3) compared to respective associations for seedling vigour I and II with seedling height I (r = -0.49 and -0.41) and seedling height II (r = -0.47 and -0.35) were observed. Similarly, the associations for seedling vigour I and seedling vigour II with pseudostem length were highly significant and positive (r = -0.30 to -0.41 and r = -0.28 to -0.44). The association of seedling vigour I and positive (r = -0.30 to -0.41 and r = -0.28 to -0.45 in E1) and seedling dry weight was significant and positive with seedling vigour I (r = -0.45 in E1) and seedling dry weight was significant and positive with seedling vigour I (r = -0.45 in E1) and seedling vigour II (r = -0.33 in E2).

Regarding association with maturity traits, seedling vigour I was significantly negatively correlated with days to 50% flowering (r = 0.15 in E3) and grain yield per plant (r = 0.22 in E1)

but significantly positively correlated with plant height (r = -0.27 in E1; r = -0.22 in E2 and r = -0.28 in E3). Similarly, seedling vigour II was significantly negatively correlated with days to 50% flowering (r = 0.22 in E1; r = 0.15 in E2 and r = 0.22 in E3) and grain yield per plant (r = 0.25 and r = 0.17 in E1 and E3, respectively) but significantly positively correlated with plant height (r = -0.21 in E1; r = -0.18 in E2 and r = -0.27 in E3).

4.2.3 Trichome density vs resistance traits

Trichome density was significantly and negatively associated with oviposition I (r = -0.38 and -0.40, respectively with trichome density on upper and lower leaf surfaces), oviposition II (r = -0.38 and -0.47), deadhearts I (r = -0.42 and -0.48), deadhearts II (r = -0.48 and -0.53). Significant and negative associations were observed between trichome density (on lower surface) and plant height (r = -0.19) over the three environments (Table 8).

The trichome density (lower surface) was significantly negatively associated with oviposition I (r = -0.19 to -0.36), oviposition II (r = -0.13 to -0.33), deadhearts I (r = -0.23 to -0.38) and deadhearts II (r = -0.30 to -0.39) in different environments. However, magnitudes of these associations were comparatively lower in environment E3 (Table 9). It was noticed that the trichome density (lower surface) was significantly negatively associated with plant height (r = -0.17 to -0.19). Similarly, The trichome density (upper surface) was significantly negatively associated with oviposition I (r = -0.15 to -0.41), oviposition II (r = -0.36 to -0.38), deadhearts I (r = -0.20 to -0.43) and deadhearts II (r = -0.26 to -0.47). However, magnitude of these associations was comparatively higher in environment E2.

4.2.4 Seedling height vs resistance traits

It was noticed that seedling height I and seedling height II were significantly negatively associated with oviposition II (-0.23 < r < -0.27), deadhearts I (-0.17 < r < -0.20) and deadhearts II (-0.26 < r < -0.30). However, significant and positive association was observed for seedling height I and II with plant height (r = 0.34 and 0.45, respectively) (Table 8).

The magnitude and direction of associations between seedling height and resistance traits were varied significantly in different environments and at different stages of observations (Table 9). The association of seedling height I was significant and negative with oviposition I (r = -0.21), oviposition II (r = -0.19 to -0.25), deadhearts I (r = -0.13 to -0.38) and deadhearts II (r = -0.26 to -0.35). Similarly, significant and negative associations between seedling height II

and resistant traits, viz., oviposition I (r = -0.18), oviposition II (r = -0.15 to -0.22), deadhearts I (r = -0.18 to -0.28) and deadhearts II (r = -0.31). In general, the magnitude of these associations at both stages of seedling growth was comparatively high in environment E3 and positively significant with resistance traits than E2 and no significant association in environment E1 was observed. Significantly positive association was observed for plant height with seedling height I (r = 0.17 - 0.35) and seedling height II (r = 0.21 - 0.42). These associations were higher in environment E1.

4.2.5 Pseudostem length and seedling dry weight vs resistance traits

Significant and negative associations between pseudostem length and resistance traits were observed only in environment E2. The magnitude of these associations with resistance traits, viz., oviposition II, deadhearts I and deadhearts II were r = 0.22, -0.23 and -0.31, respectively (Table 8).

However, significant and positive associations (r = 0.28) between pseudostem length and plant height were observed in both the environments, E1 and E2. The associations between seedling dry weight and resistance traits were significant and positive in the environment E1 (Table 9).

4.2.6 Traits at maturity vs resistance traits

Among the maturity traits, days to 50% flowering was significantly and positively associated with resistant traits, oviposition II (r = 0.21), deadhearts I (r = 0.18), deadhearts II (r = 0.51) at seedling stage, and plant height (r = 0.51) at maturity (Table 8).

No consistent associations were observed between traits at maturity and resistance traits at seedling stage (Table 9). However, the association was significant and positive for oviposition II (%) (0.15 < r < 0.27), deadhearts I (%) (0.13 < r < 0.28) and deadhearts II (%) (0.17 < r < 0.34) with time to 50% flowering. The magnitudes of association between days to 50% flowering and the resistance traits were higher in environment E2. It was observed that there was highly significant and positive association between days to 50 % flowering and plant height (r = 0.33-0.70). However, the magnitude of this association was high in environment E3.

4.2.7 Plant height vs days to 50% flowering

Significant correlation between plant height and days to 50% flowering (r = 0.51) was observed (Table 8). However, the magnitudes of correlation coefficients were varied among the environments. Strong association between these traits was observed in the environment E3 (r = 0.70). While, the association between the traits was low in the environments E1 (r = 0.33) and E2 (r = 0.41).

4.3 Inheritance of resistance

4.3.1 Broad sense heritability

The estimates of heritability for shoot fly resistance components and traits at maturity were obtained from the data collected in individual environments, viz., E1, E2 and E3. The heritability estimates were also obtained based on average performance over the environments in combinations of two, viz., E1E2, E2E3 and E1E3, and three environments (E1E2E3). The results are presented in Table 10, and are described in the following sections.

4.3.1.1 Glossiness

The heritability estimates were consistently high for the trait (>0.92) in individual and across the environment combinations. The magnitudes of heritability estimates for the trait ranged from 0.92 to 0.97.

4.3.1.2 Seedling vigour

The heritability estimates for the trait were quite high but variable in individual and across the environmental combinations and also at two stages of seedling growth. The heritability estimates in E2 and E3 were high, and consistent for both seedling vigour I and II. However, when analysed based on means over these two environments (E2E3), it was observed that the heritability estimates were reduced and were significantly differed at different stages of seedling growth. Analysis across the seasons and across the locations (E1E3) indicated the high heritability for the trait and the estimates were consistent at different stages (i.e., seedling vigour I and II). In general, heritability estimates were high in *rabi* season compared to *kharif* season.

4.3.1.3 Trichome density

The trichome density on lower surface of leaf recorded consistently high heritability estimates in individual and across the environments (>0.90). Trichome density on upper leaf surface also recorded consistently high heritability (>0.80) across the environments.

harartar	El (kharif Patancheru)	E2 (rabi Patancheru)	E3 (early rabi, Dharwar)	El (kharif E2 (rabi E3 (early rabi, E1E2E3 (across E1E2 (acr Cherescrier Patancheni) Patancheni) Dharwar) three environments) environm	E1E2 (across two environments)	E2E3 (across two environments)	E1E3 (across two environments)
Glossiness	0.92	0.94	0.95	0.97	0.95	96.0	96.0
Seedling vigour I	0.55	0.81	0.70	0.73	0.67	0.62	0.66
	0.69	0.76	0.73	0.57	0.46	0.15	0.71
Trichome density	0.93	0.94	0.92	0.96	0.94	0.93	0.96
(lower) (mm ⁻²) Trichome density	0.82	0.87	0.80	0.92	06.0	0.87	0.89
(upper) (mm ⁻)	0.56	0.68	0.47	0.67	0.72	0.42	0.52
Oviposition 1 (20)	0.46	0.66	0.21	0.51	0.68	0.02	0.22
Oviposition II (%)	0.67	0.81	0.63	0.72	0.77	0.47	0.61
Deadhearts II (%)	0.72	0.85	0.47	0.72	0.79	0.40	0.56
Seedling height I (cm)	0.57	0.61	0.55	0.70	0.68	0.52	0.58
Seedling height II (cm)		0.60	0.48	0.64	0.66	0.43	0.51
Seedling dry weight (g)	~	0.23	•	ı	0.50	•	ı
(cm)	0.48	0.81	ı	ı	0.46	•	•
Days to 50% flowering	0.87	0.95	0.74	0.89	0.85	0.85	0.82
Plant height (cm)	0.96	0.93	0.85	0.92	0.87	0.87	0.92
Grain vield (o/nl)	0.80	0.78	0.54	0.41	0.50	0.31	0.21

4.3.1.4 Oviposition

The estimates of heritability were low to moderate ($h^2 = 0.21$ to 0.68) and variable in different environments and at different stages of seedling growth. However, the heritability estimates were high in E2 and quite consistent at different stages of seedling growth. When the estimates analysed for pooled data from different environments indicate that the heritability estimates were higher across two environments (E1E2) and consistent at different stages of seedling growth.

However, the estimates were lower in E2E3, E1E3 and variable in different stages. The estimates were high in initial stage of seedling growth and reduced as the infestation level increased.

4.3.1.6 Deadhearts

The heritability estimates range from 0.50 to 0.86. The estimates were moderate and were consistent in two environments, viz., E1 ($h^2 = 0.68 - 0.72$) and E2 ($h^2 = 0.83 - 0.86$). However, lower estimates were observed in the environment E3 ($h^2 = 0.50 - 0.55$). The heritability estimate across the three environments ranges between 0.72 and 0.69. However, the estimates were high and consistent ($h^2 = 0.77$) at two stages within the location across the seasons (E1E2).

4.3.1.7 Seedling height

The estimates of heritability were moderately high and range between 0.50 and 0.86, and are variable in different environments. High estimates for the trait were observed in E2 and across the two environments (E1E2) and were consistent at two stages of seedling growth.

4.3.1.8 Seedling dry weight

The estimates of heritability of this trait observed were moderate in E1 ($h^2 = 0.40$) and E1E2 ($h^2 = 0.50$), while, low estimates were observed in E2 ($h^2 = 0.23$).

4.3.1.9 Pseudostem length

The heritability estimate observed for the trait was high in E2 ($h^2 = 0.81$) while moderate estimates were observed in E1 ($h^2 = 0.48$) and across the environments (E1E2) ($h^2 = 0.46$).

4.3.1.10 Days to 50% flowering

It was observed that the magnitude of heritability estimates was high for the trait in individual environments ($h^2 = 0.87, 0.95$ and 0.74 in E1, E2 and E3, respectively) and also across the environments (E1E2E3) ($h^2 = 0.82 - 0.89$)

4.3.1.11 Plant height

Significantly high estimates for the trait were observed in individual environments ($h^2 = 0.96$, 0.93 and 0.85 in E1, E2 and E3, respectively) and across the environments ($h^2 = 0.87 - 0.92$)

4.3.1.12 Grain yield per plant

The magnitudes of heritability estimates were significantly high in El ($h^2 = 0.80$), moderate in E3 ($h^2 = 0.54$) However, the magnitude of heritability estimates for the trait significantly reduced when the pooled data over the environment was analysed

4.3.2 Transgressive segregation

The RILs lying outside the parental limits were identified based on means across three environments The RIL population mean and individual parental mean were subjected to t-test for testing the significance of difference between the means The results obtained are presented in Table 11 It showed that RIL means differed significantly from both the parents for all the traits except trichome density (upper leaf surface) and days to 50% flowering. For trichome density (upper leaf surface), the RIL means did not differ significantly from the parent P2 (IS 18551), where as for days to 50% flowering, RIL means did not differ significantly from P1 (BTx623).

Transgressive segregants with phenotypic values outside the parental limits were observed for most of the traits except for high intensity of glossiness and low deadhearts (%) The appearance of transgressive segregants for each trait is shown in Figure 3 (A-O) In general, for the traits with RIL means less than the midparental value, the proportion of RILs were higher for those outside the low scoring parent and *vice versa*

4.3.2.1 Glossiness:

The deviation of RILs mean from midparent value observed for the trait was positive The proportion of transgressive segregants was very low and has been observed for RILs outside

over three screening environments Test of Significance Proportions outside	nents		Midnandal	1	Test of ?	Test of Significance	Proportion the nare	Proportions outside
Character	P1 (BTx623)	P1 (BTx623) P2 (IS 18551) value	value	RIL	P1/RIL	CI MEAUS	PI	P2
		-		3.65	:	:	0.008	0
Glossiness	4.9	1.1	י ר	766	*	:	0,060	0.329
Seedling vigour I	2.7	2.1	2.4	00.7				0,009
	0 (15	2.15	2.18	*	*	070.0	0.000
Seedling vigour II 2.8 Trichome density (lower) 1.07	2.8 1.07	83.96	42.515	43.14	:	:	0.012	160.0
	0.24	4.97	2.605	4.92	*	su	0.056	0.413
(mm ⁻²)			CE E1	CL 13	*	#	0.056	0.004
Oviposition I (%)	59.54	35.9	41.14	27.10	*	:	0.008	0.008
Oviposition II (%)	86.1	64.43	C07.C1	10.01	**	:	0.024	0.000
Deadhearts I (%)	68.54	27.43	47.985	0/.70	: 1		0 103	0.000
Deadhearts II (%)	82.68	38.12	60.4	66.96		;	104	0.016
Seedling height I (cm)	20.15	24.43	22.29	21.76		: :	0.087	0.012
Seedling height II (cm)	37.77	43.32	40.545	39.37	: :	: 1	0.266	0 052
Pseudostem length (cm)	3.13	5.53	4.33	3.73	*	: :	0.500	0 218
Davs to 50% flowering	75.8	79.5	77.65	75 78	su	: :	0000	0 044
Plant height (cm)	132	235.9	183.95	189.80		: 1	0.063	0.210
	20.2	13.6	16.9	15.34	:		2000	

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t data based on means averaged over two environments ... กการเอทเทียวกา

** means significantly diferent at 0.01 probability level by t-test

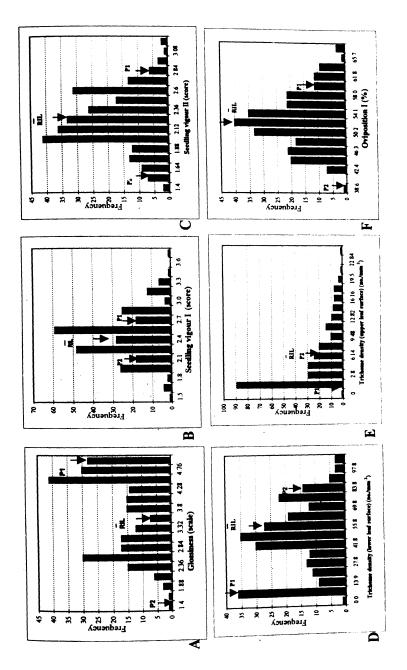
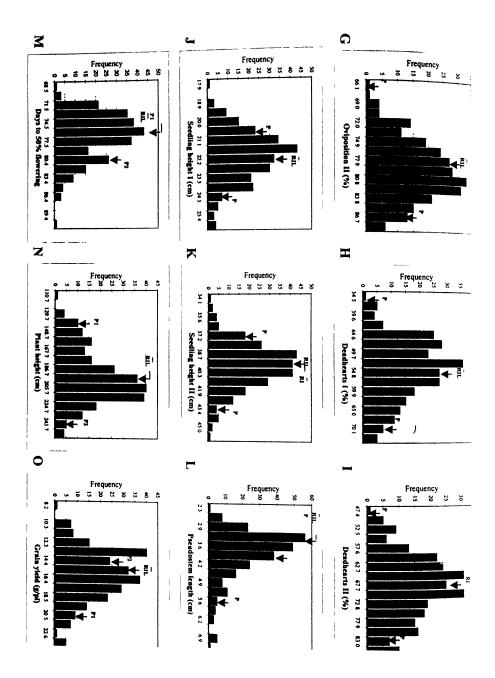


Figure 3 (A-O). Frequency distribution of 252 RILs derived from cross between BTx623 x IS 18551 for components of resistance to shoot fly, and other traits based on average performance over three screening environments.



higher scoring parent (BTx623). No transgressive segregant RILs were observed for phenotypic value outside the low scoring (or high intensity of glossiness) parent (IS 18551)

4.3.2.2 Seedling vigour:

It was observed that RIL mean values for the trait were on par with midparental values However, the proportion of transgressive segregants was high in the initial stage of seedling growth and reduced in the later stage.

4.3.2.3 Trichome density

It was observed that mean value of RILs was on par with midparental value for trichome density on lower leaf surface. However, the transgressive segregants were observed for this trait and were in higher frequency with values outside the high scoring parent. As an example, RIL (no. 252) showing trichome density higher than resistant parent is presented in Plate 16

For trichome density on upper leaf surface, mean of RILs deviates positively from midparental value. The proportion of transgressive segregants was higher than those observed for trichome density (lower leaf surface). High frequency was observed for transgressive segregation with phenotypic value lying outside the high scoring parent. As an example, RIL (no. 252) showing trichome density on upper leaf surface higher than resistant parent is presented in Plates 30 and 31.

4.3.2.4 Oviposition

The RIL means deviated from midparental value for the trait. The deviation observed was positive. The transgressive segregants were observed for the trait at both the stages. However, the proportion of RILs lying outside the high scoring parent P1 was comparatively higher for oviposition I than oviposition II.

4.3.2.5 Deadhearts

The RIL means deviated from midparental value for the trait. The deviation observed was positive. Transgressive segregation was clearly observed and for susceptibility only. The transgressive segregants were observed for the trait at both the stages. However, the proportion of RILs lying outside the high scoring parent P1 was comparatively higher for deadhearts II (%) than for deadhearts I (%).

1.3.2.6 Seedling height

The RIL mean and midparental values were recorded on par with each other. For both seedling height I and seedling height II, the proportion of transgressive segregants with phenotypic values outside the lower scoring parent was high.

4.3.2.7 Pseudostem length

Significant differences were observed for the trait between midparental values and RIL means. RIL mean for pseudostem length was lower than midparental value. While, high proportion of transgressive segregants lying outside the parental limits was observed. However, the proportion of transgressive segregants with values less than low scoring parent was high.

4.3.2.8 Days to 50% flowering

It was observed that mean of RILs did not differ significantly from low-scoring parent. Mean of RILs was also lower than midparental value. Significantly high proportion of transgressive segregants was observed for the trait. However, the proportion of RILs with values lying outside the low scoring parent was higher than those with values outside the high scoring parent.

4.3.2.9 Plant height

The two parents differed significantly from each other for plant height. The mean value for RILs was higher than the midparental value. Transgressive segregants were observed for the trait, though their proportion was low.

4.3.2.10 Grain yield

The mean value for RILs was lower than midparental value, though high proportion of transgressive segregants was observed for the trait. The proportion of RILs was high for individuals with values lying outside the lower scoring parent P2.

4.3.3 Predicted genetic gain

The predicted genetic gain (%) over mean for each component character of shoot fly resistance was calculated based on standardized selection differential at 5% selection intensity. Further, these estimates were compared with the estimates obtained considering extreme 5% individuals of the mapping population consisting of 252 RILs. The highest and lowest 5% individuals of mapping population with respect to the expression of character were selected and

data were used for the predicting the genetic gain for components of resistance. The estimates of predicted genetic gain (%) over mean obtained for different traits in individual and across the 2environments at 5% selection intensity are presented in Table 12. Means and ranges of different components of resistance to shoot fly in the highest and lowest 5% of the breeding lines (252 RILs) evaluated in individual and across the three environments are given in the Table 13. The results thus obtained are described below for the traits under study.

4.3.3.1 Glossiness

The percentage selection gain over mean predicted based on standardized selection differential was higher in environment E2 (57.01%) followed by E3 (51.36%) and E1 (48.19%). The estimates of predicted gain for high intensity of glossiness (highest 5%) ranged from -44.0 to -50.2% over mean. However, highest gain among the selected individuals was expected in the environment E2. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group was larger in environment E2 (score 1.69) followed by E1 (score 1.85) than in E3 (score 2.03). The deviation of mean of highest group from population mean was much higher than that of the lowest group. The ranges of phenotypic values for high intensity of glossiness in the environments E1, E2 and E3 were 1.4-2.1, 1.1-2.1 and 1.6-2.3, respectively.

4.3.3.2 Seedling vigour

The percentage selection gain over mean predicted based on standardized selection differential was higher in environment E2 at both the seedling stages I and II. In all the environments, the predicted gain was higher for seedling vigour II than seedling vigour I. However, in environment E2, the magnitude of estimate of predicted gain was same at both the stages of seedling growth. The predicted selection gain over mean at stage II in three environments range from -37.64 to -45.21%. The estimates of predicted gain for high seedling vigour at stage II (highest 5%) range from 19.86 - 27.81% over mean. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group for seedling stage II was larger in the environment E2 (score 1.29) followed by E3 (score 1.32) than E2 (score 1.76). The deviation of mean of highest group from population mean for seedling vigour II was larger than that of lowest group in all the environments E1, E2 and E3 were 1.4-1.9, 1.2-1.4 and 1.2-1.4, respectively.

Table 12. Predicted genetic gain (%) over mean for components of resistance to shoot fly and other traits in sorghum RIL mapping population of

mass RTV673 x IS 18551 evaluated under individual and across three screening environments	evaluate	d under ir	ndividual	and acros	s three scr	eening env	vironment	S				
	705 - 5				Highest 5% (in the desired direction)	% (in the	desired di	rection)	Lowest 5%	% (in the u	Lowest 5% (in the undesired direction)	rection)
	111	E,	F3	ELEZES EL	EI	E2	E3	E1E2E3	EI	E2	E3	E1E2E3
-	10 10	1012	51 36	53.65	43 99	-50.18	-43.76	-46.48	33.90	33.05	30.07	33.70
	40.17	10.10	28.90	20.00	-11.85	-27 88	-20.68	-20.39	14.82	44.02	21.68	26.30
	10.01	10.14		1010	-10.86	-27.81	-25.42	-17.17	23.00	38.73	30.43	18.36
Seedling vigour II	57.04	12.04	10	121 32	105.03	1	103.23	107.00	-88.43	-89.28	-89.40	-94.42
Trichome density (lower) 111/177 123.	40./11	61.671	11-071									
Trichome density (upper) 150.31 251.82	150.31	251.82	191.12	216.02	187.25	308.75	191.93	243.93	-76.69	-80.38	-78.16	-91.94
(mm ⁻²)										:		15 JU
	13 63	59.42	6.19	13.93	-11.80	-36.18	-4.08	-12.88	11.97	63.44	3.04	7/.01
	00 -	16.60	1 91	6.17	4.69	-16.69	-0.37	-6.12	4.22	14.17	0.26	5.05
	· · · ·	10.07	0001	17 80	-19 66	-50.90	-12.19	-20.81	16.78	86.16	8.15	23.08
Deadhearts I (%)	1/40	40.10	10.70	14 77	-18 12	47 59	-4 29	-18.24	14.95	55.91	2.77	17.76
Deadhearts II (%)	16.70	42.11	20.0		7 66	0 5 1	7 58	10 31	-6.41	-7.79	-6.94	-8.70
Seedling height I (cm)	18.42	6.41	66.21		00.1			105		919	-4 67	-6.28
Seedling height II (cm)	9.63	6.89	9.66	8.84	2.92	1.28	4.82	· · ·				100
Condline dry weight (0)	0.65	7.87		0.57 [†]	7.20	1.91		8.11	Τ	-1.63		-0.0-
Security ut weight (5)	3.10	62.99		1.04	14.44	68.30		31.58 [†]	-7.95	-32.30		-15.28
Pseudostem Jengui (cui)	0 70	8 71	5.67	7.66	-8.32	-8.87	-3.85	-6.81	11.79	13.09	4.67	10.42
Days to 30% HOWEILING	76.86	43.35	59.53	57.225	-33.84	-24.66	-26.66	-29.60	25.23	25.46	22.70	24.14
	10.76	16 5	10.81	5.079	53.82	31.07	27.2	17.56	-42.44	-35.2	-17.30	-14.56
Grain yield (g/pi) 110.00 100 100 100 100 100 100 100 100	are haced	on angular	transforma	tion	Glossiness ((1-5 scale):	1-high inte	nsity of glos	Glossiness (1-5 scale): 1-high intensity of glossiness, 5-non glossy	glossy		
Estimates for percentage usua = = coloction intensity					Seedling vi	gour (1-5 sc	ale): 1-higl	Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour	ow vigour			
Selection in the second second			an anniante		E1- khorif	F1. khorif. Patancheni						

t estimates based on means averaged over two environments

E1: kharif, Patancheru

E2: rabi, Patancheru

- data not available

E3: early rabi. Dharwar

Frameheru) E3 (cerki, Paiancheru)		STOLU IN	BIX07							3	
Mean and Range Interval to the probability Highest 5% Highest 5% <th>51</th> <th>SUN 202 8</th> <th>The second se</th> <th>batanchemi)</th> <th>E2 (rabi, P</th> <th>atancheru)</th> <th>E3 (early rabi , D</th> <th>(harwar)</th> <th>environments)</th> <th></th> <th></th>	51	SUN 202 8	The second se	batanchemi)	E2 (rabi, P	atancheru)	E3 (early rabi , D	(harwar)	environments)		
ge Highest 2% Lowest 2% Lowest 2% 495 19 ge 1.85 4.75 1.112.1 4.9-5.0 1.6-2.3 4.9-5.0 1.4-2.2 ge 1.42.1 4.7-5.0 1.112.1 4.9-5.0 1.6-2.3 4.9-5.0 1.4-2.2 mean 3.15 3.18 1.35 3.631 3.631 3.631 3.63 3.648 mean 3.15 2.18 1.25-1.4 3.0-3.9 1.7-2.1 3.440 1.5-1.8 ge 1.6-1.9 2.7-3.3 1.2-1.4 2.059 2.059 2.059 2.073 2.340 1.5-1.8 ge 1.41.9 3.1-3.6 2.2-1.4 2.0-3.9 1.7-2.1 2.44.0 1.5-1.8 ge 1.41.9 3.1-3.6 2.035 2.035 2.035 2.032 2.174 2.73 mean 2.3 2.1-3.1 1.229 2.035 2.035 2.072 2.177 ge 1.41.9 3.1-3.4 1.22 9.134 1.795 mean 2.45 <th></th> <th></th> <th></th> <th>207 T</th> <th>Uichest 5%</th> <th>%</th> <th>Highest 5%</th> <th>Lowest 5%</th> <th>Highest 5%</th> <th>Lowest 5%</th> <th></th>				207 T	Uichest 5%	%	Highest 5%	Lowest 5%	Highest 5%	Lowest 5%	
Xs 1.85 4.9-50 1.4-2.2 4.9-50 1.4-2.2 Range 1.4-2.1 3.15 3.631 3.631 3.78 3.648 1.7 Xs 1.78 2.85 1.35 3.631 3.631 3.631 3.631 3.633 3.631 3.648 1.7 Kange 1.6-1.9 2.733 1.2-1.4 3.0-3.9 1.72.11 3.440 1.5-1.8 Range 1.6-1.9 2.733 1.2-1.4 3.0-3.9 1.72.11 3.440 1.5-1.8 Runge 1.76 3.31 1.29 3.07 1.324 2.74 2.355 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.0-35 2.035 2.035 2.02 2.171 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.0-3 2.74 2.74 2.355 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.0-3 2.74 2.74 2.355 Runge 1.2-2 2.3-3 2.033 2.035		ge		1 of	1 69		2.03	4.98	1.9	4.92	
Range $1.4-2.1$ $4.7-5.0$ $1.1-2.1$ 3.631 3.93 3.78 3.78 3.78 3.646 3.648 Rume 1.72 3.13 $12.2-14$ $3.0-39$ $1.72.11$ $3.44.0$ 1.512 3.556 1.72 3.646 1.72 3.648 3.78 3.643 3.648 3.78 3.643 3.648 3.78 3.643 3.648 3.78 3.17 2.355 3.264279 3.264279 3.264279 $3.771-104.78$ 3.64379 3.792 3.911 1.22 9.1322 2.744 2.1322 3.777 3.176 3.777 3.161 3.791 3.791 3.791 3.791 3.791 3.791				4.07	1 2 1 2	10.50	16-23	4.9-5.0	1.4-2.2	4.9-5.0	
RL mean 3.15 3.631 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.031 3.15 3.31 1.2-1.4 3.0-3.9 1.721 3.44.0 1.5-1.8 Runge 1.6-1.9 2.7-3.3 1.2-1.4 2.059 2.059 2.059 2.059 2.059 2.055 2.74 2.355 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.035 2.035 2.035 2.03 2.172 2.173 0 1.4-1.6 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.33.2 2.035 2.035 2.032 2.177 2.352 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.83.3 1.2-1.4 2.73.0 1.4-1.6 Runge 1.64-95 1.2-1.4 2.83.3 1.2-1.4 2.73.0 1.4-1.6 Runge 76.4-95 1.2-1.4 2.83.3 <		Se		4.7-5.0	1.1-2.1	4.7-0.0		3 78	3.648	3.648	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		up		3.15	3.631	3.031	0/.C	2.58	17	3.2	
x_s $1.2-1.4$ $3.0.3.9$ $1.72.1$ $5.44.0$ $1.25.5$ Rume 1.76 3.1 1.29 $3.0.7$ 1.32 $2.36.6$ $1.52.5$ Rume 1.76 3.1 1.29 3.07 1.32 $2.36.6$ $1.52.5$ Rume 1.76 $3.1-3.6$ $1.2-1.4$ $2.83.3$ $1.2-1.4$ $2.14.5$ 2.035 2.022 2.171 $2.17-104.78$ Rume 2.45 2.035 2.035 2.022 2.032 2.022 2.171 Rume 2.45 2.035 2.035 2.035 2.022 2.171 $2.17-104.78$ Rume 7.5 83.11 2.02 2.035 2.022 2.171 2.92 Rume 7.5 $3.2.1$ 2.025 3.01 4.026 4.026 4.314 Rume $3.5.19.5$ $0.0-0.5$ $1.26.525.15$ $0.1-0.36$ $1.74.78$ 82.74 4.026 4.026 4.026				2.88	1.35	3.18	cv.1		1 5-1 8	3 0-3.7	
Autoge 1.35 2.059 2.059 2.74 2.73 2.05 \overline{X}_s 1.76 3.31 1.29 3.07 1.32 2.86 1.52 \overline{X}_s 1.76 3.1-3.6 1.2-1.4 2.8-3.8 1.2-1.4 2.8-3.8 1.2-1.4 2.7-3.0 1.4-1.6 Runge 1.4 3.1-3.6 1.2-1.4 2.8-3.8 1.2-1.4 2.7-3.0 1.4-1.6 Runge 1.4 2.45 2.035 2.035 2.02 2.7-3.0 1.4-1.6 Runge 7.45 83.1 2.02 1.32.6 1.32 91.322 Runge 7.45 16.05 0.32 16.880 2.02 2.02 2.17104.78 Runge 13.5-19.5 0.0-0.5 15.62.25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 </td <td></td> <td></td> <td></td> <td>2.7-3.3</td> <td>1.2-1.4</td> <td>3.0-3.9</td> <td>1.7-2.1</td> <td>5.4-4.0</td> <td>0.1-C.1</td> <td>2355</td> <td></td>				2.7-3.3	1.2-1.4	3.0-3.9	1.7-2.1	5.4-4.0	0.1-C.1	2355	
\bar{X}_s 1.1.6 3.1 1.29 3.07 1.32 2.86 1.1.6 \bar{X}_s 1.76 3.1.36 1.2-1.4 2.8-3.8 1.2-1.4 2.8-3.6 1.4-1.6 Runge 1.4-1.9 3.1-3.6 1.2-1.4 2.8-3.8 1.2-1.4 2.7-3.0 1.4-1.6 \bar{X}_s 83.1 2.02 2.95 85.34 1.22 91.322 2.17 \bar{X}_s 83.1 2.02 1.9 3-1.46 2.8-3.8 1.22 91.322 Runge 76.495 1.5-2.7 99.3-124.28 1.6-2.94 79.73-97.9 0.26-2.02 82.77-104.78 Runge 76.495 1.5-2.7 99.3-124.28 1.6-2.94 79.73-97.9 0.25-2.02 3.771-104.78 Runge 13.5-19.5 0.0 0.32 1.6 0.32 1.22 91.322 Runge 13.5-19.5 0.0 0.32 2.038 0.15 1.25 Runge 13.5-19.5 0.0 0.32 1.22 91.32 91				23	2.059	2.059	2.74	2.74	CCC.7	2.88	
X_s 1.2 -1.4 $2.8-3.8$ $1.2-1.4$ $2.7-3.0$ $1.4-1.6$ Runge $1.4-1.9$ $3.1-3.6$ $1.2-1.4$ $2.8-3.8$ $1.2-1.4$ $2.7-3.0$ $1.4-1.6$ RLL mean 2.45 2.035 2.035 2.02 $2.17-104.78$ Runge $76.4-95$ $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-97.9$ $0.26-2.02$ $82.77-104.78$ Range $76.4-95$ $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-97.9$ 0.152 91.322 Runge $76.4-95$ $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-97.9$ $0.26-2.02$ $82.77-104.78$ Runge $76.4-95$ $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-87.43$ $82.77-104.78$ Runge $13.5-19.5$ $0.0-0.5$ 16.82 0.32 16.82 0.32 16.82 0.32 16.82 $0.92.94.76$ 49.26 43.14 Runge $13.5-19.5$ $0.0-0.5$ $12.65-25.15$ $0.1-0.36$ 179.4		KIL mean		331	1.29	3.07	1.32	08.7		<i><i><i>c c c c c c c c c c</i></i></i>	
Range $1.4-1.9$ $5.1-5.0$ $1.21.4$ 2.45 2.035 2.035 2.02 2.17 2.17 RLL mean 2.45 2.035 2.035 2.035 2.035 2.022 2.171 $\overline{\chi}_s$ 83.1 2.02 $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-97.9$ $0.26-2.02$ $82.77-104.78$ Range $76.4-95$ $1.5-2.7$ $99.3-124.28$ $1.6-2.94$ $79.73-97.9$ $0.26-2.02$ $82.77-104.78$ Y $\overline{\chi}_s$ 16.05 0.32 16.82 0.28 20.98 0.15 17.95 Y $\overline{\chi}_s$ 16.05 0.32 16.82 0.28 20.98 0.15 17.95 Runge $13.5-19.5$ $0.0-0.5$ $12.65-25.15$ $0.1-0.36$ $17.84-24.35$ $0.0-0.41$ $15.93-24.51$ Range $13.5-19.5$ $0.0-0.5$ $12.65-25.15$ $0.1-0.36$ $17.84-24.35$ $0.0-0.41$ $15.93-24.51$ Range $13.5-19.5$	-				4101	7 8-3 8	1.2-1.4	2.7-3.0	1.4-1.6	7.6-1.7	
RIL mean 2.45 2.003 2.003 2.003 85.34 1.22 91.322 $\overline{\chi}_s$ 83.1 2.02 108.80 2.50 85.34 1.22 91.322 Range 76.495 1.5-2.7 99.3-124.28 1.6-2.94 79.73-97.9 0.26-2.02 82.77-104.78 Range 76.495 1.5-2.7 99.3-124.28 1.6-2.94 79.73-97.9 0.26-2.02 82.77-104.78 N $\overline{\chi}_s$ 16.05 0.32 16.82 0.28 0.15 17.95 Range 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Rull.mean				3.1-3.0	1.2-1.4	2.0.25	2 02	2.02	2.177	2.177	
$\bar{\chi}_s$ 83.1 2.02 108.80 2.50 85.34 1.22 91.322 Range 76.4-95 1.5-2.7 99.3-124.28 16-2.94 79.73-97.9 0.26-2.02 82.77-104.78 Range 76.4-95 1.5-2.7 99.3-124.28 16-2.94 79.73-97.9 0.26-2.02 82.77-104.78 Rull mean 39.2 39.2 50.1 50.1 40.26 40.26 43.14 Rull mean 39.2 39.2 16.82 0.28 20.98 0.15 17.95 Range 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Range 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Rull mean 3 3 36.37 74.78 87.14 4184 Rull mean 51.775 81.78 36.37 74.78 87.14 4184 Rull mean 51.75 81.78 36.37 74.78 87.14<		RIL mean		2.45	C 60.7	CC0.7	ļ			0 637	
χ_s 83.1 χ_s 83.1 χ_s 83.1 χ_s 83.1 χ_s 83.1 χ_s 15-2.7 99.3-124.28 16-2.94 79.73-97.9 0.26-2.02 82.77-104.78 83.14 RUL mean 39.2 39.2 50.1 50.1 50.1 40.26 43.14 43.14 RUL mean 39.2 39.2 16.82 0.28 20.98 0.15 17.95 RUL mean 39.2 0.00.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Range 13.5-19.5 0.00.0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Rul mean 3 3 36.37 74.78 87.14 41.84 Rul mean 3 36.37 74.78 87.14 41.84 38.56-42.79 Rul mean 51.775 81.78 36.37 74.78 87.14 41.84 Runge 67.15 81.78 36.37 74.78 81.74 <	>	ł		, 0, C	108.80	2.50	85.34	1.22	775.16	70.0	
Range 76.4-95 1.5-2.7 99.3-124.28 1.6-2.94 $79.79-97.9$ 0.20-20-20 43.14 RLL mean 39.2 39.2 50.1 50.1 50.1 40.26 43.14 \overline{X}_s 16.05 0.32 16.82 0.28 20.98 0.15 17.95 \overline{X}_s 16.05 0.32 16.82 0.28 20.98 0.15 17.95 Runge 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19.5 0.10-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 Runge 13.5-19 74.78 81.74 81.87 81.74 41.84 Runge 61.15 61.71-9.84 <t< td=""><td>_</td><td>X_{s}</td><td></td><td>70.7</td><td></td><td></td><td></td><td>0.26-2.02</td><td>82,77-104.78</td><td>\$ 0.0-0.95</td><td></td></t<>	_	X_{s}		70.7				0.26-2.02	82,77-104.78	\$ 0.0-0.95	
RL mean 39.2 50.1 50.1 40.20 70.20 <th< td=""><td></td><td>Ванае</td><td>76.4-95</td><td>1.5-2.7</td><td>99.3-124.28</td><td>1.6-2.94</td><td>19.16-61.91</td><td>40.26</td><td>43.14</td><td>43.14</td><td></td></th<>		Ванае	76.4-95	1.5-2.7	99.3-124.28	1.6-2.94	19.16-61.91	40.26	43.14	43.14	
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		nnom IId		39.2	50.1	50.1	40.20	07.01			
$\bar{\chi}_s$ 16.05 0.32 10.02 0.32 10.02 0.32 10.02 0.32 10.02 0.0-0.41 15.93-24.51 0.0-0.41 18.49 0.0-0.41 18.49 0.0-0.41 18.49 0.0-0.41 18.49 0.0-0.41 18.49 0.0-0.41 18.49 0.0-0.41 18.40 0.0-0.41 18.40 0.0-0.41 15.93-24.51 0.0-0.41 15.93-24.51 0.0-0.41 15.93-24.21 0.0-0.41 18.93 0.0-0.41 18.93<	;	IND MEMO	•		60 71	0.78	20.98	0.15	17.95	0.01	
Range 13.5-19.5 0.0-0.5 12.65-25.15 0.1-0.36 17.84-24.35 0.0-0.41 15.93-24.51 RIL mean 3 3.701 5.17 3.701 6.17 4.919 RIL mean 3 3.701 5.17 8.75 36.37 74.78 87.14 41.84 RIL mean 3 56.23 8.75 36.37 74.78 87.14 41.84 Range 41.29-44.24 6.17-69.77 6.71-9.84 31.09-45.66 70.03-76.24 86.37-88.4 38.56-42.79 Range 41.29-44.24 6.417-69.77 6.71-9.84 31.09-45.66 70.03-76.24 86.37-88.4 38.56-42.79 Runge 51.775 81.78 81.87 81.87 81.87 81.87 81.87 Runge 67.15 81.77 80.88.914 89.96-91.81 94.03-94.32 66.1-70.96 \tilde{X}_s 63.11-68.67 80.23-84.27 80.88.914 89.96-91.81 94.03-94.32 66.1-70.96 Runge 63.11-68.67 80.23-84.27 80.88.914 89.96-91.81 94.03-94.32 66.1-70.96	>	ہْدا	16.05	0.32	10.01	07.0					
Range 13.5-19.5 0.0-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 12.05-2.1.0 0.1-0.5 13.01 6.17 6.17 4.919 RIL mean 3 66.23 8.75 36.37 74.78 87.14 41.84 Range 41.29-44.24 64.17-69.77 6.71-9.84 31.0945.66 70.03-76.24 86.37-88.4 38.56-42.79 Range 41.29-44.24 64.17-69.77 6.71-9.84 31.0945.66 70.03-76.24 86.37-88.4 38.56-42.79 Run facm 51.775 81.78 81.87 81.87 81.87 51.72 Runge 67.15 81.78 50.88 82.76 91.32 94.14 69.19 Runge 63.71-68.67 80.23-84.27 44.59-53.41 80.8-89.14 89.96-91.81 94.03-94.32 66.1-70.96 Runge 63.71-68.67 80.23-84.27 44.59-55.34 80.8-89.14 89.96-91.		5			10 75 75 15	0 1-0 36	17 84-24.35	0.0-0.41	15.93-24.51	0.0-0.0	
RIL mean 3 3.701 5.701 5.701 5.701 6.17 87.14 41.84 \overline{X}_s 42.93 66.23 8.75 36.37 74.78 87.14 41.84 \overline{X}_s 42.93 66.23 8.75 36.37 74.78 87.14 41.84 Range 41.29-44.24 64.17-69.77 6.71-9.84 31.09-45.66 70.03-76.24 86.37-88.4 38.56-42.79 Rut mean 51.775 18.78 18.78 81.87 81.87 51.72 Rut 67.15 81.78 50.88 82.76 91.32 94.14 69.19 \overline{X}_s 67.15 81.78 50.88 82.76 91.32 94.03-94.32 66.1-70.96 \overline{Rux} 67.15 81.78 50.88 82.76 91.32 94.14 69.19 \overline{Rux} 67.15 81.78 50.68 82.96-91.81 94.94-93 73.55 \overline{Rux} 67.15 81.78 53.93 59.63 83.51 34.57 \overline{Rux} 67.15 57.64 97.96 53.96 <td></td> <td>Range</td> <td>13.5-19.5</td> <td>0.0-0.5</td> <td>c1.cz-c0.71</td> <td>0.1-0.00</td> <td>6 17</td> <td>617</td> <td>4.919</td> <td>4.919</td> <td></td>		Range	13.5-19.5	0.0-0.5	c1.cz-c0.71	0.1-0.00	6 17	617	4.919	4.919	
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$		nnom 110	"	3	3.701	5.701	11.0	87 14	4184	63.78	
X_s $41.29-44.24$ $64.17-69.77$ $6.71-9.84$ $31.09-45.66$ $70.03-76.24$ $86.57-88.4$ $58.50-82.4$ Range 51.775 18.78 18.78 81.87 51.72 51.72 Rut mean 51.775 51.775 18.78 50.38 82.76 91.32 94.14 69.19 Rut mean 51.775 81.78 50.88 82.76 91.32 94.14 69.19 Runge $63.11-68.67$ $80.23-84.27$ $44.59-53.41$ $80.8-89.14$ $89.96-91.81$ $94.03-94.32$ $66.1-70.96$ Range $63.11-68.67$ $80.23-84.27$ $44.59-53.41$ $80.8-89.14$ $89.96-91.81$ $94.03-94.32$ $66.1-70.96$ Rut mean 75.595 68.12 68.12 68.12 58.33 59.63 83.51 37.57 Rut mean 75.595 68.12 63.12 $53.96-62.66$ $82.44-86.41$ $34.47-40.09$ x^3 81.117 $49.2-62.04$ $53.06-62.66$ $82.46-80.13$:		47 93	66.23	8.75	36.37	/4./0		10 66 47 70	61 85-67 67	
Range $41.2944.24$ 0.175 18.78 18.78 81.87 51.72 RulL mean 51.775 51.775 51.878 18.78 81.87 51.72 51.72 RulL mean 51.775 51.775 51.878 50.88 82.76 91.32 94.14 69.19 \overline{X}_s 67.15 81.78 50.88 82.76 91.32 94.14 69.19 RulL mean 75.595 68.12 68.12 68.12 59.63 83.51 37.57 RulL mean 75.595 68.12 68.12 59.63 83.51 37.57 \overline{X}_s 41.11 72.64 9.79 $53.96.62.66$ $82.44.86.41$ $34.47.40.09$ Range $34.41-44.39$ $71.06-75.24$ $5.61.8$ 73.94 52.76 Rull mean 51.47 51.47 26.18 26.18 73.94 52.76	•			LT 97-L1 44		31.09-45.66	70.03-76.24	80.31-88.4	20.30-44.02		
RtL mean 51.775 50.19 50.19 50.13 50.13 50.13 50.14 69.19 50.19 50.13 50.13 50.13 50.13 50.13 50.13 50.13 50.13 50.13 50.53 56.170.96 78.65 73.94 73.75 66.12 68.12 68.12 68.12 68.12 68.12 53.93 59.63 83.51 37.57 73.57 Rull mean 75.595 75.24 5.54-11.7 49.2-62.04 53.06-62.66 82.44-86.41 34.47-40.09 78.06 73.94 52.76 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00 76.00		Range	41.29-44.24	300-11-00		18 78	81.87	81.87	51.72	C0.8/	
$ \overline{X}_{s} \qquad 67.15 \qquad 81.78 \qquad 50.00 \qquad 51.76 \qquad 81.78 \qquad 50.00 \qquad 51.70 \qquad 51.75 \qquad 81.78 \qquad 51.75 \qquad 51.45 \qquad 51.45 \qquad 51.47 \qquad $		RIL mean		c//.1c	10.70	82.76	91.32	94.14	69.19	86.45	
Range $63.71-68.67$ $80.23-84.27$ $44.59-53.41$ $80.8-89.44$ $63.70-71.01$ 78.65 Rull mean 75.595 68.12 68.12 92.97 78.65 Rull mean 75.595 68.12 68.12 92.97 92.97 78.65 Rull 75.595 68.12 68.12 92.93 92.97 78.65 Rull 75.595 68.12 68.12 68.12 92.97 37.57 Rull 75.96 9.79 53.93 59.65 $82.44-86.41$ $34.47-40.09$ Range $34.41-44.39$ $71.06-75.24$ $5.54-11.7$ $492.26.20.4$ $53.06-62.66$ $82.44-86.41$ $34.47-40.09$ Rull mean 51.47 51.47 51.47 52.76	~			81.78		07.70	00 06 01 81	94 03-94 32	66.1-70.96	85.54-88.2	
Rull mean 75.595 75.595 68.12 68.12 92.97 92.97 75.91 76.09 \overline{X}_{s} 41.11 72.64 9.79 53.93 59.63 83.51 37.57 \overline{X}_{s} 41.11 72.64 9.79 53.93 59.63 83.51 37.57 Range 34.41-44.39 71.06-75.24 5.54-11.7 49.2-62.04 53.06-62.66 82.44-86.41 34.47-40.09 Range 34.41-44.39 71.06-75.24 5.54-11.7 49.2-62.04 53.06-62.66 82.44-86.41 34.47-40.09 Rull mean 51.47 51.47 26.18 73.94 73.94 52.76 Rull mean 51.47 51.47 26.18 73.94 73.94 6.004 75.76	<u> </u>					80.8-89.14	10.17-04.48		10 65	78.65	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		Vange				68.12	92.97	16.76	(0.0/	0.07	
X _s 41.11 Range 34.41-44.39 71.06-75.24 5.54-11.7 49.2-62.04 53.06-62.66 82.44-86.41 34.47-40.09 Run 81.47 51.47 26.18 26.18 73.94 73.94 52.76 RIL mean 51.47 51.47 56.18 26.18 73.94 73.94 6.0004 1		KIL mean		77 64	6 <u>7</u> 6	53.93	59.63	83.51	10.15		9
Range 34.41-44.59 /1.00-73.24 3.3411.1 25.18 73.94 73.94 52.76 RIL mean 51.47 51.47 26.18 26.18 73.94 73.94 52.76	્ર	_	41.11			40 2-62 04	53.06-62.66	82.44-86.41	34,47-40.09	67.51-72.63	3
51.47 51.47 20.16 20.1			34.41-44.59			26.18	73.94	73.94	52.76		1
		RII, mea		51.47	20.10	50.10			(contd)		

(Contd)								E1E2E3 (across three	three	1
	has need	E1 (kharif, Patancheru)	Patancheru)	E2 (rabi, F	E2 (rabi, Patancheru)	E3 (early rabi, Dharwar)	Dharwar)	environments)		
Trait	Range	Highest 5%	Lowest 5%	Highest 5%	Lowest 5%	Highest 5%	Lowest 5%	Highest 5%	Lowest 5%	
Deadhearts II (%)	<u>Χ</u> ,	52.58	84.85	19.84	74.97	77.56	90.49	49.87	83.59	
	Range	55.28	82.65-87.33	14.73-23.96	71.96-80.88	70.92-79.16	89.61-92.31	47.4-51.68	81.63-85.54	_
	up		62.655	45.19	45.19	85.42	85.42	66.96	66.96	
Seedling height [(cm)			22.09	26.86	20.56	19.15	14.71	24.99	19.03	
	Ringe	27 68-29 55	21.05-22.54	26.27-28.69	19.47-21.03	18.63-19.94	13.51-15.13	24.22-25.95	17.87-19.45	
	RIL mean	25.83	25.83	23.57	23.57	16.83	16.83	21.76	21.76	
Conding height II (cr.) \overline{Y}_{-}	12	46.75	40.60	48.86	39.14	66.16	41.17	43.71	35.50	
(* 14) 11 11:STATI SITURNO	Range	47.99	39.45-41.32	47.44-50.96	34.95-40.22	33.43-35.56	26.55-28.37	42.6-45.75	34.07-36.42	
	RIL mean		44.38	43.57	43.57	30.94	30.94	39.37	39.37	
Seedling dry weight (g) Xe	X	3.67	2.65	4.81	4.13	,		4.39	3.32	
		0	7 6 7 7	4 73-4 96	4 03-4 19	,	,	4.24-4.55	3.19-3.37	
•••	DII maan	0.0-0.0 3 4	2.5-2.1 3 d	4 445	4.445			3.775	3.775	
Desidoctem length				00 8				6 77	2.50	
(cm)	X_{S}	3.26	2.30	8 90	7.90		•	17.0		
(cm)	Range	3.0-3.2	2.1-2.3	8.02-10.03	2.57-3.1		ı	5.70-7.21	2.26-2.66	
	RIL mean		3.15	4.826	4.826	1		3.731	3.731	
Days to 50% flowering $\overline{X_S}$	$g \overline{X}_{S}$	10.69	86.55	66.19	83.13	74.17	83.19	69.98	84.66	
	Range	67.03-70.12	85.23-91.79	65.17-66.76	80.44-88.15	72.85-74.97	82.68-84.2	68.5-70.5	82.8-90.9	
	RII. mean		76.27	73.03	73.03	78.25	78.25	75.78	75.78	
Plant height (cm)	X,		291.75	115.52	199.83	124.51	229.46	129.00	239.38	
	Range	136.4-160.4	283.1-311.1	101.3-122.5	190.8-217.4	104.9-131.7	222.7-248.1	110.7-135.1	232-253.2	
	RIL mean	231.2	2312	157	157	181.20	181.20	189.80	189.80	
Grain vield (g/pl)	Xs	25 48	7.17	16.14	6.33	29.20	13.12	21.86	9.93	9
	Range	23.74-30.43	5.9-8.06	15.68-17.03	4.92-7.53	25.65-38.0	10.66-14.27	20.6-23.6	8.2-10.8	4
	RIL mean 15.24	15.24	15.24	11.55	11 55	19.37	19.37	15.34	15.34	1
$\overline{X}_{\mathrm{S}^+}$ mean of selected individuals at 3% selection intensity	d individuals at	t 5% selection inte	cnsity							

4.3.3.3 Trichome density on lower leaf surface

The percentage selection gain over mean predicted based on standardized selection differential was higher in the environment E2 (123.39%) followed by E3 (120.47%) than in E1 (117.39%). The estimates of predicted gain for high trichome density (highest 5%) ranged from - 103.23 to -110.10% over mean. However, highest gain for increased density of trichomes among the selected individuals was obtained in the environment E2. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group (no./mm²) was larger in the environment E2 (108.80) than in E3 (85.34) and in E1 (83.1). The deviation of mean of highest group from population mean was much higher than that of lowest group. The ranges of phenotypic values for high trichome density (no./mm²) on lower leaf surface (in the highest group) were 76.4 - 95.0 in E1, 99.3 - 124.28 in E2 and 79.73 - 97.9 in E3.

4.3.3.4 Trichome density on upper leaf surface

The percentage selection gain over mean predicted based on standardized selection differential was much higher in environment E2 (251.82) than E3 (191.12) than E1 (150.31). The estimates of predicted gain for high trichome density (highest 5%) ranged from -187.25 to - 308.75% over mean. However, highest gain for increased density of trichomes among the selected individuals was obtained in the environment E2. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group (no./mm²) was larger in environment E3 (20.98) than in E2 (16.82) and E1 (16.05). The deviation of mean of highest group from population mean was much higher than that of lowest group. The ranges of phenotypic values for high trichome density (no./mm²) on upper leaf surface (in the highest group) were 13.5 - 19.5 in E1, 12.65 - 25.15 in E2 and 17.84 - 24.35 in E3.

4.3.3.5 Oviposition

The percentage selection gain over mean was higher for oviposition I compared to oviposition II in all the environments. It was observed that the estimates were higher in environment E2 than in other two environments. The percentage predicted gain over mean based on standardized selection differential for oviposition I in three environments range from 13.63 to 59.42. The estimates of percentage predicted gain over mean for low oviposition (%) at stage I (highest 5% group for oviposition non-preference) range from -11.80 to -36.18%. Significant differences were observed between the means of the lowest and the highest groups in each

environment. The mean of highest group for oviposition I (%) was much lower in environment E2 (8.75%) than in E1 (42.93%) and E3 (74.78%). The deviation of mean of highest group from population mean for ovipositon I (%) was lower than that of lowest group in E1 and E2. The ranges of phenotypic values for low oviposition (%) at stage I (in the highest group) were 41.29 - 44.24% in E1, 6.71 - 9.84% in E2 and 70.03 - 76.24% in E3.

4.3.3.6 Deadhearts

The percentage selection gain predicted over mean was higher for deadhearts I compared to deadhearts II in all the environments. It was observed that the estimates were higher in the environment E2 than in E1 and E3. The percentage predicted gain over mean based on standardized selection differential for deadhearts I in three environments ranged from 10.98 to 61.54. The estimates of predicted gain for low deadhearts damage at stage I (highest 5% for resistance to insect damage) range from -12.19 to -50.90% over mean. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group for deadhearts I (%) was much lower in the environment E2 (9.79) than in E1 (41.11) and E3 (59.63). The deviation of mean of highest group from population mean for deadhearts I (%) was lower than that of lowest group in E1 and E2. The ranges of phenotypic values for low deadhearts at stage I (in the highest group) were 34.41 - 44.39% in E1, 5.54 - 11.70% in E2 and 53.06-62.66% in E3.

4.3.3.7 Seedling height

The percentage predicted genetic gain over mean was low for seedling height compared to other traits. However, the estimates were higher for seedling height I compared to seedling height II in all the environments. It was observed that the estimates based on standardized selection differential for seedling height I were higher in environment E1 (18.42) followed by E3 (12.95) than E2 (6.41). The percentage predicted gain (over mean) for high seedling height at stage I (highest 5%) ranged from 7.58 to 8.51. Significant differences were observed between the means of the lowest and the highest groups in each environment. The mean of highest group for seedling height I was higher in environment E1 (28.19 cm) than E2 (26.86 cm) than E3 (19.15 cm). The deviation of mean of highest group from population mean for seedling height I was same as that of lowest group in E1 and E2. The ranges of phenotypic values for high seedling height (in the highest group) at stage I were 27.68 - 29.55 cm in E1, 26.27-28.69 cm in E2 and 18.63-19.94 cm in E3.

4.3.3.8 Seedling dry weight

The selection gain over mean predicted based on standardized selection differential was higher in environment E2 (7.87%) than E1 (0.65%). The estimates of predicted gain for high seedling dry weight (highest 5%) range from 1.91 to 7.20% over mean. However, highest predicted gain for increased seedling dry weight among the selected individuals (highest 5%) was observed in the environment E2. The mean of highest group was larger in environment E2 (4.81 g/pl) than in E1 (3.67 g/pl). The ranges of phenotypic values for high seedling dry weight (in the highest group) were 3.6 - 3.8 g/pl in E1 and 4.73 - 4.96 g/pl in E2.

4.3.3.9 Pseudostem length

The selection gain over mean predicted based on standardized selection differential was significantly higher in environment E2 (62.99%) than in E1 (3.10%). The estimates of predicted gain over mean for high pseudostem length (highest 5%) ranged from 14.44% to 68.30%. Highest predicted gain for increased pseudostem length among the selected individuals was observed in the environment E2. The mean of highest group of RILs was significantly higher in the environment E2 (8.90 cm) than in E1 (3.26 cm). The ranges of phenotypic values for high pseudostem length (in the highest group) were 3.0-3.2 cm in E1 and 8.02-10.03 cm in E2.

4.3.3.10 Days to 50% flowering

The selection gain over mean predicted based on standardized selection differential was significantly higher in environment E1 (9.79%) followed by E2 (8.71%) than in E3 (5.67%). The estimates of predicted gain for early maturity (highest 5%) ranged from -3.86 to -8.87% over mean. However, highest predicted gain for early maturity among the selected individuals was observed in the environment E2. The mean of highest group was high in environment E2 (66 days) followed by that for E1 (69 days) and E3 (74 days). The ranges of phenotypic values for early maturity (in the highest group) were 67 - 70 days, 65 - 67 days in E2 and 73 - 75 days in E3.

4.3.3.11 Plant height

The selection gain over mean predicted based on standardized selection differential was ^{significantly} higher in the environment E1 (76.86%) than in E3 (59.53%) and E2 (43.35%). The ^{estimates} of predicted gain for reduced height (highest 5%) range from -24.66 to -33.84% over mean. However, highest predicted gain for reduced plant height among the selected individuals was observed in the environment E1. The mean of highest group was smaller in environment E2

(115.2 cm) than in E3 (124.51 cm) and E1 (150.01 cm). The ranges of phenotypic values for reduced plant height (in the highest group) were 136.4 - 160.4 cm in E1, 101.3 - 122.5 cm in E2 and 104.9 - 131.7 cm in E3.

4.3.3.12 Grain yield per plant

The selection gain over mean predicted based on standardized selection differential was higher in environment E1 (10.76%) and E3 (10.81%) than in E2 (5.91%). The estimates of predicted gain for high yield (highest 5%) ranged from 27.2 to 53.82% over mean. However, highest predicted gain for high grain yield among the selected individuals (highest 5%) was observed in the environment E1. The mean of highest group for grain yield per plant was high in environment E3 (29.2 g) followed by E1 (25.48 g) and E2 (16.14 g). The ranges of phenotypic values for high grain yield per plant (in the highest group) were 23.74 - 30.43 g in E1, 15.68 - 17.03 g in E2 and 25.65 - 38.0 g in E3.

4.3.4 Correlated genetic gain

Genetic correlations were assigned a positive sign if direct and correlated responses were in the same direction and they were assigned a negative sign if responses were in the opposite direction. The results obtained are presented in Table 14.

It was observed that correlated response for glossiness with oviposition (%) and deadhearts (%) was negative. The correlated genetic gain was higher for glossiness with trichome density followed by deadhearts and oviposition in the initial stage of seedling growth. Correlated genetic gain for the glossiness was lower with traits seedling height (at both I and II) followed by seedling vigour (at both the stages). However, correlated genetic gain for glossiness with seedling height was higher at later stage of seedling growth (seedling height II). Among the adult traits correlated genetic gain for glossiness with plant height was high and low with days to 50% flowering.

Trichome density recorded with negative genetic gain with deadhearts (%) and all other components except with glossiness. The correlated genetic gain of seeding vigour with deadhearts (%) was negative in environments E1 and E3. Seedling height and pseudostem length recorded negative selection gain with oviposition II, deadhearts I and deadhearts II. However, correlated genetic gain of plant height at maturity with shoot fly damage was negative in E3 and positive in E1.

rait	Env	g	vigl	vigII	Trait Env gl vigl vigII trlw tn	trup	*ovil	*ovill	thb*	II4b*	shtl	shtII	drywt	pslg	daf
130	Ξ	1.82													
	E3	2.07													
	E3	1.94													
vigl	EI	-0.05	0.51												
	E3	-0.17	0.95												
	E	0.56	0.79												
vigIl	EI	0.83	0.33	0.76											
	E2	-0.06	0.75	0.92											
	E3	1.17	0.53	0.83											
trlw	EI	-0.17	0.20	-0.10	47.26										
	E2	-0.15	0.23	0.24	62.02										
	E	-0.25	0.14	-0.05	48.50										
trup	EI	-0.11	0.17	-0.10	30.24	9.28									
•	E2	-0.24	0.15	0.03	33.30	9.32									
	. E 3	-0.19	0.21	-0.01	34.87	I·1.80									
*ovil	EI	0.71	-0.15	0.36	-13.67	-3.11	7.43								
	E2	0.66	-0.24	-0.22	-23.34	4.54	11.16								
	E3	0.94	-0.05	0.34	-10.48	-2.42	5.07								
*ovill	EI	0.53	0.01	0.43	-16.50	-4.30	5.69	5.98							
	E2	0.41	-0.02	0.03	-17.07	-3.85	9.24	11.37							
	E	0.91	0.13	0.39	-6.93	-0.98	3.72	1.78							
ttp*	EI	0.91	-0.13	0.44	-16.28	-	7.84	6.89	10.11						
	E2	0.78	-0.11	-0.03	-25.10	4.51	12.08	11.46	16.11						
	E3	1.20	0.07	0.58	-11.90	-2.79	5.22	3.38	8.12						
*dhil	EI	0.96	-0.11	0.51	-18.59	-4.07	8.11	7.66	10.22	11.73					
	E2		0.00	0.12	-25.65	-4.96	11.48	12.07	16.12	19.33					
	53		0.07	0.48	-15.53	-3.51	5.05	2.51	6.77	4.97					

to shoot fly, and other traits in sorghum RIL mapping population of cross BTx623 j

(contd)															
Trait	Env	5	lgiv	llgiv	trlw	trup	*ovil	*ovill	[प[₽	11db*	shtl	shtll	drywt	bslg	daf
shtl	E	-0.39	-0.40	-0.47	-7.01	-1.15	1.37	-1.21	-0.66	-1.14	3.10				
	E2	-0.21	-0.50	-0.66	4.19	0.28	0.55	-3.16	-3.01	-5.67	3.00				
	B	-0.89	-0.43	-0.63	0.03	0.04	-2.10	-1.79	4.68	-3.51	2.18				
shtll	EI	-0.17	-0.32	-0.40	-2.66	0.30	1.45	-1.71	-0.66	-0.93	2.68	2.98			
	E2	-0.20	-0.39	-0.56	-5.20	0.25	-0.73	4.26	-3.67	-7.41	2.81	4.62			
	E	-0.77	-0.42	-0.60	5.01	1.22	-2.07	-2.01	-3.65	-2.84	1.87.	2.99			
drvwt	El	0.12	-0.27	-0.13	-6.43	-1.34	4.64	0.42	2.57	2.74	1.60	1.68	0.51		
	E	0.14	-0.04	-0.32	-10.33	-0.77	2.69	-1.16	0.13	-2.45	1.28	1.66	0.35		·
nelo	El	-0.18	-0.24	-0.34	-2.00	-0.48	2.04	-1.06	-0.15	-0.15	2.23	2.43	0.46	09.0	
0	E3	-0.33	-0.32	-0.47	-7.60	-0.84	-0.66	-3.86	-4.33	-6.70	2.09	3.89	0.98	3.04	
daf	E	0.18	0.09	0.23	-12.86	-0.20	1.14	1.71	1.61	2.83	0.15	0.18	-0.01	0.08	9.16
1	E E	034	0.0	0.15	-2.75	0.48	3.26	4.63	5.65	7.41	-0.88	-0.93	-0.14	-1.44	8.48
	E E	0 38	0.14	0.21	2.70	2.22	-0.58	-0.19	0.65	-0.76	-0.03	-0.52	,		4.88
nlht	Ē	-0.24	-0.26	-0.24	-9.08	-1.37	1.23	0.36	0.50	0.68	1.86	2.79	0.24	21.31	3.59
hund	E	-0.15	-0.27	-0.21	-12.51	-0.86	2.32	1.77	2.08	1.75	0.83	3.12	0.13	1.01	3.73
	E E	-0.35	-0.31	-0.29	-8.55	-1.61	-0.77	-0.14	-0.48	-0.06	1.00	1.23	,	,	5.46
vlđ	Ξ	0.19	0.17	0.28	11.20	2.23	0.18	0.39	0.44	0.36	-1.56	-2.27	-0.25	-3.79	-2.10
	E2	-0.29	0.10	-0.02	4.28	2.07	-2.27	-2.11	-2.41	-3.57	0.24	0.39	0.14	0.36	-0.90
	E	0.06	0.08	0.19	-7.42	-0.20	1.68	1.55	1.86	1.67	-0.09	0.21		,	2.21
	e .														

El: kharif, Patancheru. E2: rabi, Patancheru. E3: early rabi, Dharwar. *Estimates for percentage data are based on angular transformation.

4.4 G x E interaction

To test the consistency and similarity in rankings between environments for particular trait, Spearman rank correlation coefficients were estimated for all possible paired combinations among three environments, viz., E1 and E2; E2 and E3; E1and E3. The results obtained are presented in Table 15. Variances due to $G \times E$ interactions for the traits are given in Table 6.

4.4.1 Glossiness

Highly significant rank correlation coefficients were observed for all the paired combinations of environments. The magnitudes of correlation coefficients for glossiness were high $(\mathbf{r}(s) = 0.89 \text{ to } 0.90)$ and consistent among all paired combinations of environments. However, significant variances due G x E interaction were observed for the trait, the magnitude of these variances was low across the environments. Based on the rankings in the order of high to low intensity of glossiness at 5% selection intensity, it was observed that 4 to 5 lines were found in common between two environment combinations. Three lines, viz., RIL 111, 114 and 176 were found in common among the three environments (Table 16a). And the RILs, 12, 119, 252, 90 and 166 were found exclusively in common between the two environments. These lines also showed high intensity in the highest group at 5% selection intensity of glossiness, none of the lines showed dead-heart damage less than the resistant parent.

4.4.2 Seedling vigour

The rank correlation coefficients were significant for all the paired combinations of environments except for seedling vigour II between E2 and E3. The magnitudes of correlation coefficients were low (seedling vigour II) to moderate (seedling vigour I) for all paired combinations of environments. This indicates lack of consistency of magnitudes of these estimates observed among the pairs of environments. And variances due to $G \times E$ interaction were highly significant for both seedling vigour I and seedling vigour II across the environments.

4.4.3 Trichome density on lower leaf surface

Highly significant rank correlation coefficients for this trait were observed for all the paired combinations of environments. The magnitudes of correlation coefficients for trichome density were significantly high (r(s) = 0.85 to 0.90) for all paired combinations of environments. However, the magnitude of these estimates was consistent between two pairs of environments, viz., E1 and E2; E1 and E3 but reduced for the pair E2 and E3. While, significant variances due

veen screening	Trichome density (lower) Trichome density $(1000000000000000000000000000000000000$		EI EZ	+ 00 -	0.82*	0 83*	0.82	•		
structure to shoot fly and other traits between screening	ne density (lower)		E2				0.85*		4	
shoot fly	Trichon		21	51	*00	>	*00			
stance to	NT V C70X	vigour II		F2	-	-	0 0 M	20.0		
ats of resi	CLOSS D 1	Seedling vigour II		EI		0.25	* * *	- rc.0		
for componen	population of	1	Seedling vigour 1	Г.)			*0 U su v V V su v O d su v O			
on coefficients	RIL mapping	-	Seedling	:	ΕI	101 0	0.49	0 A5*	C+-0	
correlatio	sorghum		Gloceiness		E2				0.89* 0.9*	
man's rank	stimated in		Sloce	CEDID	F.I	1	0 89*		0.89*	
	Table 15. Speanments estimated in sorghum RIL mapping population of cross B1X02. A to the renvironments environments estimated in sorghum RIL mapping population of the transmission of transmission of the transmission of transmissi		Environmenut		rait		5	52	L3	5

Seedli	EI E2	13	0.49*	0.41* 0.36*		
	Deadhearts I (%) Deadhearts II (%)	EI E2	0.67* 1	0.41* 0.31*	0.04 ^{ns} 0.44* 0.33* 0.41	
	Deadhearts I (%)	EI E2	-	1.00.0	0.44* 0.33*	
	(%) 11 (%)		EI EZ	0.53*	0.04	<u>cr.0</u>
		Oviposition I (%)	EI E2	0 504	-75-0	0.36* 0.28* 0.10
Table (contd.)		Environmenut	rait		E2	. F.3

 			_	
Grain yield (g/pl)	EI E2	0.4*	0.79* 0.11** 0.28*	
plant height (cm)	EI E2	10 00*	0.00* 0.79*	1 00.0
	tlowering		0.42* 0.73*	0.71*10.01.01.01
Pseudostem	length (cm)	EI	0.42*	•
Seedling dry	weight (g)	EI	0.28*	,
Pseudostem	Security induction of (cm)	EI E2	E1 E1	0.29* 0.23*
Table (contd.)	Environment/t	rait		E3

E1 : kharif, Patancheru

E2: rabi, Patancheru

E3 : early rabi , Dharwar

* Significant at p = 0.01** Significant at p = 0.05- data not available

IS 18551	s at 5%	veen the		TITICIES
Table 16a. The RILs of cross BTx623 x IS 18551	selected for highest intensity of glossiness at 5%	selection intensity found in common between the	screening environments	

EIEZE3		-	77	7			~			
EIE3	-	7			-	7			2+3	
E2E3							-	7	1+3	
E1E2	7				7				2+3	
Entry	12	6	111	114	119	166	176	252	Total	

Table 16b. The RLLs of cross BTx623 x 1S 1850 selected for highest trichome density at 5% selection intensity found in common between the		ELES ELEZE
Table 16b. The RLLs of cross BTx623 x IS selected for highest trichome density at 5% selection intensity found in common betwee		E7E3
The RIL: r highest ntensity fo		E1E2
Table 16b. The RLLs of selected for highest trich selection intensity found	Current of	

	E1E2E3	-	*			> -	~	7		7	-	>	9	
	E1E3		-	7			-	~					2+6	nvironments
2	E2E3	7	•							-	7		2+6	: common between environments
INTI OTITICI	E1E2				7								2+6	V : comme
screening environments	Entry	16	20	53	64	67	129	134	166	172	213	252	Total	

Table 16c. The RILs of cross BTx623 x IS 18551 selected for lowest dead hearts I (%) at 5% selection intensity found in common between the eccention environments

	E1E2E3		-	7-	7		7		3	
	E1E3	-	7		-	7			2+3	
nts	E2E3								÷	
environmen	E1E2	~						7	2+3	
screening environments	Entry	232	243	213	53	166	51	170		

Table 16d. The RLLs of cross BTx623 x IS 18551 selected for lowest deadhearts II (%) at 5% selection intensity found in common between the screening environments

E1E2E3	1-	7												
E1E3	7				-	7		-	7		2+1			
E2E3			-	7						7	2+1			
E1E2			7		7		7	7			4+1	Patancheru	atancheru	E3: early rahi. Dharwar
Entry	51	53	88	108	114	166	221	232	243	252		E1: kharif. Patancheru	E2: rahi, Patancheru	E3: carly ra

SS 4-:	igu surface		IIHb	63.86	51.25	48.92	66.32	58.88	67.44	56.37	47.65	50.79	61.35	66.17	61.23	55.08	38.12
Ls of cro	1 WILLI II n lower		lhb	52.63	41.57	36.75	53.77	46.91	57.36	42.24	36.31	34.47	53.67	51.18	49.11	44.2	27.43
Table 17b. The RILs of cross	B1x023 x IS 18331 With fugit sichteme density on louver surface	e density o ection)	trlw	104.78	103.79	98.35	95.5	93.56	92.09	88.37	87.73	86.24	86.19	84.92	82.89	82.77	IS 18551 83.96
Table 1	B1X023	(5% selection)	entry	172	252	166	64	67	129	20	53	213	16	28	17	145	IS 1855
			1	1									1				
	SS L	ug	IIHb	47.4	62.74	51.25	56.15	64.05	48.92	56	60.09	38.12					
•	Table 17a. The RILs of cross	BTx623 x IS 18551 with high intensity of alossiness	Idb	39.35	49.67	41.57	42.53	49.16	36.75	42.54	45.65	27.43					
	7a. The R	BTx623 x IS 18551 w intensity of glossiness	ol Broom	1.4	1.5	17	1 8	8	01	61	0 6						
	Table 1	BTx62	entry	114		252	176	1 2	166	0	011	IS 18551					

	nance of lines for different characters is based on average performance over three environments
	flin
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	lote:
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			10)4
yld	13.4	17.5	11.8	13.6
plht	218.7	183.2	192	235.9
daf	77.5	73.0	70.1	.79.5
shtII	43.63	42.69	43.26	43.32
shtI	24.42	22.26	23.01	24.43
IIHb	47.4	48.92	51.25	38.12
lhb	39.35	36.75	41.57	27.43
ovill	72.8	70.43	76.02	64.43
ovil	43.79	44.75	45.66	35.90
trup	15.36	17.85	14.68	4.97
trlw	73	98.35	103.79	83.96
vigll	1.5	2.0	1.7	1.5
lgiv	2.4		2.3	2.1
وا	1.4	1.9	1.7	1.1
entrv	114	166	252	IS 18551 1.1 2.1 1.5 83.96 4.97 35.90 64.43 27.43 38.12 24.43 43.32 79.5 235.9 13.6
	entry gl vigl vigll trlw trup ovil ovill dhl dhll shtl shtll daf plht yld	entry gl vigl trlw trup ovil ovil dhl dhl shtl shtl daf plht yld 114 1.4 2.4 1.5 73 15.36 43.79 72.8 39.35 47.4 24.42 43.63 77.5 218.7 13.4	entry gl vigl trlw trup ovil ovil dhl dhl shtl shtl daf plht yld 114 1.4 2.4 1.5 73 15.36 43.79 72.8 39.35 47.4 24.42 43.63 77.5 218.7 13.4 166 1.9 2.4 2.0 98.35 17.85 44.75 70.43 36.75 48.92 22.26 42.69 73.0 183.2 17.5	

to G x E interaction were observed for the trait across two and three environments. Based on ranking in the order of high to low density of trichomes at 5% selection intensity, it was observed that 8 and 6 lines were found in common between two and three environment combinations, respectively (Table 16b). These lines (except RIL no.134) also showed high density of trichomes in highest group at 5% selection intensity based on average performance over the three environments (Table 17b). Despite the observation that number of lines were recorded with trichome density higher than resistant parent, none of these lines showed deadheart damage lesser than the damage in resistant parent.

4.4.4 Trichome density on upper leaf surface

Highly significant rank correlation coefficients were observed for all the paired combinationss of environments. The magnitudes of these correlation coefficients for the trait were high (r(s) = 0.82 to 0.83) and consistent among all paired combinations of environments. In addition, the trait was recorded with non-significant variances due to G x E interaction across the environments.

4.4.5 Oviposition

The rank correlation coefficients were significant for all the paired combinations of environments except for oviposition II between E2 and E3. The magnitudes of correlation coefficients were low ($\mathbf{r}(s) = 0.13$ to 0.53) to moderate among all paired combinations of environments indicating lack of consistency. The estimates were high between E1 and E2. These results are further supported by significant and high magnitude of variances due to G x E interaction observed across the environments except between E1 and E2.

4.4.6 Deadhearts

The rank correlation coefficients were significant for all the paired combinations of environments. The magnitudes of these correlation coefficients were low to moderately high (r(s) = 0.31 to 0.67). However, the range of estimates shows the lack of consistency of magnitudes across the pairs of environments. While, significant and high estimates of rank correlation coefficients were observed between E1 and E2. In support of these results significant and high magnitude of variances due to G x E interaction was observed across the environments.

Based on the rankings in the order of low to high deadhearts I (%) at 5% selection intensity, it was observed that 5 lines were found in common between two environment

combinations (Table 16c). Three RILs, viz., 213, 53 and 166 were found in common among the three environments. However, for deadhearts II (%) 2-3 lines were found in common between two-environment combinations, while, only one line (RIL no. 53) was found in common between three environments. These lines recorded lowest damage in the group of 5% extreme individuals (based on means over three environments) (Table 17d and 17e).

Three lines, viz., RIL 114, 166 and 252 that were recorded with lowest damage (based on means over three environments) possessed high intensity of glossiness, high seedling vigour, high trichome density (upper and lower surface), maximum seedling height and plant height (Table 17c).

4.4.7 Seedling height

The rank correlation coefficients were significant for all the paired combinations of environments. However, the magnitudes of correlation coefficients were low (r(s) = 0.23 to 0.49). The range of estimates shows the lack of consistency of magnitudes of estimates across the pairs of environments. While, the estimates were high between E1 and E2 for both seedling height I and seedling height II. These results were further supported by significant and high magnitude of variances due to G x E interaction recorded across three environments.

4.4.8 Seedling dry weight

The rank correlation coefficients were significant between E1 and E2. However, the magnitude of correlation coefficients was low (r(s) = 0.28).

4.4.9 Pseudostem length

The rank correlation coefficient was significant between E1 and E2. However, the magnitude of correlation coefficient observed was low (r(s) = 0.42). It was observed that the variances due to G x E interactions were highly significant across the two environments.

4.4.10 Days to 50% flowering

Highly significant rank correlation coefficients were observed for all the paired combinations of environments. The magnitudes of correlation coefficients for time to 50% flowering (days) were moderately high (r(s) = 0.71 to 0.73) and consistent among all the paired combinations of environments. However, significant variances due G x E interaction were observed for the trait across two and three environments.

Table 17d. The RILs of cross
BTx623 x IS 18551 with
deadhearts I (%) (5% selection)

Table 17e. The RILs of cross
BTx623 x IS 18551 with
deadhearts II (%) (5% selection)

Entry	Deadhearts I (%)	Entry	Deadhearts II (%)
213	34.47	114	47.4
51	35.36	53	47.65
243	35.44	24	48.51
232	36.19	232	48.72
53	36.31	166	48.92
166	36.75	51	49.23
197	37.53	2	50.52
24	38.4	197	50.71
37	39.28	213	50.79
114	39.35	252	51.25
170	39.56	153	51.42
233	39.7	88	51.52
207	40.09	243	51.68
IS 18551	27.43	IS 18551	38.12

Note: The performance of lines for different characters is based on average performance over three environments

4.4.11 Plant height

Highly significant rank correlation coefficients were observed for all the paired combinations of environments. The magnitudes of these correlation coefficients for the trait were high $(r_{...}) = 0.79$ to 0.88). However, the range of estimates observed shows the lack of consistency of magnitudes existed across the pairs of environments. While, significantly high magnitude of variances due to $G \times E$ interaction were observed for the trait across two and three environments.

4.4.12 Grain yield

Rank correlation coefficients observed for the trait were significant for all the paired combinations of environments. The magnitudes of correlation coefficients for the trait were low $(r(\bullet) = 0.11$ to 0.4) and inconsistent among all the paired combinations of environments. However, variances due to G x E interaction observed for the trait were significant across two and three environments.

4.5 Molecular analysis

4.5.1 Parental polymorphism

The parental lines BTx623 and IS 18551 were surveyed against SSR markers to identify polymorphism at the DNA level. A total of 96 SSR primer pairs were used for parental screening. An image of a silver-stained polyacrylamide gel (Plate 10) shows the results of parental lines screened against some of these SSR primer pairs. The results of this parental survey for SSR marker polymorphism are presented in Appendix III. Approximately 80% of the primer pairs detected polymorphism between the parental lines. However, only 49% of these markers were showed clear polymorphism that can be scored reliably on a silver-stained gel A total of 44 markers that showed clear polymorphism were used to screen a random subset of the RIL mapping population consisting of 93 individuals.

4.5.2 Marker segregation and segregation distortion

The segregation patterns of 44 marker loci for 93 RILs obtained from the cross (BTx623 x IS 18551) were compared with the expected ratio of 1:1 (1 homozygote of parent P1: 1 homozygote of parent P2). The calculated χ^2 values were compared with table values at 1 degree of freedom for each marker locus and are presented in Table 18. A total of 28 markers out of the 44 co-dominant marker loci recorded non-significant χ^2 values when compared with table values at 5% and 1% probability levels. Twelve markers out of 16 remaining markers

Table 18. Test of significance for segregation distortion relative to the expected 1:1 ratio for 44 SSR markers screened in the mapping population subset consisting of 93 RILs derived from cross between BTx623 (susceptible) and IS 18551 (resistant)

Sl.no	Marker	P1 alleles	P2 alleles	χ^2
1	Xtxp3	42	51	0.87
2	Хтхрб	35	56	4.85*
3	Xtxp7	34	55	4.96*
4	Xtxp8	42	50	0.70
5	Xtxp9	43	47	0.18
6	Xtxp10	60	18	22.62**
7	Xtxp12	40	50	1.11
8	Xtxp15	40	51	1.33
9	Xtxp18	51	40	1.33
10	Xtxp21	53	40	1.82
11	Xtxp25	41	52	1.30
12	Xtxp32	36	47	1.46
13	Xtxp34	42	51	0.87
14	Xtxp37	33	56	5.94*
15	Xtxp40	61	26	14.08**
16	Xtxp41	38	48	1.16
17	Xtxp43	34	55	4.96*
18	Xtxp55	48	45	0.10
19	Xtxp56	33	60	7.84**
20	Xtxp57	51	39	1.60
21	Xtxp65	57	35	5.26*
22	Xtxp69	43	44	0.01
23	Xtxp75	37	56	3.88*
24	Xtxp94	51	42	0.87
25	Xtxp96	34	57	5.81*
26	Xtxp141	39	45	0.43
27	Xtxp205	32	56	6.55*
28	Xtxp210	56	35	4.85*
29	Xtxp228	48	45	0.10
30	Xtxp229	34	50	3.05*
31	Xtxp248	41	45	0.19
32	Xtxp265	42	45	0.10
33	Xtxp285	42	47	0 28
34	Xtxp286	34	55	4.96*
35	Xtxp289	37	56	3 88*
36	Xtxp295	49	43	0.39
37	Xtxp298	39	50	1.36
38	Xtxp302	50	43	0.53
39	Xtxp312	50	37	1.94
40	Xtxp316	49	40	0.91
41	Xtxp319	48	45	0.10
42	Xtxp357	30	58	8.91**
43	XSbKAFGKI	46	40	0.42
44	Xgap342	47	36	1.46

*: 0.05 0.01 P1: BTx623

**: p < 0.01 P2: IS 18551

Note: Marker data for 93 RILs in this table includes only parental alleles excluding the data points scored as heterozygotes. offtypes and missing

deviated significantly from expected ratios at probability levels between 1% and 5%. Four markers showed the higher degrees of distortion with χ^2 values ranging from 7.84 to 22.62, significant at probability levels <0.01. The order of these most significantly deviating marker loci (from most distorted to least distorted) was Xtxp56, Xtxp357, Xtxp40 and Xtxp10.

It was observed that a large number of markers showed higher proportions of alleles from BTx623 (P1) (51.6 to 58.8%) than from IS 18551 (P2) (41.2-48.4%) as shown in Figure 4A and 4B. A total of 34.1% of loci deviated significantly (p<0.05) from the expected 1:1 ratio between the two homozygous genotype classes. Out of these markers showing segregation distortion, 68.2% of the markers showed a higher proportion of alleles from IS 18551. The strongest deviations from the expected 1:1 were observed for Xtxp10 followed by Xtxp40 which both exhibited a preponderance of BTx623 alleles (Table 18).

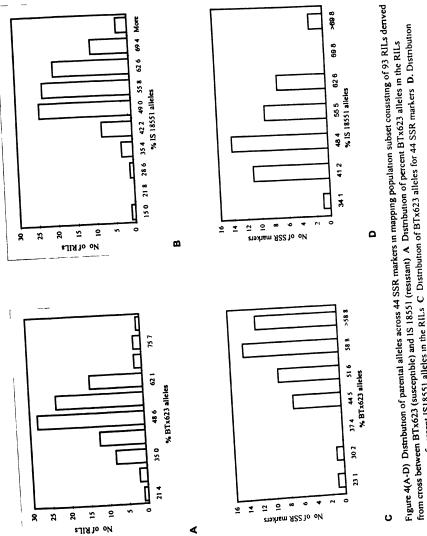
4.5.3 Genome Composition of RIL population

Though skewed segregations of markers were observed, the average RIL was comprised of comparatively equal proportion of the parental genomes (P1 and P2) (Figure 4C and 4D). The average RIL was homozygous BTx623 and homozygous IS 18551 at 48.9 and 49.0% of the scored markers, respectively. The distributions of RILs (%) for these homozygous classes range from 21.4 to 82.5 and 15.0 to 76.2. The details on percentage of parental alleles and heterozygous loci in the 93 genotyped RILs are presented in Appendix IV.

It was observed that 24.7% of individuals were heterozygous for 2.5-2.6% of the loci. The average RIL was heterozygous for 0.7% of the loci with a range of 0-4.7%. The observed heterozygosity percentage in the RIL population after six generation of selfing was close to the expected 1.56%.

4.5.4 Construction of Linkage map

Loci detected by a total of 44 SSR markers were used to construct a skeleton map for the subset RIL mapping population based on cross BTx623 x IS 18551. The size of mapping population used for construction of this linkage map was 93 RILs. Linkage analysis was accomplished using the program JOINMAP (Stam, 1993; Stam and Van Ooijen, 1995). Linkage distances in terms of centimorgan (cM) values were calculated using the Kosambi function. Linkage map was constructed for 23 linked markers out of 44 markers screened in the population for use in QTL analysis. The details on linkage groups to which 23 markers were assigned and



B Distribution of percent IS18551 alleles in the RILs C Distribution of BTx623 alleles for 44 SSR markers D. Distribution of IS 18551 alleles for 44 SSR markers linkage distances are given in Table 19. The rest of the SSR markers were found unlinked because of lack of additional SSR markers to link these. Since the order of markers was same as expected, all the markers (linked and unliked) were assigned to linkage groups as per the map of Bhattramakki *et al* (2000) (Fig. 5). The linked markers used in the present study were assigned to 5 linkage groups: A, B, C, H and J. The number of SSR loci mapped per linkage group (LG) ranges from 2 (LGs J and H) to 9 (LG A). The linkage map thus constructed had a length of 188.2 cM. The mapped SSR loci are distributed unevenly throughout the five linkage groups and the average distance between the markers is 8.2 cM (Table 19).

Nine SSR loci mapped to the linkage group A. The low distances were observed between the markers, viz., *Xtxp75* and *Xtxp229* (4 cM); *Xtxp248* and *Xtxp316* (6.8 cM). Seven and three markers were mapped to linkage groups B and C, respectively.

4.5.5 QTL mapping

For QTL mapping and Q x E interaction analysis, the linkage map constructed for the subset of 93 individuals from the RIL population of cross (BTx623 x IS 18551) was used. The software package PLABQTL was used to analyze the data by composite interval mapping (CIM) procedures, the CIM method was implemented using a LOD of 2.5 as threshold value for QTL significance. The software calculates additive effects and estimates the portion of phenotypic variation explained by individual QTLs.

In this mapping population, the female parent (the marker allele homozygote scored as A) was BTx623 (susceptible to shoot fly damage) and the male parent (marker allele homozygote scored as B) was IS 18551 (resistant to shoot fly damage). The results are described below from CIM analysis for identification of QTLs with significant effects that were detected in individual screening environments and across screening environments for the RIL mapping population subset of 93 lines for shoot fly resistance components.

Among the components of shoot fly resistance analysed, QTLs were identified for glossiness, seedling height, seedling vigour, deadhearts (%) and grain yield.

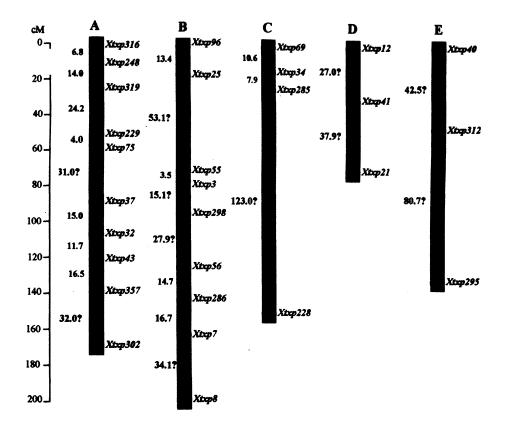
Sl.no	Linkage group ⁽¹⁾	Marker interval	Linkage distance (cM)
1	В	Xtxp96 -Xtxp25	13.42
2	В	Xtxp55-Xtxp3	03.48
3	B	Xtxp56 -Xtxp286	14.73
4	В	Xtxp286 -Xtxp7	16.68
5	Α	Xtxp75 -Xtxp229	04 00
6	Α	Xtxp229 -Xtxp319	24.20
7	Α	Xtxp319 -Xtxp248	14.00
8	Α	Xtxp248 -Xtxp316	06.80
9	Α	Xtxp357 -Xtxp43	16.50
10	Α	Xtxp43 -Xtxp32	11.70
11	Α	Xtxp32 -Xtxp37	15.00
12	C	Xtxp69 -Xtxp34	10.59
13	С	Xtxp34 -Xtxp285	07.90
14	н	Xtxp18-Xtxp210	17.71
15	J	Xtxp94 -Xtxp65	11.52

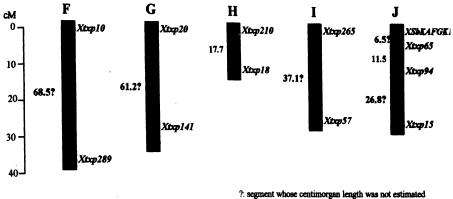
Table 19. Linkage distance between the SSR markers screened in subset mapping population of 93 RILs derived from cross between BTx623 x IS 18551

⁽¹⁾: Linkage group nomenclature as per Peng et al (1999)

No. of unlinked markers: 21; they are

Xtxp298, Xtxp8, Xtxp228, Xtxp9, Xtxp205, Xtxp12, Xtxp57, Xtxp265, Xtxp302, Xtxp21, Xtxp15, Xtxp289, Xtxp295, Xtxp312, Xtxp41, Xtxp141, Xtxp6, Xgap342, Xtxp40, XSbKAFGK1, Xtxp10





?: segment whose centimorgan length was not estimated in the present study but depicted with linkage distances shown in the map of Bhattramakki *et al* (2000).

Figure 5. Linkage map of the S. bicolor BTx623 x IS 18551 subset RIL population (93 individuals) consisting of 44 SSR loci ordered at LOD score 4.0 using JOINMAP.

4.5.5.1 QTL analysis in single environments

The phenotypic data from three environments and genotypic data for 93 RILs were subjected to QTL analysis. The results of single environment analyses are presented in Table 20 and Figure 6.

Analysis with composite interval mapping revealed 2-3 QTLs for components of resistance in single environments, and all together 8 QTLs were identified.

PLABQTL identified a single QTL for glossiness located on Linkage Group J with significantly high phenotypic variance explained in each environment. The QTL identified on this linkage group was consistent across the three screening environments. Adjusted phenotypic variance (%) explained by this QTL for glossiness ranged from 34.4 to 46.5. The phenotypic variance explained by the QTL was higher in E2 (46.5%) followed by E3 (39.7%) and E1 (34.3%). In all the environments, the resistant parent IS 18551 contributed alleles for glossiness.

Based on analysis of data from environment E3 one QTL mapped to the interval Xtxp94-Xtxp65 for deadhearts I (%) was identified on linkage group J, which explained 15.8% of phenotypic variation. It was observed that BTx623 alleles in this genomic region were associated with increased level of deadhearts damage. Resistant parent IS 18551 contributed alleles for lower deadheart damage. This QTL for deadheart I (%) mapped to the same chromosomal region as glossiness on linkage group J.

For seedling vigour II, two different QTLs were identified in environments E1 and E2. The QTL detected for seedling vigour II in environment E1 (in interval Xtxp18-Xtxp210) and E2 (in interval Xtxp229-Xtxp319) were mapped to LG H and LG A, respectively. The direction of additive effects of these QTLs was differed. In the environment E1 the alleles of IS 18551 in the LG H interval were associated with increased seedling vigour. Whereas in the environment E2 the alleles of BTx623 in the LG A interval was associated with increased vigour. In other words, for seedling vigour II, positive alleles for increased seedling vigour were contributed by the resistant parent in E1 and by the susceptible parent in E2.

For seedling height, two different QTLs mapped to LG A were identified in environments E2 and E3. One QTL for seedling height I was detected in the environment E3 in chromosomal region Xtxp34-Xtxp285 (LG C). For seedling height II, a QTL detected in environment E2 was

environments) based on composite interval mapping (PLABQTL, LOD >2.5) using 93 RILs derived from a Table 20. Characterstics of QTLs associated with components of resistance to shoot fly (in three screening cross between BTx623 (susceptible) and IS 18551 (resistant)

Environment/trait	Marker interval ¹⁾	Distance between the markers (cM)	Linkage group	Peak LOD ²⁾ Adj. R ² % ³⁾ Effect ⁴⁾	Adj. R ² % ³⁾	Effect ⁴⁾
Kharif, Patancheru (E1)	(E1)					
Glossiness	Xtxp94 - <u>Xtxp</u> 65	10	ц Н	8.82 2.76	34.4 05.7	-0.584 -0.138
Seedling vigour 11	Seedling vigour 11 Atxp16 -Atxp210	þ				
Rabi, Patancheru (E2)	(E2)					
	5 yurriy - Lourriy	10	ſ	13.43	46.5	-0.722
	Virun 70 - Yrrn 319	24	A	02.91	04.8	+0.113
Seedling height II	Xtxp32 -Xtxp37	14	V	02.80	03.1	+0.582
Early rabi, Dharwad (E3)	ad (E3)					
	3 yurry - Lourry	10	ſ	11.31	39.7	-0.639
	Syurix- Fourix	01		04.30	15.8	-2.499
Seedling height I	Xixp34 - <u>Xixp</u> 285	5	C	02.57	02.7	-0.261
Glossiness (1-5 scale)	Glossiness (1-5 scale) : 1-high intensity of glossiness, 5-non glossy	iness, 5-non glossy				

b · (ATTOM (-I) SCATTISSOLD

Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour

1) Underlined markers are the nearest markers to the QTLs.

2) Log10 likelihood.

3) Percentage of adjusted explained phenotypic variance.

4) + sign indicates that the homozygous IS 18551 allele genotype has a numerically greater value for the trait than does the homozygous Btx623 allele genotype.

- sign indicates that the homozygous BTx623 altele genotype has a numerically preater value for the trait than the does the

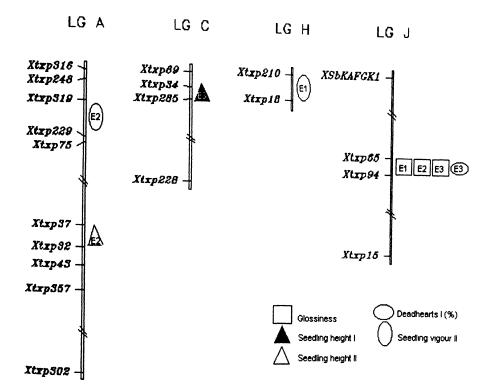


Figure 6. QTLs affecting shoot fly resistance and its components in theBTx623 x IS 18551 subset RIL population (93 individuals) under three screening environments, E1 (*kharif*, Patancheru), E2 (*rabi*, Patancheru) and E3 (early *rabi*, Dharwar).

located in chromosomal region Xtxp32-Xtxp37 (LG A). The direction of additive effects of these QTLs was also differed in the respective environments. In the environment E2 the alleles of IS 18551 were associated with increased seedling height at the later stage of seedling growth, where as in the environment E3 the alleles of BTx623 were associated with increased seedling height in the initial stage of seedling growth. In other words, in the environment E3, the susceptible parent BTx623 contributes alleles for increasing seedling height in the initial stages of seedling growth, however, in the environment E2, resistant parent IS 18551 contributed alleles for increasing height in the later stages of seedling growth.

4.5.5.2 QTL analysis across environments

In order to determine chromosomal regions that are important for the expression of the trait under different environmental conditions, QTL analysis was done based on the phenotypic values averaged over the environments. To detect the $Q \ge E$ interaction effect the analysis was made for two-environment combinations and across all three test environments. The results obtained are presented in Table 21 and Figure 7.

One QTL for glossiness (Xtxp94-Xtxp65) was detected that mapped to linkage group J across all two-environment combinations and across the three environment combination. The portion of phenotypic variance explained by this QTL was consistently high across the environments ranging from 39 to 45%, highest across environments E2 and E3 (45%).

QTLs explaining lower proportion of phenotypic variance for other traits, seedling vigour, seedling height and grain yield were identified and are described below.

Across environments E1 and E2, one QTL each was detected for seedling vigour I, seedling height I and grain yield. The QTL for seedling vigour I had an overlapping support interval with the QTL for seedling height I at region *Xtxp69-Xtxp34* on linkage group C. For all these traits, alleles of BTx623 contributed positive phenotypic effect relative to alleles of IS 18551.

Analysis across environments E2 and E3 detected one QTL each for seedling vigour II and grain yield, while two QTLs were identified for seedling height I. The QTL for seedling vigour II had an overlapping support interval (*Xtxp229-Xtxp319*) with one QTL for seedling height I. QTLs for all traits (seedling vigour II, seedling height I and grain yield) had negative Table 21. Characterstics of QTLs associated with the components of resistance to shoot fly (across the environments) based on composite interval mapping (PLABQTL, LOD >2.5) using 93 RILs derived from cross between BTx623 (susceptible) and IS 18551 (resistant)

															ſ				
					E1E2			E2E3			E1E3			E1E2E3			Q×Ei	Q x E interaction	g
Trait	Marker	51	ν			Effect	LOD R ² %	R ² %	Effect LOD R ² %	LOD	R ² %	Effect	LOD	Effect LOD R ² %	Effect E1E2		E2E3	EIE3	E1E2E3
Dess D	Xtxp94 - Xtxp65			12.3 42.9		-0.656 13.34 45.3	13.34	45.3	-0.681	11.12 39.2	39.2	-0.616 12.49 43.1	12.49	43.1	-0.659	รน	22	22	SI SI
Seedling vigour I	Xtxp69- Xtxp34	с	6	2.59	4.6	+0.105										su			
Seedling vigour II	Xtxp229- Xtxp319	۲	24				3.67	6.1	+0.095							```	SI		
1	Xtxp18- Xtxp210	Н	6							12.5	6	-0.142						:	
Seedling height I	Xtxp69- Xtxp34	J	4-7	3.07 3.3		-0.402							3.43	3.6	-0.347 **	:			:
	Xtxp229- Xtxp319	۷	24				2.96	13.6	-0.519				12.5	5.7	-0.394		*		:
	Xtxp34- Xtxp285	υ	5				3.27	15.6	-0.467							<u> </u>	:		
Seedling height II	Xtxp18- Xtxp210	Н	12							3.07	6.3	+0.519						:	
Xtxp18- Grain vield Xtxp210		H	14-15 2.99	2.99	7.8	7.8 -1.151 2.69 7.1	2.69		-0.986 3.96 13.3	3.96		-1.594 4.19 12.8	4.19		-1.351 ns		u su	- su	
	Ι.		1			aloeev													

Glossiness (1-5 scale) : 1-high intensity of glossiness, 5-non glossy

Seedling vigour (1-5 scale): 1-high vigour, 5-low vigour

+ sign indicates that the homozygous IS 18551 allele genotype has a numerically greater value (or lower score) for the trait than the does the homozygous Btx623 allele genotype.

- sign indicates that the homozygous BTx623 allele genotype has a numerically greater value (or score) for the trait than the does the homozygous [S 1855] allele genotype.

LOD: Log10 likelihood.

R²%. Percentage of adjusted explained phenotypic variance.

** significant ns: nonsignificant

LG: linkage group cM: centimorgan distance

E1 : kharif, Patancheru

E2 : rabi, Patancheru

E3 : carly rabi, Dharwar

E1E2, E2E3, E1E3 - across two environments

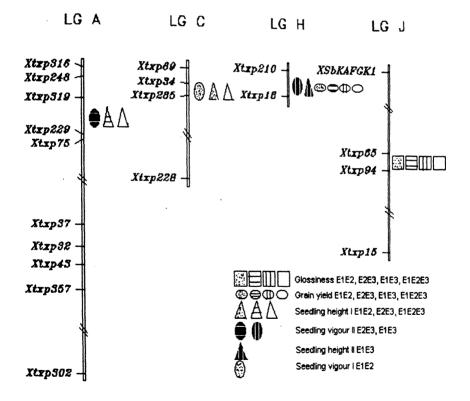


Figure 7. QTLs affecting components of resistance to shoot fly in the BTx623 x IS 18551 subset RIL population (93 individuals) across two environment combinations and three environments.

additive effects. In other words alleles of BTx623 had more positive phenotypic effect for these traits than did the alleles of IS 18551 across the environments E2 and E3.

Across the environments E1 and E3, one QTL each were detected for seedling vigour II, seedling height II and grain yield. The QTL for seedling vigour II had an overlapping support interval (*Xtxp18-Xtxp210*) on LG H with the QTL for seedling height II. The QTL for grain yield was also mapped to this genomic region. Positive additive effects were observed for seedling vigour II and seedling height II across this pair of environments. In other words IS 18551 alleles had more favorable phenotypic effects than BTx623 alleles across E1 and E3. However, for grain yield BTx623 alleles had more positive phenotypic effects than did IS 18551 alleles.

QTL analysis across the three environments detected two QTLs for seedling height I mapping to linkage group A (LG A) and C (LG E) and one QTL for grain yield mapping to linkage group H (LG H).

Q x E ANOVA revealed that Q x E interaction for glossiness was nonsignificant across all the two-environment combinations and the three-environments combination. QTL detected for seedling vigour I across E1 and E2, and QTL detected for seedling vigour II across E2 and E3 showed nonsignificant Q x E interaction. One QTL detected for grain yield across twoenvironment combinations and the three environments combination also showed nonsignificant Q x E interaction in all environmental pair combinations. Other QTLs for seedling vigour II and seedling height II showed significant Q x E interaction variances across two environment combinations and three environments.

The percentage phenotypic variance explained by single QTL ranged from 4.6% to 6.1% for seedling vigour, from 3.3 to 15.6% for seedling height, and from 7.1% to 13.3% for grain yield per plant. The QTLs identified for seedling vigour and seedling height mapped to LG A and LG C.

Greatest additive effects were observed for grain yield (-0.986 to -1.594) followed by that for glossiness (-0.616 to -0.681) across the environments. Two QTLs for seedling height I, viz., *Xtxp229-Xtxp319* (on LG A) and *Xtxp34-Xtxp285* (on LG C) were recorded with large portions of the phenotypic variance explained (13.6 and 15.6%, respectively) and high additive effects (-0.519 and 0.467, respectively).

Discussion

V. DISCUSSION

Shoot fly is major insect pest of sorghum. Though genetic studies have been made on host plant resistance to shoot fly by number of workers under different genetic backgrounds, the genetic information available is limited and available in piece meal. There is need to study the genetic architecture of shoot fly resistance and its component traits in appropriate breeding material. Shoot fly resistance is quantitative in nature, involving number of components, which are quantitative in nature and influenced by of G x E interaction. Therefore, phenotypic trait selection for this trait will be difficult. Despite efforts made since last two decades by utilizing the existing cultivated sources of resistance, the level of resistance achieved so far is limited Marker-assisted selection will increase the efficiency of breeding for such traits. As an initial step of this program, genomic regions associated with resistance and its components are to be detected.

The recombinant inbred lines (RILs) obtained from cross BTx623 (susceptible) x IS 18551 (resistant) were characterized for components of resistance to shoot fly and for agronomic traits. The parental polymorphism for both phenotypic traits and SSR markers was also studied. A subset of the mapping population was genotyped with a number of polymorphic SSR markers. The phenotypic and genotypic characterization of the RILs and their parents are discussed under following headings with the intention of improving understanding the genetic architecture of shoot fly resistance in sorghum and its component traits.

5.1 Phenotypic and genotypic variability

Characterization of variation (phenotypic and genotypic) for components of resistance to shoot fly is a prerequisite to application of molecular genetic knowledge to broader understanding of the genetic control of resistance to shoot fly in sorghum. For quantitative traits, the effects of genotype and environment often cannot be readily distinguished. Quantitative genetic theory has made major contribution to analysis of such traits by providing methods for separating genetic effects from environment effects. As a first step of analysis of shoot fly resistance traits that are quantitative in nature, genetic expectations of means and variances were obtained using 252 recombinant inbred lines (RILs) evaluated along with their parents. The genetic variability was assessed under three levels of shoot fly infestation in RILs derived from a

cross between resistant and susceptible inbred lines. Estimates of genetic variance components thus obtained have been used to estimate the degree of variation, to predict genetic gains from selection and to compare the heritabilities of different traits.

The pooled analysis of variance for different components of shoot fly resistance at the crops seedling growth stage and traits at maturity revealed highly significant differences for genotype and $G \times E$ interaction effects (Tables 5 and 6). This analysis not only depicts the variability that existed in the different test environments but also reflects the presence of genetic variability among the tested genotypes for shoot fly resistance and its component characters. Highly significant differences detected among the RILs and phenotypic differences recorded in the parents for various resistant parameters and phenotypic characters suggested that sufficient variability existed in the experimental material.

Based on the varying range of phenotypic values for deadhearts I (%) in susceptible check CSH 9 in the three test environments, these environments were categorized as moderate shoot fly pressure (E2), optimum (E1) and high pressure (E3). The first deadhearts damage ratings in susceptible check in these environments were 54.0, 73.7 and 76.8%, respectively. According to Borikar et al (1982) shoot fly tolerant genotypes can be selected by growing the breeding material under optimum shoot fly pressure with 67-70% seedling mortality on the susceptible check. Rana et al (1975) also suggested selection for shoot fly resistance under conditions when mortalities ranged between 6.7 to 67.0 percent. According to Rao et al (1974), the level of seedling mortality in a field crop due to shoot fly deadhearts is a function of the intensity of insect infestation, plant growth rate and inherent genotypic differences. Borikar and Chopde (1980) studied genetics of shoot fly resistance under three levels of shoot fly infestation and indicated that variation between and within genotypic groups became more apparent under high shoot fly population. It appears that the extent of deadhearts observed is primarily related to the level of shoot fly pressure This observation is supported by Rao et al (1974). Since the rate and level of shoot fly population build up varies with season and location, sorghum genotypes also show variable degree of shoot fly damage in different environments. Therefore, evaluation of genotypes and breeding populations in a range of environments should provide opportunities for development of adaptive genotypes.

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The high incidence of deadhearts (%) observed in environment E3 suggests that it should be considered as most favorable environment for shoot fly infestation. The possible reasons for high level of infestation observed in environment E3 could be low seedling vigour due to drought stress occurred during seedling stage. Notably there was no other sorghum crop at similar stage of growth as the experimental material nearby.

The two parents differed phenotypically for shoot fly resistance components and other traits (Table 3). The contrasting features of the parents for resistance components satisfy the requirement of parental polymorphism for phenotypic characters in mapping experiments. Sufficient polymorphism between the parents for the phenotypic traits of interest and at the DNA level is essential to trace the recombination events. Young (2001) emphasized that in the absence of DNA polymorphism, segregation analysis and linkage mapping are impossible. The development of a linkage map and genetic resolution of quantitative traits depends on the degree of recombination. In this direction, use of RILs as the mapping population is appropriate as it allows breakage of even tightly linked genes through occurrence of frequent meetic events during development of the mapping population. The contrasting features of the parents are responsible for the range of variability observed among the RILs for the characters studied.

Parental performance and RIL mean performance (Table 3 and 4) for glossiness were consistant across the environments. This suggests that evaluation of this trait was reliable The recovery of seedling vigour at later stage of seedling growth clearly shows the response of genotypes to environment. The differences between the parents for deadhearts percentage were observed in each environment with IS 18551 being more resistant than BTx623.

The expression of some traits was highly unstable across the environments, resulting in shift in environmental means (Figure 2), demonstrating clearly that the expression of these characters is highly sensitive to the environment to which the genotypes were exposed. The continuous variation observed is due to simultaneous segregation of a large number of loci conditioning metric traits and due to variable microenvironment variation interacting with these genes, resulting in higher experimental error estimates of treatment means for these traits. The amount of variation brought about by allelic substitution at any single locus in such cases is not experimentally recognizable. The variation observed for shoot fly resistance components and other traits (Table 7 and 4) is discussed in the following paragraphs.

5.1.1 Glossiness

The high level of variability observed based on high PCV and GCV values, and the wide range of phenotypic values indicate that selection for glossiness will be efficient. The marginal difference between PCV and GCV values for glossiness suggests the predominance of genetic factors in controlling this trait. The consistency of variability across the environments indicates that the character is little influenced by environmental factors. Higher variability noticed for the trait in postrainy season suggests that selection for the trait will be more effective in the postrainy season. The glossy trait has been reported to be the characteristic of most of winter (*rabi*) sorghum varieties of India (Blum, 1972; Rao *et al.*, 1978). According to Taneja and Leuschner (1985), glossiness contributes less to shoot fly resistance during the *rainy* season. In addition, this source of shoot fly resistance is apparently limited and largely confined to *rabi* sorghums of India (Nimbalkar and Bapat, 1987). This suggests the importance of screening for and incorporation of a high intensity of glossiness in *rabi* breeding material.

5.1.2 Seedling vigour

The lack of consistency in variation across the test environments and the difference in the magnitude of PCV and GCV for seedling vigour indicate that this character is greatly influenced by environmental factors. The recovery of seedling vigour has implications for recovery (i.e., tolerance) and escape of the plants from damage by larvae emerging from second round of egg laying, since these larvae will be unable to move to the growing point. The high level of variability observed for the trait at seedling growth stage II suggests that it would be appropriate to carryout selection at this later stage of seedling growth.

5.1.3 Trichome density

The high variability observed based on high PCV and GCV values indicate that selection for trichome density would be efficient. The high phenotypic variability in E2 (*rabi*, Patancheru) and E3 (early *rabi*, Dharwar) indicate that selection can be efficiently carried out in postrainy season. The marginal difference between GCV and PCV observed for trichome density shows that this character is predominantly under genetic control. Consistency of high level of variation across test environments indicates that influence of environment on the expression of this character is minimal.

5.1.4 Oviposition

The range of phenotypic values for oviposition varied significantly across the three test environments indicating influence of environment on shoot fly egg laying. Though the variability was high for related traits, glossiness and trichome density, the low variability for this trait indicates that favorable alleles for these traits related to oviposition are not in association but might be in dispersion. The variability observed for oviposition depends upon the level of shoot fly pressure prevailing in the test environment and upon breeding material evaluated. In the present study the variability was high at moderate insect pressure (E2) and at optimal insect pressure (E1). As the shoot fly pressure increased, the observable variability for resistance decreases, which explains the limited variation in oviposition observed in test environment E3. It is reported by several authors that the efficiency of the oviposition non-preference mechanism of shoot fly resistance is not stable and is ineffective under heavy shoot fly pressure (Singh and Jotwani, 1980a; Borikar *et al.*, 1982 and Sharma *et al.*, 1997). Borikar *et al* (1982) reported that estimates of genotypic coefficients of variability were higher for oviposition when the material was tested under optimum shoot fly population levels (i.e., not too high and not too low).

Resistance in terms of oviposition non-preference is due to the component traits that prevent egg laying. Therefore, high levels of resistance in terms of oviposition non- preference will be due to the combination of characters with expression in favorable direction. It is reported that leaf colour, texture and width (Raina, 1982) and hairiness (Bapat and Mote, 1982b) are important factors in selection of the oviposition substrate by female flies. In the present study the material screened was a population of RILs. Each individual line has combination of alleles controlling the trait received from two parents due to recombination events that occurred during the process of their development. Therefore, low variability may be expected in RILs lacking set of favorable alleles controlling component traits required to bring about high level of resistance.

5.1.5 Deadhearts

The lower variability observed for this trait indicates that the alleles controlling the component traits responsible for preventing egg laying or subsequent damage in the resistant parent IS 18551 are dissociated from resistant parent in the RILs. This is due to frequent recombination events during the selfing process. In other words, the parental association of resistance alleles was broken by genetic recombination and the frequency of parental recombinations was too low to permit their observation in the RIL population used in this study resulting in observed reduced variability for resistance. Reduced variability in terms of deadhearts damage indicates superoptimal pest population pressure in the test environment. The lack of consistency of variability across the three test environments also indicates that the deadhearts damage level is greatly influenced by environmental factors. Higher levels of deadhearts damage and low frequency of RILs with reduced damage noticed in E1 and E3 indicate these test environments might be favorable for multiplication of shoot fly. Under increased shoot fly pressure, the resistance is broken down and hence low variability observed for the trait. In contrast to these results, Borikar et al (1982) have reported that estimates of GCV were higher for seedling mortality when the material was tested under optimum shoot fly population.

For effective selection of the trait, the seedling stage at which variability is high is taken in to consideration. This criteria again depends on level of shoot fly pressure prevailing in the environment. It is observed in the present study that the variability (both phenotypic and genotypic) is high for deadhearts I (%). However, the magnitude of variation is higher at moderate insect pressure (E2) and optimum (E1) pressure than high pressure (E3) prevailing in the environment. This suggests that initial selection may be carried out under moderate insect pressure and final selection may be practiced in screening environments with high insect pressure.

5.1.6 Seedling height

Significant differences between PCV and GCV values for seedling height indicate the influence of environmental factors on observed variability. Consistent and lower magnitude of variability observed for this trait across environments might be due to masking of gene effects through epistasis and G x E interaction.

5.1.7 Seedling dry weight

The significant difference between PCV and GCV for seedling dry weight indicates the role of environmental factors in the observed variability. This was further evidenced by differences in phenotypic ranges observed in two screening environments.

5.1.8 Pseudostem length

Significant differences between PCV and GCV values and varying levels of variation across the screening environments indicate the influence of environment on pseudostem length. Greater variation for pseudostem length than for seedling vigour and seedling height suggests that height and vigour can be measured in terms of pseudostem length which actually plays a role in the movement of shoot larvae to the host plant growing point. The major draw back with this is that recording observations on pseudostem length is tedious, labour intensive and time consuming. The identification of markers linked to such traits is appropriate and use of linked markers could improve the efficiency of selection for this trait.

5.1.9 Days to 50% flowering, plant height and grain yield per plant

Marginal differences between PCV and GCV values for the traits plant height and days to 50% flowering indicate that the characters are predominantly under genetic contro¹. However, the higher estimate of PCV than GCV for grain yield suggests the influence of environment for the trait. Significantly high levels of variation observed for the traits observed at maturity in E1 suggest that selection for these characters may be effective in *kharuf* season. For plant height though GCV and PCV are on par with each other the wider range of variability observed in E1 and E3 might be due to gene interaction with environment.

5.2 Correlations

Shoot fly resistance is a complex character and its expression depends on the interplay of several to many component characters, which finally sum up in the expression of resistance. Correlation studies provide a basis to decide upon suitable selection criteria for use in genetic improvement of resistance to shoot fly in sorghum.

It was established from previous studies that glossiness (Omori et al., 1983), trichome density (Maiti and Bidinger, 1979) and seedling vigour (Karanjakar et al., 1992) were favorable selection characters for conferring resistance to A. soccata. The association of these traits with shoot fly resistance in terms of oviposition (%) and deadhearts (%) was found to be significant and negative in the present study (Table 8). However, these correlations were low. Similar observations were made by Maiti and Gibson (1983) for correlations between trichome density and the percentage of main culms with deadhearts, which ranged from -0.29 to +0.24 (all nonsignificant at p = 0.10). However, high degrees of correlation were reported by Omori *et al* (1983) for association of shoot fly resistance with two resistance component characters, viz., trichome intensity (abaxial surface) (-0.730 < r < -0.817), and glossiness intensity (-0.811 < r < -0.935) as significant and negative. Similarly, there were negative and highly significant correlations between deadhearts percentage and trichome density (r = -0.58) (Jadhav et al., 1986). Significantly negative and strong associations for shoot fly egg laying with trichomes (-0.697 < r < -0.752) and glossiness (-0.747 < r < -0.825) were also reported by Omori et al (1983). According to Omori et al (1983), although correlation coefficients for these two component traits with shoot fly resistance were high, these resistance components do not play any direct role in building up the total variability in shoot fly resistance. The negative correlation between egg laying and glossiness intensity may be caused by the tight positive association of glossiness with trichomes being a deterring factor (0.815 < r < 0.833) (Omori et al., 1983). However, in the present study, the association between glossiness and trichome density is nonsignificant.

In addition, lack of consistent relationships between component traits and resistance traits in different test environments was noticed in the present study (Table 9). In test environment E3 where the shoot fly pressure was high, the magnitude of correlation coefficients was high for association of glossiness, seedling vigour and seedling height with resistance traits including oviposition I (%), oviposition II (%), deadhearts I (%) and deadhearts II (%).

In this study, time to 50% flowering was positively associated with shoot fly resistance in terms of oviposion (%), deadhearts (%) and plant height at maturity.

Based on the above observations the following inferences were made.

- The association of components of resistance established so far depends on the linkage between the genetic factors controlling these traits with shoot fly resistance. The lack of strong association of components of resistance with shoot fly resistance noticed in the present study suggests that genes controlling the resistance component traits are not linked to each other. However, association of seedling vigour and seedling height indicates linkage of genetic factors controlling these traits.
- 2. No significant association was found in environment E2 in which high variability was observed for the traits. This clearly shows that additional components might be involved in controlling resistance.
- 3. Lack of consistency of trait associations in different environments suggests that the associations of components observed depend on the levels of insect pressure.

The degree of association of glossiness with deadhearts damage and oviposition was greater than the associations of trichomes with deadhearts (%) I and II and oviposition (%) I and II.

In the present study there was significant positive correlation between plant height and time to 50% flowering. However, the values of correlation coefficients for this pair of traits varied in different environments (r = 0.33 in E1 and r = 0.70 in E3) indicating the effect of environmental factors. Similarly, high phenotypic correlations of plant height and flowering were previously reported in sorghum (r = 0.76: Rao and Goud, 1979; r = 0.5: Wenzel, 1990; r = 0.79: Lin *et al.*, 1995) and maize (r = 0.40: Koester *et al.*, 1993).

5.3 Inheritance of components of resistance to shoot fly

The continuous distribution of RILs for various shoot fly resistance component traits revealed that most of the traits studied were polygenic except for trichome density (upper surface) that consistently showed skewed segregation across environments. According to Menendez and Hall (1995), the absence of discrete segregating classes for a trait suggests that its inheritance could be determined either by a large number of genes with small effects or a few major genes with substantial environmental effects. The observations made in the present study are supported by previous workers that resistance to *Atherigona soccata* is a quantitatively inherited (Agrawal

and Abraham, 1985) and polygenically controlled (Goud *et al.*, 1983; Halalli *et al.*, 1983) trait. Sharma *et al* (1977) and, Borikar and Chopde (1980) observed continuous variation in different generations and indicated that shoot fly resistance is due to gradual accumulation of resistance genes. For oviposition (%) and deadhearts (%) traits, the high frequency of progenies with high percentages of egg laying or deadhearts damage indicates that shoot fly resistance is under the control of recessive genes. However, varying degrees of damage in the RILs suggests that each line has some favorable and some unfavorable alleles fixed and the more favorable resistance alleles are said to be in dispersion.

The genetic analysis of components of resistance to shoot fly is discussed in detail below.

5.3.1 Heritability

The effectiveness of selection for a trait depends on the relative levels of the genetic and non-genetic causes leading to phenotypic differences among the genotypes in a population and is expressed as the heritability of the trait. The variability observed in quantitatively inherited characters is controlled primarily by genetic constitution of different individuals and due to the interaction of these genes with each other and with environment. Heritability is a useful quantitative statement of the relative importance of heredity and environment in determining the expression of characters (Allard, 1960). The estimates of heritability help the plant breeder in selection of genotypes from diverse genetic populations. Effective selection can be achieved when additive effects are substantial and environmental effects are small, so that heritability estimates are high.

5.3.1.1 Glossiness

Consistently high heritability estimates observed for glossiness in individual test environments and across three test environments indicates that contributions to phenotypic variance due to environmental factors and $G \times E$ interaction are less than genotypic factors. The expression of this character varies little across test environments, and therefore selection for glossiness may be conducted in any environment.

These results are generally consistent with the finding that variances due to $G \times E$ interaction effects for glossiness were low (Table 6). This observation also supports previous

reports that glossiness is simply inherited (Agarwal and House, 1982) and therefore could be used as a simple and reliable selection criteria for resistance (Omori *et al.*, 1983).

5.3.1.2 Seedling vigour

The variability in heritability estimates in individual test environments and across these environments, and at different stages of seedling growth indicates that the character is influenced by environmental factors. Heritability estimates for seedling vigour were moderate to high in individual test environments and across test environments. However, lack of consistency of heritability estimates observed across the environments indicates that selection for seedling vigour can be improved when data from diverse seasons and locations are considered in the analysis. Higher heritability in *rabi* season compared to *kharif* season offers an opportunity to increase the efficiency of selection and this in turn would be helpful in developing materials to withstand shoot fly damage which is severe in postrainy season.

5.3.1.3 Trichome density

Consistently high heritability for trichome density on lower leaf surface $(h^2 > 0.9)$ and upper leaf surface $(h^2 > 0.80)$ in individual test environments and across these test environments indicates that the contributions of environmental factors and G x E interaction towards total phenotypic variance is limited. In other words, high heritability for this trait shows that much of the variation for trichome density is genetically controlled and this character is little influenced by changes in environmental factors. Gibson and Maiti, (1983) reported high heritability estimates for this trait. High heritability estimates observed for trichome density in the present study could be high genetic variance component for this trait.

5.3.1.4 Oviposition

The estimates of heritability were low to moderate ($h^2 = 0.21$ to 0.68) for oviposition percentage and the varying levels of heritability estimates in each test environment and across these environments indicate that ovipositional non-preference is clearly environmentally dependent. It was reported by previous workers that the heritability estimates for total egg count per plant in the F₃ generation was 50% (Halalli *et al.*, 1983) to 80-93% (Borikar and Chopde, 1981a). According to Halalli *et al* (1982), heritability for eggs/plant was low to medium. Under

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high levels of infestation the variability for oviposition is reduced drastically and therefore heritability estimates also decline. Nimbalkar and Bapat (1992) have also reported low heritability for oviposition. However, it was reported that estimates of heritability for this trait were high when material was tested under optimum shoot fly population levels (Borikar and Chopde, 1982; Borikar *et al.*, 1982).

5.3.1.5 Deadhearts

In general, the heritability estimates observed for this trait range from medium to high The results are in conformity with those results obtained by Nimbalkar and Bapat (1992) The moderately high and consistent heritability estimates in two seasons within the same location $(h^2=0.67 \text{ and } 0.85 \text{ for E1} \text{ and E2}, \text{ respectively})$ shows that selection for lower damage levels can be conducted within the same location across the seasons. In other words, selection for shoot fly resistance can be practiced under moderate levels of shoot fly infestation. It is observed that the estimates for shoot fly resistance in terms of deadhearts were reduced as the level of insect pressure increased.

Borikar and Chopde (1980) noticed similar results with heritability of 15% recorded under high to medium infestation. According to Blum (1969b) seedling mortality is dependent on the intensity of insect infestation and hence any data on sorghum reaction to shoot fly must be interpreted with reference to shoot fly population level. Borikar *et al* (1982) reported that the estimates of heritability were higher for seedling mortality when the material was tested under optimum shoot fly population levels. Similarly, Borikar and Chopde (1981a) observed high heritability estimates (75-77%) conditioning this trait. However, Rana *et al* (1975) reported low heritability for resistance to shoot fly. According to Halalli *et al* (1983), the heritability estimate observed in BC₁F₃s on the eighteenth day after germination was 36%.

5.3.1.6 Seedling height

The estimates of heritability for this trait were moderate and quite variable across the screening environments ranging from 0.40 to 0.61. This indicates expression of seedling height is influenced greatly by environmental factors. Halalli *et al* (1983) also reported moderate heritability for this trait.

5.3.1.7 Seedling dry weight

Low heritability observed for this trait across test environments was attributed to low variability observed for the trait in the environments E1 and E2.

5.3.1.8 Pseudostem length

High heritability observed for pseudostem length in environment E2 was attributed to the high genotypic variances noticed for this trait.

5.3.1.9 Days to 50 % flowering

Consistently high heritability observed for this trait in each screening environment and across these environments was attributed to high genotypic variances, low $G \times E$ interaction variances, and low influence of environmental factors.

5.3.1.10 Plant height

Significantly high estimates for plant height at maturity were observed in individual screening environments and across these environments could be owed to high genotypic variances observed for the trait. The high heritability observed for the trait indicated that it was less subjected environmental influences than other characters.

5.3.1.11 Grain yield per plant

The low heritability estimates observed for grain yield across three screening environments was attributed to epistatic gene interactions, $G \times E$ interactions and influence of environmental factors on grain yield.

5.3.2 Transgressive segregation

For any continuously varying character, the expected mean and variance of all possible pure breeding lines derived by inbreeding by single seed descent following an initial cross between a pair of pure breeding lines can be specified in terms of components of means and variances of biometrical genetics (Mather and Jinks, 1971). According to Jinks and Pooni (1976), if an additive (D) and dominance (H) genetic and additive environmental (E) model of gene and environmental action is adequate, then the expected mean is m, the midparent value, and the expected variance is D+E. By adding further components to these simple expectations, we can accommodate the effects of non-allelic interaction, G x E interactions and linkage. Jinks and Pooni (1981) hypothesized that in the absence of epistasis and in the presence of linkage equilibrium, the mean of RILs will be the midparental value (average of the two parents). Epistasis leads to asymmetry in the distribution of derived inbreds relative to the initial inbred parental means. In other words deviation of the mean of the population derived inbreds from the midparental value (either positive or negative) indicates the presence of epistasis. In the present study, an attempt has been made to elucidate the genetic constitution of parental inbreds BTx623 and IS 18551, and the nature of gene action involved in controlling shoot fly resistance components in the RILs based on means (of the parents and their derived RILs) and the appearance of transgressive segregants.

From the predicted mean and variance, Jinks and Pooni (1976) made an attempt to determine the probability of obtaining inbreds that fall outside of the parental range. According to Jinks and Pooni (1976), in the presence of epistasis the mean of inbreds is still m, the mean of the original pair of parents now being m+i. If i is positive the probability of a derived inbred exceeding the higher scoring parent P1 will be less than the probability of a derived inbred falling short of the lower scoring parent P2. This situation matches that observed in the present study. In general, for traits with RIL means less than the midparental value, the proportion of RILs for those outside the low scoring parent was greater than that outside the higher scoring parent and vice versa. In no case was the expectation of equal frequency of inbreds lying outside the parental limits of P1 and P2 observed. This shows that for each trait observed there were epistatic interactions influencing trait expression. Further the occurrence of transgressive segregants indicates that the two parental lines of the RIL population both carried desirable and undesirable alleles at various proportions of loci governing the various traits observed.

For most of the traits studied the mean of RIL population resembled more closely the midparental value than the means of either parent indicating polygenic inheritance with no major genes. The nonsignificant difference observed between the RIL population means and the higher scoring parent for trichome density (upper surface) was due to the high proportion of transgressive segregants falling outside the higher scoring parent. Similarly, for time to 50% flowering, nonsignificant differences between the RIL population mean and the lower scoring parent were due to a high proportion of observed transgressive segregants falling outside the lower scoring parent segregants falling outside the NIL population mean and the lower scoring parent were due to a high proportion of observed transgressive segregants falling outside the lower scoring parent.

Using extreme segregants from the RIL population in an applied breeding programme, it would be possible to develop genotypes with levels of resistance comparable to or even higher than that of the resistant parent.

5.3.2.1 Glossiness

Though the distribution of the glossiness trait is continuous, the roughly bimodal distribution of phenotypes observed indicates that this glossiness trait is quantitative in nature and is controlled by major loci. However, it was reported by previous authors that the character glossiness is controlled by a single recessive gene (Tarumoto, 1980). A major gene will have major effect that will be larger than that arising from non-heritable agencies, their effects will be well precipitated in the phenotypic expression of a trait. This is also supported by the consistency of high heritability estimates observed for glossiness across the environments.

The observation that no transgressive segregant RILs were recorded with phenotypic scores falling outside the high intensity (low scores) of glossiness of the resistant parent indicates that the alleles for this trait are predominantly in coupling phase. The positive deviation of the RIL population mean for glossiness from the midparental value indicates the presence of epistasis. The high mean value of RILs, approaching that of the high scoring parent (BTx623) indicates that the frequency of RILs with high scores (indicative of low intensity of glossiness) was greater than that of intensly glossy individuals (Figure 3).

The frequency distribution of RILs approximated to the ratio of 9: 6: 1 (non-glossy: intermediate: high intensity of glossiness) suggests the glossiness trait is under the control of major loci and high intensity of glossiness is due to recessive loci. The varying intensity of glossiness indicates action of both favorable and unfavorable alleles at different loci. The varying degrees of intensity of glossiness also indicates allelic substitution has occurred by recombination. Deviation from the expected near normal frequency distribution of RILs in these three phenotypic classes clearly indicates the presence of epistasis. The lower frequency of RILs with a high intensity of glossiness is therefore due to recessive nature coupled with epistatic gene interactions. Because, as the number of genes controlling the trait increases the probability of obtaining the individuals homozygous for favorable alleles at all the concerned loci will be

reduced. The low probability of getting recombinants with favorable alleles at all the loci could be the major reason for non-recovery of transgressive segregants for even higher intensity of glossiness than that found in glossy shoot fly resistant parent IS 18551.

5.3.2.2 Seedling vigour

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The equality of the means of the RIL population and their midparental value indicates that additive gene action is controlling the trait and the genes are in linkage equilibrium (i.e., they are not linked). The higher proportion of transgressive segregants observed in the initial seedling growth stage and their reduction in proportion in the later growth stage might be due to gene interaction (additive x additive) and $G \times E$ interaction.

5.3.2.3 Trichome density on lower leaf surface

One parent of the cross from which the RILs have been derived has high trichome density and the other has no or very few trichomes. The frequency distribution of RILs for trichome density (no./mm²) approximated to the phenotypic ratio of 1: 7 (no trichomes or very low trichome density ranging from 1-4: low to high trichome density). These results indicate that trichome density is under the control of major loci. High trichome density is due to presence of excessive favorable alleles coupled with epistatic interaction, and no trichomes or few trichomes are due to homozygous unfavorable alleles at several loci. The varying density of trichomes indicate the action of both favorable and unfavorable alleles at different loci. However, Ayyangar (1942) listed hairiness of the leaf tip and of midrib edges in sorghum as each being controlled by a single locus with hairiness being dominant.

The mean of the RIL population was nearly equal to the midparental value indicating the presence of additive gene action and that the alleles are in linkage equilibrium. The appearance of transgressive segregation in RILs might be due to complementation of favorable and unfavorable alleles received from both the parents. In other words, both types of alleles (favorable/unfavorable) are in association in each of the parents. Due to complementation of positive and negative alleles in the F_1 and subsequent recombination events, the individuals with higher than the parental proportion of favorable alleles have been observed as transgressive segregant RILs having trichome density higher than that of the high scoring parent. The presence of additive gene action supplemented by additive x additive gene interaction in these RILs might

be the main reason for occurrence of these transgressive segregants with trichome densities higher than the high scoring parent.

Occurrence of transgressive segregants might also be due to overdominance of heterozygous loci. It is probable that some small (circa 3%) amount of heterozygous loci still remain in the F_6 generation. The possible explanation for retaining more heterozygosity even in the later stage of selfing generations could be occurrence of crossover suppressors due to inversion.

5.3.2.4 Trichome density on upper leaf surface

The frequency of lines with no trichomes (similar to susceptible parent BTx623) was high. This and the appearance of the transgressive segregants, indicates the presence of alleles in repulsion in F_1 . Following are some of the inferences drawn based on the results observed:

- 1. The discontinuous distribution or non-normality of distribution indicates that the character is under the control of major genes. Jinks and Pooni (1981) also reported that kurtosis will occur if few gene loci contribute to the phenotypic distribution.
- 2. High trichome density in same individual RILs might be due to the presence of multiple loci with favorable alleles. Genotypes with medium density might be having both favorable and unfavorable alleles at different loci, so that some will have increasing and others having decreasing effect and thus giving the expression of medium trichome density.
- 3. The deviation of the RIL population mean from midparental value indicates the presence of epistatic gene action. The equality of means of the RIL population and the resistant parent (P2) clearly shows the epistatic gene interaction occurs in a positive direction, favoring high trichome density.
- 4. Using simulation studies, Pooni et al (1977) demonstrated that directional epistasis and G x E interaction are the two major causes of non-normality in the distribution of homozygous breeding lines. However, the consistency of the frequency distribution

across screening environments and nonsignificance of variances due to $G \times E$ interaction observed in the present study further confirms that expression of this character is mainly under the control of genetic factors and influence of environmental factors on it is less. It also shows that inter-allelic gene interaction for expression of this trait is little influenced by changes in environmental conditions.

- 5. It is reported by Pooni *et al* (1977) that non-normal distributions demonstrate the effect of epistasis if they are based on a heritability of one $(h_n^2 = 1.00)$ and therefore do not take into consideration the important influence of the environment and G x E interactions. In the present study the heritability was less than 1.00 and the observation of nonsignificant G x E interaction variances clearly shows that the decrease in heritability estimates below 1.00 for this trait is due to gene interaction.
- 6. The presence of few trichomes in the resistant parent can be explained in terms of preponderance of favorable alleles and the presence of a few unfavorable alleles and their interaction have resulted the expression of low trichome density.
- 7. The occurrence of transgressive segregants is due to additive x additive gene interaction. This indicates that favorable alleles might be in dispersion in the parents. The degree of transgressive segregation depends on the degree of dispersion of the interacting alleles in the parental alleles. The performance of parents and RILs in the present study shows that there is preponderance of unfavorable alleles in susceptible parent and preponderance of favorable alleles in the resistant parent. When the favorable alleles from both the parents are inherited together the interaction between them might have resulted in higher trichome density than the high scoring resistant parent.

5.3.2.5 Oviposition

The continuous distribution observed for this trait suggests the action of polygenes. According to Halalli *et al* (1983) observed that F_3 progenies were more variable and F_4 progenies less variable for egg count per plant. It was observed in the current study that the mean of the RIL population was higher than the midparental value for observations in both seedling growth stages. This indicates the operation of epistatic gene interaction in the controlling the trait, and that this epistasis favor high oviposition levels at both growth stages. In addition, the appearance of transgressive segregants for both oviposition I (%) and oviposition II (%) indicates the presence of favorable loci in both of the parents. As expected the proportion of transgressive segregants with values higher than high scoring parent (P1) was high, which is due to additive genetic effects, and additive x additive gene interaction effects.

5.3.2.6 Deadhearts

The deviation of RIL population means from the midparental values was observed for this trait at both the stages of observation. This indicates that epistatic gene interaction operating for the trait. It was observed that none of the RIL entries showed deadhearts (%) values lower than the resistant parent. However, the appearance of transgressive segregants with values higher than the high scoring (susceptible) parent (P1) shows that epistatic gene interaction favors higher degree of susceptibility. Therefore, lower magnitudes of variances observed for this trait are due to any one or combination of the following:

- 1. The genes in linkage equilibrium and dispersion
- 2. Involvement of many genes each with small effects;
- 3. The environmental influence;
- 4. G x E interaction;
- 5. Combination of any of the above

The reasons for lack of transgressive segregation for deadhearts (%) values lower than the resistant parent could be, firstly, the two parents might be differing for few genes at resistance loci. Secondly, the resistance alleles in the resistant parent are not in association. In other words, resistant parent contains alleles similar to the susceptible parent at some resistance loci. Alternate reason could be many genes of small effect followed by epistatic interactions favoring susceptibility. However, Hallalli *et al* (1983) reported transgressive inheritance of shoot fly resistance in which five BCF₃ progenies, one F₃ progeny and three F₄ progenies were more resistant than the highly resistant parent IS 5604. F₃ progenies were more variable and F₄ progenies the least variable for deadhearts incidence (Halalli *et al.*, 1983). The extent of damage varied depending on the number and strength of favorable genes for each of the components present in an individual RIL (e.g., glossiness) and interaction between these factors.

5.3.2.7 Seedling height

The equality of the population mean of RIL population and the midparental value indicates that alleles are in linkage equilibrium and variation is due to additive gene action. The high proportion of transgressive segregants observed for seedling height indicates that the favorable alleles are dispersed in the two parental lines. The transgressive segregation is due to complementation of alleles received from both parents and subsequent recovery of individuals with larger number of favorable alleles in coupling phase due to recombination events during the process of inbred line development. The narrow range observed for seedling height also suggests that favorable alleles might be in repulsion phase coupled with low frequency of recombination in RILs.

5.3.2.8 Pseudostem length

The lower mean of RIL population compared to the midparental value for pseudostem length indicates the presence of epistasis favoring short pseudostem length. This is further evidenced in the high proportion of transgressive segregants with values lying outside that of the low scoring parent P1 (BTx623). The results also show that the alleles for this trait are in linkage equilibrium and are in repulsion phase. Due to epistasis and recombination of unfavorable alleles in repulsion phase from the two parents, these short pseudostem length transgressive segregants have been observed.

5.3.2.9 Days to 50% flowering

The nonsignificant difference between the population mean of flowering time of the RILs and the phenotypic value of the lower scoring parent (BTx623) indicates that a higher proportion of RILs had flowering time values near to earlier flowering parent. This in turn shows that control of maturity involves epistasis. The appearance of transgressive segregants indicates that alleles controlling flowering time might be present in repulsion phase in the two parents and expression is due to accumulation of favorable alleles for early maturity in the RILs during the process of inbreeding. Because of this genetic nature, the proportion of RILs with flowering time similar to or less than the earlier flowering parent was high. The high proportion of these individuals is therefore due to recombination of alleles favoring early flowering from both the parents and also some amount of epistatic gene action favoring early flowering.

5.3.2.10 Plant height

The higher mean plant height of RILs may be due to transgressive segregants with values lying outside the taller parent P2 (IS 18551). The low frequency of appearance of such transgressive segregants indicates that most of the alleles might be associated in coupling phase. Some parental alleles might be in repulsion phase, the result of which is limited opportunity for transgressive segregants.

5.3.2.11 Grain yield

The results indicate that alleles for grain yield are in linkage equilibrium, present in repulsion phase and that are acting epistatically. The appearance of transgressive segregants might be due to recombination of alleles received from the parents.

5.3.3 Predicted genetic gain

Improvement in the mean genotypic value of selected plants over the parental population is known as genetic advance. This is the measure of genetic gains from selection. The success of genetic gain under selection depends on three major factors: genetic variability, heritability and selection intensity (Allard, 1960). It was also suggested by Johnson *et al* (1955) that heritability estimates along with genetic advance would be more useful in predicting the performance under phenotypic selection than heritability estimates alone.

The speed of selective advance depends on the number of units of inheritance that contribute to additive genetic variance and non-additive genetic variance. The greater the proportion of additive heritable variation, the more effective is the selection (Mather and Jinks, 1982). In RILs, since they are homozygous, additive component of genetic variance that contributes to total genotypic variation for which selection should be effective. Gain from selection estimates revealed that single trait selection could improve various components of shoot fly resistance. However, a form of multiple trait selection would likely be necessary to improve shoot fly resistance itself.

The selection gain (%) over the RILs mean was estimated for each trait and the results obtained are discussed below.

5.3.3.1 Glossiness

High heritability alone does not guarantee large gain from selection unless sufficient additive gene action is present to permit genetic advance (GA). High heritability coupled with moderate GA observed for glossiness revealed the importance of both additive and non-additive effects for this trait. It was observed that the genetic gain predicted based on a standardized selection differential of 5% was higher during the postrainy season. The predicted genetic gain for high glossiness intensity was higher in screening environment E2 based on standardized selection differential as well as when the top 5% of RIL individuals for high glossiness were considered. This is also corroborated by low mean value (i.e., high intensity of glossiness) observed for the trait in screening environment E2.

A high percentage gain was estimated for glossiness intensity when RIL mean performance over the three environments was considered in the analysis. This high selection gain was due to high estimates of heritability observed for the trait. In general, the predicted genetic gain for this trait was higher in E2 followed by E3 and E1.

5.3.3.2 Seedling vigour

The estimates of predicted genetic gain for seedling vigour were higher in screening environment E2 at both seedling growth stages. However, high genetic gains predicted for seedling vigour II were attributed to high genotypic variances observed for this trait. Significant differences observed between the means of the lowest and the highest groups for this trait in each environment suggests improvement of the character by selection will be effective.

5.3.3.3 Trichome density on lower leaf surface

The estimates of predicted genetic gain (%) over the mean of RILs for this trait were high and consistent in each of the screening environments. High genetic advance estimates for the trait is attributed highly significant genetic variation. Highly significant differences were observed between the means of the lowest and the highest groups in each screening environment which also suggests that improvement should be possible by selecting extreme individuals. Single-trait selection based on mean performance apparantly will be useful in accumulating favorable genes for the trait under selection.

5.3.3.4 Trichome density on upper leaf surface

The estimates of predicted genetic gain were high and significant in the postrainy season. Significant differences were observed between the means of the lowest and the highest groups in each screening environment. This suggests that improvement in this trait should be possible by selecting extreme individuals.

5.3.3.5 Oviposition

Oviposition non-preference is the prevalent mechanism of resistance to shoot fly in sorghum and leaf trichomes on the seedling leaves contribute to this (Maiti *et al.*, 1980; Maiti and Gibson, 1983). The percentage selection gain over RILs mean was higher for oviposition I compared to oviposition II in all the screening environments. This suggests that selection would be most efficient for ovipositional non-preference in the initial stage of seedling growth. Significant differences observed between the means of the lowest and the highest groups in each screening environment indicate that improvement should be possible for the trait by selection of extreme individuals. The estimates of predicted genetic gain for oviposition I were significant in selective environment E2 (with moderate shoot fly pressure), where as Borikar *et al* (1982) reported that estimates of genetic advance were higher for oviposition when the material was tested under optimum shoot fly pressure. Blum (1972) opined that there has been skepticism on the practical value of non-preference in developing resistant varieties. However, Busbice *et al* (1968) reported a successful example where non-preference has been made use of in the development of weevil resistant alfalfa varieties inspite of environmental interaction.

5.3.3.6 Deadhearts

The percentage selection gain predicted over mean was higher for deadhearts I than deadhearts II in all three screening environments. This suggests that selection should be carried out in the initial stage of seedling growth where the genetic variation is high for this trait. Highest estimates of genetic advance were observed for this trait in the screening environment E2 with moderate shoot fly pressure, where as Borikar *et al* (1982) reported that the estimates of genetic advance were higher for seedling mortality when the material was tested under optimum shoot fly population. These observations suggest screening under several levels of shoot fly population during selection for improved levels of resistance.

The moderate heritability estimate for deadhearts (%) was higher than expected for field evaluation of this complex trait, indicating that selection for improved resistance levels based on phenotypic response in the field should be successful for this population than the general literature sorghum shoot fly resistance suggests.

5.3.3.7 Seedling height

The higher estimates of prediction genetic gain percentage observed for seedling height I compared to seedling height II in all the environments suggests selection for seedling height should be more effective in the initial stage of seedling growth. The relatively low estimates of predicted genetic advance observed for this trait can be attributed to low variability observed for the trait in both seedling growth stages.

5.3.3.8 Seedling dry weight

In general, predicted genetic gain observed for seedling dry weight was low. The low estimates can attributed to low genetic variation and low heritability estimates observed for the trait. However, higher estimates were observed in screening environment E2 than in E1.

5.3.3.9 Pseudostem length

Highest predicted gain for increased pseudostem length among the selected individuals was observed in screening environment E2. This is attributed to high genetic variation and heritability observed for the trait in this screening environment with relatively low shoot fly pressure.

5.3.3.10 Days to 50% flowering

Though the heritability estimates observed for flowering time were high, the predicted genetic gain for early maturity was low. This can be attributed to the limited genetic and phenotypic variation observed for the trait.

5.3.3.11 Plant height

High estimates of predicted gain (%) over mean were observed for the trait. However, higher estimated gains were observed for plant height among the selected individuals from the environment E1 than those selected in E2 and E3.

5.3.3.12 Grain yield per plant

The estimates of selection gain (%) over the RILs mean predicted based on standardized selection differential were low. This could be attributed to low phenotypic variation.

5.3.4 Correlated genetic gain

It was reported in previous studies that glossiness is a simply inherited trait and can be used as an indirect selection criterion for shoot fly resistance. It is evidenced in present study that glossiness has high heritability, has a significant negative correlation with shoot fly resistance, and is genetically controlled by major loci. The predicted correlated genetic gain was negative and high for shoot fly resistance (measured in terms of deadhearts) when glossiness was used as an indirect selection creiterion. In addition, predicted correlated genetic gain when using glossiness as an indirect selection criterion was positive with other shoot fly resistance components such as seedling vigour, trichome density, seedling height and plant height. In general, correlated genetic gain was high in screening environment E3 (with high shoot fly pressure) when glossiness was utilized as a selection creiteria. This shows the need to practice selection under high shoot fly population pressure. The importance of shoot fly population in selection for resistance was also supported by previous authors (Borikar *et al.*, 1982; Rana *et al.*, 1975 and Rao *et al.*, 1974).

However, with respect to trichome density, although the estimates of GCV, heritability and predicted genetic gain were high, the correlated genetic gain using indirect selection criteria was negative with other components like seedling vigour II, seedling height and plant height, while correlated genetic gain was positive for glossiness with these traits. Therefore,

1. For improvement of shoot fly resistance, glossiness may be used as reliable indirect selection criterion.

- 2. For maximum gain for shoot fly resistance phenotypic selection for glossiness combined with marker-assisted selection for other components with low heritability will be effective.
- 3. For incorporation of both high intensity of glossiness and high trichome density, lines with high trichome density and high intensity of glossiness may be intercrossed followed by selection for both the traits. For this individual trait selection may be feasible as first step in breeding programme.
- 4. The correlated genetic gain for glossiness with other traits was high in screening environments E1 and E3, which represent optimum to high shoot fly pressure. Therefore glossiness appears to be more important than trichome density. High heritability of glossiness would facilitate selection for this component of resistance. If the intensity of glossiness is controlled by major loci the segregates with high glossiness intensity could be selected easily in a breeding programme. This would be an effective way to recover improved resistance to shoot fly in segregating generations involving one parent with high glossiness intensity. By backcrossing, the high glossiness intensity could be transferred to shoot fly susceptible genotypes having other desired characters (components of resistance and agronomic characters).

5.4 G x E interaction

The evaluation of genotypes in different environments plays an important role in breeding programmes in the environmental adaptability of genotypes. The factor that dictates testing of genotypes in a plant breeding programme is the existence of $G \times E$ interaction. The detection of significant $G \times E$ interaction is common where diverse genotypes are tested under a wide range of environmental conditions. While determining selection strategy, $G \times E$ interaction is considered as an important genetic parameter because it reduces average progress across environments from selection in many single environments. Selection involves ranking of genotypes. The ideal situation for the plant breeder is that the rank orders of genotypes are constant across the environments, so that best genotype in one environment is also best in all other environments. In real applications, this is generally not the case. Any deviation from the ideal situation of identical rank orders is the result of $G \times E$ interactions or imprecision in measurements as a result of experimental errors. If the environments are similar, the more similar the rankings of the tested genotypes are also similar. That means the distance between the two

environments can be quantitatively expressed in terms of Spearman's rank correlation coefficient between the rankings of genotypes in these environments. The present study is an effort to analyse the RILs for relative shoot fly resistance under three levels of infestation. Planting the material in three environments (involving three seasons in two locations) created three levels of infestation. The three environments provided a sufficient range of shoot fly infestation for evaluation of genotypes.

G x E interaction from the analysis of variance were found to be significant for all the traits studied except trichome density on upper leaf surface (Table 6). This indicated that the genotypes reacted differently with changing levels of shoot fly population (or other aspects of the test environments such as temperature and photoperiod). Therefore, ranking of the genotypes was not consistent over the three screening environments.

Spearman's rank correlation coefficient measures the similarity of the ranking of RIL genotypes pairs of screening environments. Closer inspection of $G \times E$ interaction revealed that the causes were primarily related to magnitude changes but also included genotype rank changes. However, the interaction variances observed were small relative to the genetic variance in all the cases.

The rank correlations were significant among all paired combinations of screening environments for all observed traits except seedling vigour II and oviposion II between E2 and E3. This indicates similarity of rankings of genotypes for all the traits except seedling vigour II and oviposition II between E2 and E3. However, the varying magnitudes of rank correlation coefficients reveal the change in degree of similarity in rankings between the environment combinations. As expected the magnitudes of correlation coefficients were low to moderate for direct measures of shoot fly resistance (oviposition% and deadhearts%). It is clear that these traits are much influenced by environmental factors. However, significantly high correlation coefficients were noticed for glossiness, trichome density on upper and lower leaf surfaces, and plant height measured in the different screening environments.

To identify the most resistant RILs, they were ranked based on mean performance across the three screening environments (Table 17d and 17e). The desired genotypes will be those that

5.5 Molecular analysis

5.5.1 Parental polymorphism

The two parents of the mapping population were screened for polymorphism using SSR primers. A total of 96 SSR primer pairs were used to identify the DNA polymorphism. About 80% of the primer pairs detected polymorphism between the parents, BTx623 and IS 18551 However, only about 49% of the markers detected gel scorable polymorphism (Appendix III) It is clear from these observations that the amount of polymorphism detected by SSRs satisfies the criteria of parental polymorphism at the DNA level. For in the absence of DNA polymorphism, segregation analysis and linkage mapping are impossible (Young, 2001).

5.5.2 Marker segregation and segregation distortion

Skewed segregation of alleles at marker loci detected throughout the genome. About 36% of the markers showed the deviation from 1:1 ratio of each of homozygous classes for most of the markers in the RIL population as a result of overabundance of homozygous IS 18551 alleles (Table 18). This indicates that the selection was favored for IS 18551 alleles even though care was taken during the development of the RILs by randomly selfing the plants without subjecting to any kind of selection. The segregation distortion probably resulted from cumulative effect of selection against alleles of one of the parent (i.e., BTx623) during the generation advancement of the RILs. This might be due to differential survival of the individuals in response to natural infestation by shoot fly due to the action of alleles of the resistant parent IS 18551. However, strongest deviation was observed for Xtxp10 followed by Xtxp40 containing preponderance of BTx623 alleles, which could be due to selective abortion of gametes containing IS 18551 alleles

The skewed segregation favoring IS 18551 (male parent) alleles observed for majority of markers. A similar observation was reported in an RFLP map using RILs obtained from a wide cross in cultivated rice (Wang *et al.*, 1994) in which an intraspecific recombinant inbred population (Co39/Moroberekan) was reported to have 98.8% of marker loci showing skewness towards the indica parent. Segregation can occur due to gametic selection especially among male gametes of the selfed F_1 plant that contribute to F_2 seed production through selective influence of gynoecium including genetic incompatibility, environmental effects and differential competitive ability of genetically variable pollen (Lyttle, 1991; Xu, 1997). This kind of segregation distortion with skewed marker biased towards the alleles from a male parent has also been reported in pearl

show low mean for deadhearts (%) and oviposition (%) and minimum sensitivity to changing shoot fly infestation. Since the line that shows low damage with respect to deadheart percentage is a desirable character in sorghum, the information on the best line in terms of consistency of low damage across the environments could be drawn from mean values and complicated statistical computations could be avoided. These lines could be used in the breeding programme. Sharma *et al* (1977) reported that susceptible parents were generally poor combiners while resistant parents were better combiners for eggs/plant and deadhcarts (%).

The performance of lines for glossiness and trichome density in different environments was closely related (r(s) = 0.9) (Table 15). Though variances due to G x E interaction variances were significant for these traits, it may be concluded that G x E interaction for these traits was small and probably of limited importance. These observations are also supported by the fact that the lines found in common between the environments (at highest 5%) were also ranked high (at highest 5%) when averaged over three environments (Table 16a and 16b). These lines may be utilized in crossing programmes to incorporate high level of expression of these resistance components traits in other cultivars. In addition, the lines that were ranked high in all the screening environments will be highly useful in shoot fly resistance breeding programmes. Considerable variation in rank correlation coefficients was observed for seedling vigour in different environment combinations. This suggests that the phenotypic expression of polygenic characters is subjected to considerable modifications by differences in the intangible environments to which the members of the RIL population were exposed.

It is clear that the effect of environmental factors and presence of $G \ge E$ interaction reduce the estimates of heritability. However, based on simulation studies, it was suggested by Piepho (1996) that low repeatability may be partly due to statistical errors in the stability estimates, which are best reduced by including in the analysis as many genotypes and environments as possible. This was also supported by Becker (1987) who demonstrated based on Eberhart and Russell (1966) that heritability may be improved by increasing the number of years and number of locations. millet cross LGD x ICMP 85410 (Liu *et al.*, 1994). Segregation distortion is most commonly observed in interspecific crosses, however this study supports the occurrence of the phenomenon in sorghum intraspecific crosses.

The occurrence of skewed segregation is one of the limitations in the map obtained in these RILs. Because it may affect both the establishment of linkage groups and estimation of recombination frequencies. Calculations of linkage distance usually assume no segregation distortion and use recombination fraction of 0.499 for rejection of linkage. According to Wang *et al* (1994) skewed segregation decreases the recombination fraction used to reject linkage and limits RILs to detect linkage among closely linked markers. Skewed segregation could also cause over-estimation of recombination frequency between linked markers (Paran *et al.*, 1995).

5.5.3 Genome Composition of RIL population

Distribution of parental alleles for each marker locus and each line were roughly symmetrical around 0.5 (Figure 4C-D) suggesting no overall bias towards either parent. Huang *et al* (1997) also reported similar results for IR 64 alleles in rice.

The average RIL was heterozygous for 0.7% of the scored marker loci with a range of 0.0 - 4.7%. The observed heterozygosity percentage in the RIL population after six generation of selfing was closed to the expected 1.56%. Heterozygosity of 1.6% and 2.7% was reported for maize RIL populations (Burr and Burr, 1991) and 0.42% for *Arabidopsis* RILs (Lister and Dean, 1993). According to Burr and Burr (1991), although lines will become homozygous, some regions of genome tend to stay heterozygous longer than expected from theory (Burr and Burr, 1991).

5.5.4 Construction of Linkage map

Though the SSR markers were initially selected based on clear polymorphism between the parents, final selection of markers for use in mapping was based on linkage distance of 20 cM in the map of Bhattramakki *et al* (2000) since most of the SSR markers available at present have been mapped previously. The reason to select the markers with linkage distance roughly of 20 cM as a limit of resolution that what is detected as QTL is a segment of choromosome of this length that may contain several loci affecting the trait not necessarily in the same direction. Thus, more QTLs are likely to be detected when the alleles are in association than when they are in dispersion (Tanksley, 1993). However, the marker analysis could not establish linkage relationships for most of the marker loci. This is because of small population size used, coverage by the markers used, lack of markers linked to these marker loci and limited genome coverage by the markers used.

5.5.5 QTL mapping

Assessing co-segregation of the markers (based on recombination frequency of marker loci) and the phenotype gives the information on the location of a QTL between these flanking markers. If the proportion of recombinants with higher or and lower phenotypic values is less, this indicates the tight linkage of flanking markers and QTL is indicated.

5.5.5.1 QTL analysis in single environment

Single-environment analysis was made to detect QTLs for components of resistance to shoot fly. QTL analysis detected chromosomal regions controlling glossiness, seedling height, seedling vigour, deadhearts (%) and grain yield. The results obtained are discussed here under.

5.5.5.1.1 Mapping QTLs for glossiness

A QTL detected for glossiness on LG J within marker interval Xtxp94-Xtxp65 (10 cM) had a large effect on glossiness in each screening environment explaining 34.4 to 46.5% of the observed phenotypic variation for this trait. Therefore, it is assumed that this QTL represents a major gene for glossiness. Strikingly, this region mapped to QTL for deadhearts (%) in the environment E3 (that represents high shoot fly pressure). The QTL identified for glossiness may therefore be considered to have candidate gene for shoot fly resistance. The identification of a QTL explaining a high proportion of the phenotypic variance indicates a strong association between genotype and phenotype. According to Terwilliger (2001), if the test locus genotype-phenotype relationship is strong the power of QTL identification is solely a function of the strength of linkage relationships.

To assess the effects of a specific locus on the trait, the percent of phenotypic variation under the control of each locus is used as a parameter. According to classification of major and minor gene traits in rice by Mackill and Junjian (2001), QTLs controlling 25-50% of variation would be classified as a major QTLs. In the present study the QTL controlling glossiness accounting for 34.4 to 46.5% of phenotypic variation will be considered a major QTL (Table 22) The rest of the QTLs controlling other traits (seedling vigour, seedling height, deadhearts (%) and grain yield) explaining 2.5 to 15.8% phenotypic variation may be categorized as minor QTLs.

The identification of a QTL for glossiness explaining a high proportion of observed phenotypic variance in different environments confirms the high heritability of this character and the low degree of influence on it by environment and G x E interaction. This is also in agreement with observation of genetic control by a recessive gene with major effect for glossiness and observation of few RIL individuals with high levels of glossiness. The results are also partly in conformity with reports by previous workers that the character glossiness is simply inherited (Agarwal and House, 1982), controlled by a single recessive gene (Tarumoto, 1980) and highly heritable. Therefore, glossiness can be used as simple and reliable selection criteria for resistance (Omori *et al.*, 1983).

The negative additive effects by QTLs for glossiness show that alleles from IS 18551 have an effect towards increasing the intensity of glossiness. The variance explained by QTL was higher in screening environment E2 (46.5%) followed by E3 (37.9%) than in E1 (34.4%) This was also confirmed by high phenotypic variation explained by QTL based on combined analysis across E2 and E3. It may be concuded that since expression by the QTL is high in postrainy season across the two testing locations, the screening for glossiness may be conducted effectively during the postrainy season. It was reported previously that the glossy trait is a characteristic of most of the winter (*rabi*) sorghum varieties of India (Blum, 1972; Rao *et al.*, 1978) and is associated with shoot fly resistance (Blum, 1972; Maiti and Bidinger, 1979; Taneja and Leuschner, 1985; Omori, *et al.*, 1988). In addition, Taneja and Leuschner (1985) reported that glossiness contributed less to shoot fly resistance during the *rainy* season.

Since glossiness is highly heritable and can be assessed visually and economically, the marker-assisted selection for this trait may be questioned. However, the use of marker-assisted selection can be justified for following two reasons:

			% Phenotypic variance	
Character	Trait	Segregation	explained	Classification
Glossiness	Quantitative	Continuous (bimodal distribution)	34.4 - 46.5	Major QTL
Seedling height	Quantitative	Continuous	2.7 - 5.1	QTL
Seedling vigour	Quantitative	Continuous	4.8 - 5.7	QTL
Deadhearts (%)	Quantitative	Continuous	15.8	QTL
Grain yield (g/pl)	Quantitative	Continuous	7.8 - 13.3	QTL

 Table 22. Classification of quantitative shoot fly resistance traits and component traits

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1. As observed in this study, glossiness is under the control of recessive loci, that interact together in controlling this phenotype. Therefore, the expected frequency of individuals with a high intensity of glossiness will be low, as evidenced in the present study. Previous studies on screening for glossiness also found low frequencies of glossy individuals in germplasm (Maiti et al., 1984) and breeding material or derived lines. In breeding for improved shoot fly resistance in sorghum male-sterile lines at ICRISAT, Patancheru, among the 153 and 206 genotypes evaluated in kharif and rabi seasons 2001, none was recorded with score 1 (highest glossiness intensity). Out of 206 advanced breeding lines evaluated in rabi season 2001, only 8 lines were scored as 2. During phenotypic screening of germplasm lines or segregating lines for glossiness, non-glossy (including lines heterozygous for one or more glossiness genes) may be rejected. In such a case, screening with the co-dominant SSR markers linked to genes controlling the trait could be followed to separate heterozygous non-glossy from homozygous non-glossy individuals. Recovery of lines with a high intensity of glossiness is needed because of the strong association of this trait with shoot fly resistance (observed in the present study) and its role in abiotic stress tolerance (e.g., drought tolerance) (reported by Maiti, 1996). Heterozygous non-glossy lines may be selfed or intercrossed to recover glossy lines.

2. Based on phenotypic performance, RILs were grouped as lines with high intensity of glossiness, lines with intermediate levels of glossiness and non-glossy lines. Despite the oligogenic nature of this trait, these true breeding homozygous lines could be so categorized based on phenotypic evaluation alone. Eliminating the non-glossy individuals, the glossy lines can be used further in the breeding programme to incorporate characters lacking/or having low levels of expression. However, because its expression is under the control of several genes that interact and appropriate alleles at each of the loci concerned must be homozygous, and the expected frequency of individuals with high glossiness intensity will be low (as observed in the present study). The RILs with intermediate glossiness expression that are having other desired characters could there fore be used as recurrent parents in a backcross breeding programme to recover lines with a high intensity of glossiness as well as other desirable traits. In these lines the dispersion of QTLs for glossiness is expected. Marker-assisted backcross breeding will therefore be helpful to recover lines with favorable alleles of all the QTLs for glossiness in addition to these QTLs for other desired traits.

5.5.5.1.2 Mapping QTLs for other component traits

It was proposed that if there are large numbers of QTLs controlling the trait, individual QTLs each account for smaller proportions of genetic variation (Kearsey and Farquhar, 1998). The low phenotypic variance explained by QTLs detected for seedling vigour, seedling height, deadhearts (%) and grain yield clearly indicate polygenic inheritance of these traits.

Varying levels of expression observed for the traits in different environments suggests action of different genetic factors controlling the trait in different environments. This is supported by identification of different QTLs controlling the traits seedling vigour and seedling height in different environments. Positive and negative additive effects of QTLs controlling these traits indicate that alleles causing increased expression were inherited from both the parents. The action of alleles from both the parents towards increasing the trait expression in different environments supports the concept of allelic dispersion. The presence of allelic dispersion in turn supports the occurrence of transgressive segregants for these traits. Xu (1997) reported that when genetic stocks with dispersed QTLs are used as the parents to produce a segregation population, a part of progeny of the resulting progeny will have transgressive phenotypes; that is, they are phenotypically outside the range of the parents because these progeny associate more alleles of similar directional effects as the result of recombination of different QTL alleles.

Mapping QTLs for deadhearts percentage and glossiness to the same region (Xtxp94-Xtxp65) on linkage group J supports the significant and high degree of correlation coefficients observed for the two traits. In this region, alleles of IS 18551 increased both the intensity of glossiness and shoot fly resistance (measured in terms of deadhearts percentage) in screening environment E3 where high shoot fly pressure was noticed.

5.5.5.2 QTL analysis across the environments

The aim of multi-environment trial analysis is to obtain the most precise estimates of 'genotype performance to use in the subsequent mapping procedure. It was opined by Tao *et al* (2000) that multiple environment testing was very helpful for correctly identifying QTLs associated with the stay-green trait (a component of terminal drought tolerance) in sorghum. The success of marker- assisted selection will be influenced by the consistency of expression of the genes linked to markers across the environments. It was also suggested by Kang (1998) that it is

desirable to use multi-environment testing to determine the phenotype of the trait, for which expression varies amongst the environments. According Xu (1997) the larger the environmental effect on the character (i.e., low heritability), the less likely a QTL will be detected. It was also opined by Xu (1997) that estimates of heritability can be improved by controlling environmental error. Importance of use of multiple environments for QTL mapping was demonstrated by Paterson *et al* (1991) who showed that only four out of 29 QTLs identified for fruit characters in tomato were detected in all three screening environments used. These results point to the need for a mapping population can be replicated and evaluated in multiple environments.

Many studies of QTL mapping have used only one or a limited number of screening environments for evaluation of mapping progeny phenotype. These studies have therefore ignored the G x E interaction that exists for quantitative traits. The Q x E interaction analysis provides an estimation of the percentage of the genetic variance explained by the QTL and an estimation of Q x E interactions if the number of screening environments is sufficiently large (>5) (Utz and Melchinger, 1995). The preliminary study on identification QTLs for seedling vigour showed nonsignificant Q x E interaction indicating the possibility of identifying QTLs showing consistent expression across the three screening environments. Genotyping the whole mapping population of 252 progenies with a number of markers on linkage group A should make this possibility a reality. Tao *et al* (2000) opined referring to the stay-green trait in sorghum that QTLs with consistent effect across a set of screening environments would increase the efficiency of selection because of the relatively low heritability of the trait.

The portion of phenotypic variance explained by QTLs for glossiness was high across E2 and E3 indicating good expression of glossiness in the postrainy season. Mapping QTL for seedling vigour I and seedling height I to the same chromosomal region across E1 and E2 supports the strong association between the traits and hence linkage between (or perhaps pleiotropy of) the alleles controlling the two traits.

The level of phenotypic variance explained across pairs of screening environment combinations was improved compared to estimates obtained from analysis individual screening environments. This indicates the importance of inclusion of a number of environments in the analysis. Favorable phenotypic effect by BTx623 alleles at QTLs for seedling vigour and seedling height across the screening environment combination pairs supports the high frequency of transgressive segregation observed for these traits. This observation supports the opinion of presence of favorable alleles in dispersion for the traits in the parental lines of the RIL population.

It was observed that there were only small difference in seedling vigour I, seedling vigour II in E2, seedling height I and seedling height II between the two parents (Table 3) of the segregating population used in this study which is not ideal case for genetic mapping. However, the identification of genomic regions associated with these traits in this population indicated the power of the approach of detecting QTLs by the application of molecular markers on RILs through multi-environmental testing.

5.5.5.3 Evidence of pleiotropy or linkage

CIM analysis revealed chromosome regions where more than one trait mapped. According to Hemamalini et al (2000) this co-segregation may be due to tight linkage, pleiotropy or a causal relationship between the traits. Based on single environment study it was found that the chromosomal segment flanked by Xtxp94 and Xtxp65 on linkage group J contain OTLs for glossiness and deadhearts (%). Based on analysis across-environments it was found that the chromosomal region flanked by Xtxp69 and Xtxp34 on linkage group C contain QTLs for seedling vigour I and seedling height I and a chromosomal segment close to this region flanked by Xtxp34 and Xtxp285 contain a QTL for seedling height I. The chromosomal region flanked by Xtxp18 and Xtxp210 on linkage group H contains QTLs for seedling vigour II, seedling height II and grain yield (Table 21). These traits have been found to possess strong correlations with each other (Table 9). The region on linkage group J flanked by Xtxp94 and Xtxp65 was found to be associated with glossiness and deadhearts (%) in E3. These two traits were found to show a strong negative correlation (r = 0.59) in this environment. The effect of substitution of IS 18551 alleles in place of BTx623 is favorable for both the traits. Fine mapping of such chromosomal regions will help to reveal the genetic basis of the correlation of these traits. If markers common to these regions are identified for such traits, combined selection may be expected to be successful.

The results showed that the susceptible parent BTx623 also contained at least some of the alleles responsible for increasing seedling vigour and seedling height. This indicates allele dispersion (Xu, 1997) among the parents and hence the occurrence of transgressive segregation. And the mean of $F_{5.6}$ RILs for these traits and midparent did not vary significantly, indicating predominance of additive gene action. These observations are in conformity with Messmer *et al* (2000) who opined based on QTL analysis results, and nonsignificant difference between RIL mean and midparental value for leaf rust resistance that additive effects were the predominant mode of inheritance for leaf rust resistance. Also, they reported that both the parents contributed positive alleles for leaf rust resistance in winter wheat thereby allowing favorable transgressive segregants to be selected on applied breeding program.

5.6 General discussion

5.6.1 The allele association/dispersion and transgressive segregation

The frequency distribution pattern of RILs for a trait and non-appearance of transgressive segregants support existence of alleles in association (i.e., association of positive alleles in one parent and negative alleles in other parent). However, Xu (1997) reported that the extreme phenotypes of quantitative traits come from the association of favorable OTL alleles, while a preponderance of intermediate phenotypes usually indicates allele dispersion. The extreme phenotypes of parents for glossiness and trichome density, and distribution pattern of phenotypes in RILs support the concept of association of alleles for these traits in the RIL population parents. According to Xu (1997), for the traits naturally selected towards intermediate phenotype alleles of similar effect at multiple loci are more likely dispersed than associated. This holds good for seedling vigour, seedling height and deadhearts (%). This is further supported by opinion by Xu (1997) that genetic stocks with dispersed QTL alleles usually show similar phenotype, making it difficult to identify genetic differences only by phenotypic evaluation. Further, positive and negative transgressive individuals will arise from the associations of positive and negative alleles, respectively. The observation that the appearance of transgressive segregants lying outside both the parental limits and the contribution of QTL alleles from both the parents towards increasing the expression of various traits (viz., seedling vigour and seedling height) clearly substantiate the concept of allele dispersion for such traits in the parents of this RIL population. The lack of common genotypes across the environments also supports the concept of allele dispersion. It could be that the genotypes might be in dispersion (alleles in opposite direction) so that allelic interaction and interaction with environment might have brought about differential expression in different environments.

Classical genetic analysis provides some examples of allele dispersion. The first in plants may come from *Nicotiana rustica*. Transgressive segregants for plant height and and flowering time (B2 and B35) were obtained from a cross between two cultivars (1×5) (Jinks and Perkins, 1969; Perkins and Jinks, 1973). Simultaneous analysis of the two contrasting crosses $(1 \times 5 \text{ and } B2 \times B35)$ indicated the allele dispersion in the original cultivars (Jayasekara and Jinks, 1976). Appearance of transgressive segregation was also reported in rice for tiller angle (Xu and Shen, 1992).

The deviation of RIL mean from mid-parental value was observed for a number of components of resistance to shoot fly. According to Holland (2001), any deviation from predictions based on the additive model can be attributed to epistasis. It appears that genetic control of most of the components of shoot fly resistance involves epistatic gene interaction. However, for some components (viz., seedling height and seedling vigour), no significant deviation of RIL mean from mid-parental value has been observed, so epistatic gene interactions appear to be of limited importance for these traits in this RIL population. Xu (1997) opined that if no significant epistasis can be detected by biometrical analysis, transgressive segregation in the populations derived from two genetic stocks provides evidence for allele dispersion. This opinion corroborates a high proportion of transgressive segregants noticed in the present study for seedling vigour and seedling height. This observation is further supported by QTL analysis that revealed contribution of alleles from both the parents for increasing the expression of these traits.

5.6.2 Breeding for shoot fly resistance

It is revealed from phenotypic data analysis that glossiness is under the control of major loci, recorded with high heritability estimates, high rank correlation coefficients across all the screening environment-combinations, and low levels of G x E interactions. Molecular analysis detected a major QTL explaining a consistently high proportion of total phenotypic variation for this trait across the screening environments and non-significant Q x E interaction was observed

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for this chromosomal region. Based on these observations it may be concluded that breeders should be able to handle the manipulation of glossiness trait in routine breeding programs. Xu (1997) pointed out that the major QTLs would be ones with high heritability, easily manipulated through traditional breeding practices and may already be fixed in many breeding lines. The opportunity to develop elite lines with increased expression of glossiness therefore appears promising. The estimated genetic parameters indicate that single trait selection may be used to rapidly accrue favorable genes for shoot fly resistance component traits. Glossiness appears to have the potential to be used for indirect selection for shoot fly resistance.

The moderate to high heritability estimates for shoot fly resistance components traits indicated that improvement should be possible for these traits by phenotypic selection of lines in this population. Single trait selection based on progeny mean performance apparently will be useful in accumulating favorable genes for the trait under selection. The present study thus indicates that shoot fly resistance could be improved through selection and breeding giving emphasis on highly heritable traits (viz., glossiness and trichome density). Selection for these traits allows progress by selection in environment E3 where the level of resistance is more influenced by environment and hence efficiency of selection of genotypes only based on deadheart damage is reduced.

The significant deviation (p < 0.05) of the population mean towards BTx623 from its mid-parental value in screening environment E3 may reflect a difficulty in recovering breeding lines with the same level of resistance as that of IS 18551. The transgressive segregation observed for trichome density in this population suggests that the trichome density can be increased above the level found in the resistant parent. However, the expression of varying trichome density on either surface in the present study indicates a role of epistatic gene interaction for this trait. Though the frequency of lines showing consistent performance for low deadheart damage or high level of resistance is observed to be low in the present study, it is expected that additive gene action and additive x additive gene interaction might be fixed in these lines. These lines could be exploited in a breeding program to recover lines with higher levels of resistance. The estimates of genetic advance indicated that the best screening environment for improving individual shoot fly resistance components is E2. However, genetic advance was predicted to be greater through indirect selection in screening environment E3 where high shoot fly pressure is prevailing. This is due to the fact that the components of resistance in E3 are highly significantly and negatively correlated with direct measures of shoot fly resistance. In addition, the levels of correlation coefficients were significantly higher in E3 than in the other two screening environments. Because of differential response of the RILs in different environments, selection based on any single environment will be less efficient than indirect selection based on a combination of environments. It is considered that evaluation and selection should be conducted under optimum shoot fly pressure to take advantage of correlated responses. In addition, evaluation and selection under optimum shoot fly pressure ensures the preservation of genotypes for shoot fly resistance.

The lack of segregation for high level of resistance (or low deadheart damage) even in genetically variable RILs may be cited as the reason for reasearchers have been unable to increase level of shoot fly resistance in sorghum. The reason also holds good for non- recovery of high frequency of glossy lines. However, by selecting extreme progenies for the component traits in RILs across the environments it would be possible to develop cultivars with comparative or even higher level of shoot fly resistance possessing higher yield potentiality. In addition, based on the concept that lines with alleles present in *trans* lead to transgressive segregants due complementation, intercrossing between moderately susceptible may be practiced to recover lines with high level of resistance. This is supported by Snijders, (1990) who reported that crosses with moderately susceptible genotype for head blight caused by *Fusarium culmorum* resulted in offspring with very high resistance levels and transgression for resistance wheat.

5.6.3 Detection of QTLs for components of resistance to shoot fly

Identification of individual genes contributing to shoot fly resistance could lead to several applications. First it could improve the efficacy of selective breeding, especially for traits with low heritability (Soller and Beckmann, 1988; Lander and Thompson, 1990). Glossiness was observed to have high heritability, genetic control by major loci with epistatic gene action and a major QTL for this trait was detected explaining a high proportion of the observed phenotypic variance for this trait across screening environments. It appears that selection in a single

environment for this trait may be adequate to separate of resistant and susceptible lines based on glossy and non-glossy leaf blades.

Screening of genotypes for shoot fly resistance component traits other than glossiness have rarely been incorporated into applied breeding programs due to the time consuming nature of trichome counting (microscopic) and the low heritability of other traits. Despite genetic variability observed for various traits in the present study, their genetic improvement using conventional selection based on phenotype alone is difficult. Application of marker-assisted methods for such traits has the potential to greatly enhance the efficiency of selection.

However, in the present study, a few QTLs for the resistance components with low heritability (viz., seedling vigour and seedling height) were detected. And no QTLs for other components of resistance have been detected. Reason for lack of identification of QTLs for other traits could be lack of linkage disequillibrium between the markers used and QTLs for the traits. Because the identification of markers linked to QTLs depends on the amount of linkage disequillibrium present in the population. According to Terwilliger (2001) the correlation between the observed marker locus genotypes and trait phenotypes is a convolution of the correlation between marker and resistance locus genotypes and the correlation between trait locus genotypes. If the linkage relationships are very strong, then the power of a test is a function of the trait locus genotype-phenotype relationship. In the present study lack of identification of QTLs for other components of resistance indicates that both the relationships are weak for the portions of the genome that were marker genotyped in the subset of 93 RILs. Failure to identify QTLs for other traits despite number of markers genotyped could also be due to smaller population size used in the present study and incomplete genome coverage of the skeleton map.

Rapid advances in molecular marker technology have helped to develop highly saturated sorghum molecular maps (Bhattramakki *et al.*, 2000) and have made it possible to identify some of the map locations of gene blocks contributing to shoot fly resistance components in the present investigation. QTLs described in the present study would provide initial tools for testing MAS approaches to retaining and combining quantitative resistance trait loci for the control of shoot fly resistance in sorghum. If the cost and time required in DNA extraction of large number of samples are minimized through advanced technologies, the markers linked to QTLs for glossiness may be used as reliable tool for selection for resistance to shoot fly.

The availability of a permanent mapping populations such as the RILs used in this study will greatly facilitate the mapping of new DNA markers in the sorghum genome. In concurrent studies, this RIL population may be used to develop a public genetic linkage map (based on SSRs) and for mapping QTLs responsible for genetic variation described in the present study. Seeds of these lines may be distributed to different laboratories and mapping data may be added to the existing database, and markers may be available freely to the public. In addition to the mapping of new DNA markers, the RILs are being evaluated for various quantitative shoot fly resistance traits in multiple environments in order to test the significance of G x E interaction for these traits (personal communication with scientists at ICRISAT, Patancheru). The experimental material also provides the ideal material to study the real contribution of each resistance component to the target trait, for example the association of glossiness with deadhearts, and trichome density with deadhearts. Also RILs ca be used to study G x E interaction for the target trait.

5.6.4 Population size

Greater population size is needed to trace more recombination events (occurrence of single and double cross-overs). For example, in a small population size the small number of recombinants observed does not necessarily mean that the two markers are tightly linked. Confirmation of putative linkage associations detected in this population can be obtained when associations are tested in large population size. Small population bias the linkage distance and hence QTL mapping (i.e., for resolution of map and even marker order). The reason behind the estimation of linkage distance in turn depends on recombinants or cross-overs. The accuracy lies with the estimation of linkage distance including double cross-overs. With higher population size the chance of tracing the double cross-overs will be greater and therefore the accuracy of estimated linkage distance. Beavis *et al* (1994) and Young (2001) recommended the use of large population size in mapping experiments. In the present study effective population size used for mapping QTLs for shoot fly resistance components was small (i.e., only 93 RILs). However, the results obtained can be verified with increased population size (up to 252 RILs) and more

number of markers. Further, QTL analysis with full RIL population can be expected to provide significant estimates of QTL effects even for the traits with low heritability.

5.6.5 Correlations

In the present study none of the resistance components were highly correlated. Some of the resistance components varied significantly across the environments and showed no correlation or non-significant correlation with deadhearts percentages. The weak associations observed between components of resistance with established shoot fly resistance traits suggest that the genes controlling the traits are different and are not linked. They may be located on same chromosome at independently segregating locations or on different chromosomes. Therefore, selection has to be conducted separately for each trait. Low levels of phenotypic correlations among the traits also indicate that selection for higher or lower levels of one trait would not adversely raise the levels of another trait. Though seedling vigour is significantly correlated with deadhearts percentages, its value to the plant breeder in the field conditions is questionable unless this relationship is repeatedly demonstrated. Although breeding for improved resistance to shoot fly will have certain difficulties because of low heritability, a proper screening program using the available resistant germplasm should minimize this problem.

5.6.6 The selection environments

In the present study, three environments were categorized (based on level of deadheart damage ratings in susceptible checks) as having medium shoot fly pressure (in E1), optimum (in E2) and high (in E3). However, from the point of view of selecting breeding material for shoot fly resistance, these environments may be classified as:

More favorable selection environment - E2 Less favorable selection environment - E1 Least favorable selection environment - E3

This categorization of test environments is based on two criteria: first, the relative levels of differences between resistant parent and susceptible parent, and resistant parent and susceptible control; second the levels of heritability and genetic advance estimated for the target

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trait. The ability to distinguish a resistant progeny from a susceptible one depends on the deviation of the performance of resistant parent (with respect to deadheart damage ratings) from susceptible parent (P1-P2) and susceptible check (C-P2) in the test environments. In test environment E2, these deviations were greater in the initial stage and increased further in second stage of seedling observations (Figure 8A-B). In E1, these deviations were comparable to those observed in E2 for deadheart I (%), but decreased marginally for deadheart II (%) Further the deviations were significantly lower in E3 at stage I than that observed in E2 and E1 and further decreased observation stage II.

The estimates of broad sense heritability and prediction genetic gain were significantly higher in E2 that, those obtained for data sets from E1 and E3 (Tables 10 and 12)

5.6.7 Inconsistency of measurements on plant height in different environments

It was reported by Quinby and Karper (1945) that a maturity gene Mal and plant height gene Dwl in sorghum are closely linked. Lin *et al* (1995) reported stronger association between phytochrome and height QTLs in sorghum. It was observed that *phyb* was associated with height QTLs in maize and sorghum (Lin *et al.*, 1995). The region near *phyb* on chromosome 3 is associated with QTLs for height and flowering in rice. In addition, major QTLs detected with relatively large effects for heading date in most of the studies correspond well with the photoperiod sensitivity genes identified previously (Yu *et al.*, 2002). Yano *et al* (2000) further defined a genomic region as a candidate for the *Hd-1* locus, which is allelic to *Sel* (photoperiod sensitive gene) and has high homology with *CONSTANTS*, a gene for flowering time in *Arabidopsis*. In addition, epistatic interactions for plant height and heading date are often reported in rice (Yu *et al.*, 2002). Therefore these observations on association between genes for plant height and maturity; heading date and photoperiod sensitivity support the lack of consistency observed for plant height among RILs in different screening environments (involving *kharif* and *rabi* seasons) in the current study. However, results of further mapping experiments with the existing population in future may confirm these observations.

5.6.8 A synthesis

Shoot fly has been problematic since 1970s as exotic materials were introduced Since then several studies have been made regarding genetics and mechanisms of resistance, and

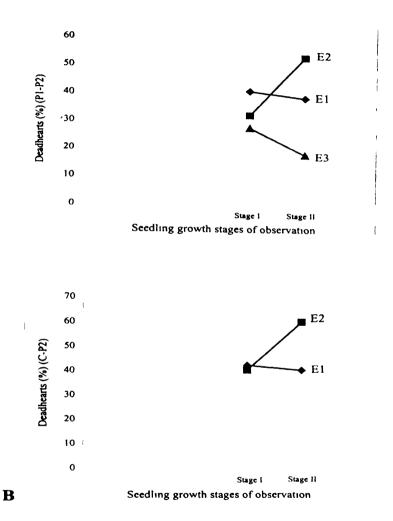


Figure 8 (A-B). Relative deadhearts ratings (%) due to shoot fly in the susceptible parent (P1) and susceptible control (C) deviated from that of the resistant parent (P2) during two stages of seedling observations in three environments, E1 (*kharif*, Patancheru), E2 (*rabi*, Patancheru) and E3 (early *rabi*, Dharwar).

P1: BTx623 (susceptible); P2: IS 18551 (resistant); C: CSH 1 (susceptible)

screening for resistance. It is observed that frequency of germplasm resources identified with high levels of resistance is low (see 2.1.1.3.1) with none showing immunity. Despite efforts made utilizing the existing sources with high level of resistance, the genetic improvement achieved to date is limited. The reasons could be:

- The genes controlling shoot fly resistance are dispersed through out the genome and hence segregate independently. Therefore, change in gene frequency and genotype frequency may be low unless very large segregating populations and efficient screening methods can be employed. Hence, lower frequency of genotypes with higher level of resistance in breeding populations of the sizes typically used in applied sorghum breeding in India.
- 2. The genes controlling the character might be in dispersive condition rather in association, and in addition, interactions between the alleles and loci in dispersive condition make the conventional transfer and selection difficult.
- The genotypes with desired alleles might have been eliminated during the course of breeder selection only based on deadhearts damage which could be due to Due to interaction with environment
- 4. Insufficient replication (at individual screening sites as well as in space and time) based selection procedures that are required for success with a low heritability trait like shoot fly deadhearts damage (%

Published reports show that sorghum shoot fly resistance breeding programs have been concentrated for more than 3 decades on the following:

- 1. Evaluation of germplasm resources to identify superior resistance sources.
- 2. The transfer of resistance from source material to agronomically superior line usually by single cross hybridization followed by repeated backcross hybridization.
- 3. Improvement of resistance by pedigree breeding. The inbreds obtained by selfing followed by selection during each generation.
- 4. The lines are mostly derived for resistance in addition to high yield.
- 5. Subsequent utilization of these derived lines in breeding for improved levels of resistance in agronomically superior genetic backgrounds.

The breeding for shoot fly resistance might have led to a decrease in number of favorable alleles available in the derived breeding populations. The situation is quite contrast to the one in wild species, where the species is cleistogamous, completely self-pollinated and crossincompatible or non-interfertile with the cultivated sorghum. But these wild relatives are immune to shoot fly. Because the alleles are fixed due to continuous self-pollination the alleles are in association and are being maintained. The genetic structure thus being maintained in wild species is said to be co-adaptive complexes. However, the alleles for shoot fly resistance in cultivated accessions might be in dispersion (which is evidenced in the present study for some resistance components), and therefore the expression of resistance cannot be predicted to be high in subsequent generations. The situation has become so worse that even the cultivated resistance sources also do not show the same levels of performance in each season or location or in the next generation due to outcrossing with susceptible geneotypes. This is because of interaction between favorable and unfavorable alleles or alleles responsible for high level of resistance might not be sufficient enough to have desired effect and thereby allowing the plants susceptible to shoot fly when infectation levels are severe. If the favorable genes present are not in association the chance that individuals with all the genes responsible for high levels of resistance in IS 18551 has been reduced in the RILs since the number of latter is small relative to the size of the perfect population in which one could reasonably expect one such perfect segregant. The component characters may not have been liked to shoot fly in these plants therefore may not be strongly rejected by shoot fly. During the process of natural and applied pedigree selection, it might not be possible to retain the genes for high level of resistance. Alternatively, it might be possible that the genes for resistance in cultivated and wild species are different. In either case, population breeding with a recurrent selection approach is considered to be useful in pyramiding such diverse but large number of genes.

The results obtained in the present study differ from previous studies made by several authors. This might be due to previous lack of assessment of the traits in proper breeding material and diverse environments. The number of genotypes used in previous studies were small thus limiting the information provided about the potential for genetic improvement of shoot fly resistance. Therefore, in the present study a reasonably large number of diverse recombinant inbred lines of sorghum have been utilized. Their evaluation in additional diverse environments could be used to study further the genetics of shoot fly resistance.

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None of the recombinants with high levels of expression of individual resistance components (even higher than the resistant parent e.g., trichome density) were found to have resistance levels higher than the resistant parent. It is possible that genes controlling individual components are not linked to each other and high levels of resistance (i.e., low deadhearts%) is very rare, so a very large RIL population would have to be expected to find such segregants. This is corroborated by the observations of negative or very low degrees of association existing between the component resistance traits. Even in previous studies using different breeding material it was also concluded that genes for all of the components are important to the final expression of resistance.

It appears that the breeding strategy has to be changed to look for other components of resistance at biochemical level. In other words resistance components and mechanisms operating in the wild relatives of sorghum need to be studied detail. The results obtained from study of wild species could then be extended to the breeding material used in the present study. While simultaneously generating the data the experiment may be evaluated for resistance at diverse environments. And also may be studied for other components of resistance. The lack of high levels of resistance noticed in the present material, supported by similar previous reports indicates that antibiosis factors that are detrimental to shoot fly even at high level of infestation are lacking in cultivated sorghum accessions. It remains to be seen whether such antibiosis factors can be documented in wild relatives of this crop.

In formulating future breeding strategies for resistance to shoot fly in sorghum, the following issues may be addressed seriously:

- 1. Why do the susceptible checks show consistently high susceptibility across the environments, while the "so called resistant cultivars" show variable levels of resistance in same set of environments?
- 2. Why are some wild accessions immune to shoot fly attack? What are the resistance components strongly associated with this immunity that are not present in cultivated sorghum and why the level of expression of the trait is less or variable in cultivated types?

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These queries are raised with the opinion that the mechanism of antibiosis contributes more to higher levels of resistance than do mere non-preference mechanisms that may fail under nochoice conditions and high pest population levels. It is necessary to look for identification of the antibiosis factor(s) operating in wild accessions. Breeding for such characters should be concentrated in future to identify the resistant lines and intercrossing number of such lines with agronomically elite sorghums to develop a population that can be subjected to selection to increase frequency of desirable alleles. Further breeding may be continued to isolate the lines with high level of expression of the desired characters (both resistance and agronomic eliteness).

The problem may be posed to the experts in different disciplines to look for the solution in different ways For utilization of wild species, the expertise is needed in different fields, including cytogeneticists, molecular biologists, biochemists and statisticians in addition to plant breeders and entomologists to bring meaningful collaboration. However, recent advances in genetic engineering approaches may substitute conventional approaches in sorghum Efforts are being made to develop transgenic sorghum with resistance to shoot fly (personal communication with Scientists at ICRISAT, Patancheru)

3 Why the resistant parent even under choice condition is highly infested by the shoot fly? Does it mean that the limited (if any at all) antibiosis factors present in the resistant parent are not strong enough to bring about stable resistance?

The study of pest resistance in crops has entered a new era. The new technology of molecular tools can afford the genetic dissection of resistance to pests. Yet significant challenges remain especially in understanding the complex resistance traits. In contrast to Mendellian traits, one seeks the genes that influence the entire complex of traits rather than that cause individual components of the complex. Newer and more imaginative approaches are needed to achieve this task. We realize that complex traits arise from interactions among the multiple genes and environments, but most studies ignore these interactions because past methods have not been able to deal with them. We need to understand how genes and environments work together to produce the complex traits. However, genetic dissection of these traits needs multiple approaches and collaborative efforts and much research as suggested earlier. Regardless of the approach taken, however, reliable phenotypic assessment of large number of segregants will be critical to success of the program.

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The actual cost required to identify genomic region is a function of higher costs incurred in genotyping the individuals of mapping population rather than phenotyping However, accuracy in phenotyping, and the required skills and manpower cannot be sacrificed to achieve efficacy of application of markers in any plant breeding program

5.7 Future line of work

1. The success of transfer of QTLs depends on the close linkage of associated marker(s) with the target QTL. Towards this following points are to be considered

- a) Development of high density linkage maps that are essential to screen the population with a number of linked markers.
- b) Once QTLs with high LOD score explaining large portions of phenotypic variance are identified, the fine mapping of the region is needed
- c) An independent sample of RILs derived from the same cross is needed to check the association; this should also be larger than the current 92-entry subset, because it is expected that small size population biases the estimation of QTL effects.
- d) A large number of screening environments is to be considered to reduce the error due to environmental effects and biases due to G x E effects. However, for the component traits with high heritability and for which environmental effects are less important (e g, glossiness and trichome density), data from appropriate environments are to be clubbed together to identify chromosomal regions associated with these component traits But the phenotypic values for the target trait (with low to moderate heirtability) may be taken across the diverse environments
- e) Most importance has to be given to accuracy in phenotyping for unbiased estimation of QTL effects as well as identification of QTL effects
- f) Consistency of the estimated QTL positions, effects across different environments are essential if marker assisted selection is to be most cost effective since the costs of detecting QTLs are high and might be most rapidly recovered if the QTLs detected are effective in many production environments and many genetic backgrounds. Similar studies in other mapping populations may help to confirm the inferences drawn in the present study.

If markers are tightly linked to QTL, even after a number of backcross generations, the linkage should be retained. Such markers are potentially useful in even screening wild species and further cloning of the resistance gene(s) responsible for the QTL.

2. Since the lines are inbreds, a line x tester study using a range of 3-5 testers differing markedly in their shoot fly resistance may be conducted. The lines with high glossiness intensity may be backcrossed with IS 18551 to recover all the alleles for glossiness. Similar approach may be followed for trichome density. For selection for glossiness an additional generation of selfing is needed since it is under the control of the recessive genes. Selection in BC_1F_2 may be done for trichome densities higher than the resistant parental line (IS 18551) on both lower and upper surfaces, and for lines with highly glossiness intensity.

3. The present study has identified some molecular markers linked to components of resistance to shoot fly under a range of shoot fly pressures that will help in molecular fine mapping tightly linked markers. Genomic regions in which these QTLs have been detected now need to be saturated with markers to achieve an average marker density greater than 1 per cM in fine mapping populations derived from crosses of selected RILs and their elite susceptible parent BTx623. This can help to understand the genetic interaction among traits contributing to shoot fly resistance and pleiotropy or tight linkage of genes controlling the component traits.

4. The component traits may be tested in the presence or absence of stress, because the expression of resistance or susceptibility by the individual depends on interaction between host and insect. The differential expression in different environments cannot be explained only based on G x E interaction, but has to be explained in terms of interaction between the host plants and insect pests.

5. One reason for low frequency of lines with high intensity of glossiness might be due to maternal effects. However, this has to be confirmed by progeny of the reciprocal cross.

.6. Research efforts may be concentrated on tissue-specific expression for antibiosis factors, since shoot fly attacks during 10 to 30 days after emergence, and that too targeting growing point. Studies on induced expression and meristematic tissue specific expression of the biochemical factors that deter growth and development of larvae may be conducted in addition to phenotypic components of resistance.

7. The money spent on screening the material only for deadhearts (%) can be diverted to look for other components, that bring about resistance in wild species but are lacking in cultivated accessions. These might be structural or biochemical and are nothing but antibiosis factors responsible for deterring shoot fly attack in non-host relatives of cultivated sorghum.

8. The possibility of overdominance of loci as a reason for transgression of trichome density in the RILs can be excluded by successively selfing the transgressive individuals to determine if they maintain the same phenotype in advanced generations.

Summary

VI. SUMMARY

Sorghum is an important cereal crop in semi-arid areas of Asian and Africa. Major constraints in sorghum production include crop damage due to insect pests. Shoot fly is one of the serious pests attacking sorghum crop during seedling stage. Progress in genetic manipulation is hindered by lack of understanding of inheritance of quantitative resistance to shoot fly attack in appropriate material. Conventional breeding supplemented by marker based selection, could ease in improving the level of resistance. Detection of markers linked to QTLs associated with quantitative insect resistance (target trait and component traits) is the first step in application of molecular markers in plant breeding. Therefore, present investigation was initiated with the following objectives.

- 1. Phenotyping RILs for the components of resistance to shoot fly under three different environments.
- 2. Study of genetic architecture of component characters of resistance in relation to varying levels of shoot fly infestation.
- 3. Survey of parental polymorphism at DNA level using SSR markers.
- Genotyping the subset of RIL population with the number of SSR markers showing polymorphism.
- 5. Construction of genetic linkage map and identification of QTLs for resistance to shoot fly and its component traits using the marker genotyped subset of the RIL population.

A set of 252 RILs ($F_{5.6}$) derived from the cross between BTx 623 (S) and IS 18551 (R) were used for phenotypic evaluation and a subset of this mapping population consisting of 93 RILs ($F_{6.7}$) was used for genome mapping with SSR marker.

The experimental material consisting of 252 RILs, two parents and two susceptible controls was evaluated for shootfly resistance and its component traits, and agronomic traits under artificial infestation in three environments viz., E1 (*kharif*, Patancheru), E2 (*rabi*, Patancheru) and E3 (early *rabi*, Dharwar). Based on the varying range of deadhearts (%) observed in susceptible control CSH 9 in three screening environments, these environments were categorised as moderate shoot fly pressure (E2), optimum (E1) and high pressure (E3).

The two parents differed phenotypically for shoot fly resistance traits (oviposition% and deadhearts%), the component traits and agronomic traits in individual environments. However,

the range of parental mean values for these traits varied across the environments. The resistant parental line IS 18551 was recorded with high leaf glossiness, high seedling vigour, high trichome density (lower and upper leaf surfaces), maximum seedling height and high pseudostem length. It was late flowering with maximum plant height at maturity and low grain yield per plant. The susceptible parental line BTx623 was non-glossy, with low seedling vigour, no trichomes, and minimum seedling height and pseudostem length. It was early in flowering with lower plant height and higher grain yield. It was highly susceptible to shoot fly damage.

Variances due to genotypes (F_6 bulk RILs) were significant for all the traits studied based on performance in individual environments and averaged over these environments. The estimates of genotypic variance and genotype x environment interaction variance were significant for all the observed traits except trichome density (upper leaf surface).

Variability was observed for all the traits studied and high variability was noticed in screening environment E2 except for seedling dry weight, plant height and grain yield per plant. In general, the level of PCV (%) was higher than GCV (%), as is expected for the traits affected by the environment. Consistently high levels of variability were observed for highly heritable traits [glossiness and trichome density] in all the screening environments. However, for other traits [oviposition (%), deadhearts (%) and seedling height] the levels of percentage variation were varied across the environments, while the estimates for coefficient of variation for shoot fly resistance traits, [oviposition (%) and deadhearts (%)] were higher in the initial stage of seedling growth than at the later stage in which observations were collected. However, for seedling vigour highest variability was noticed in the later stage of seedling growth.

Highly significant and negative correlation coefficients were observed for shoot fly resistance measured as oviposition percentage or deadhearts percentage and component traits such as glossiness, trichome density and seedling vigour. The two component traits glossiness and trichome density had significantly high heritability in individual and across the three screening environments. The shoot fly resistance traits [oviposition (%) and deadhearts (%)] had only moderately high heritability estimates.

Averaged over all the screening environments, the 252 RILs showed continuous distribution for most of the traits indicating quantitative nature of these traits. The skewed distribution observed for the traits glossiness (bimodal distribution) and trichome density on upper leaf surface (non-normality), and continuous distribution for trichome density on lower leaf surface with range of phenotypes indicate the genetic control of these traits under the control

of major loci. The low frequency of individuals with high intensity of glossiness suggests the recessive nature of the trait. High frequency of RILs with presence of trichomes indicate the dominant nature of trichome density. Varying range of phenotypes for intensity of gossiness and trichome density indicate the action and interaction of favorable and unfavorable alleles at different loci.

Deviation of the RIL population mean from midparental values was observed for most of the traits indicating the role of epistatic gene action for the expression of these traits. The equality of the mean of RILs and mid-parental value for seedling height and seedling vigour indicates that genes for these traits are in linkage equilibrium and variation is predominantly due to additive gene action. Transgressive segregants with phenotypic values outside the parental limits were observed for most of the traits except for high intensity of glossiness and low deadhearts (%). In general, for the traits with RIL means less than the mid-parental value, the proportion of transgressive RILs were higher for those outside the low scoring parent and vice versa. In no case was the expectation of equality of frequency of inbred lying outside the parental limits of Btx623 and IS 18551 observed. This shows that there were different proportion of desired and undesired alleles in both the parents for all of the traits observed in this study.

In general, predicted percentage gain estimates over RIL population mean was high in environment E2 for all the traits studied except for seedling height, time to 50% flowering and plant height. High genetic gain estimates observed in screening environment E2 were attributed to high levels of genotypic variance and high mean of selected individuals (highest 5%) over the population mean in comparison to estimates observed in other screening environments. For most of the traits measured at two stages (oviposition, deadhearts and seedling height), genetic gain estimates were higher in the initial stage of seedling growth compared to the later stage of seedling growth. In contrast, for seedling vigour, predicted genetic gain was higher at the later stage of seedling growth. However, the levels of prediction gain for this trait were comparable at both the stages in environment E2. Prediction genetic gain estimates for glossiness and trichome density were higher in postrainy season (across the locations) than in the *rainy* season. Prediction correlated genetic gain was high for shoot fly resistance when highly heritabe traits such as glossiness and trichome density were used as indirect selection criteria

Highly significant rank correlations were observed for all the traits among all the pairs of environmental combinations, except for seedling vigour II and oviposition II, indicating similarity in the rank orders for all the traits in all the environments. However, varying levels of rank correlation coefficients observed across the pairs of environmental combinations for the components with low heritability indicate the effect of environmental factors in their expression. Consistency of rank correlation coefficients with high levels was observed for the traits glossiness and trichome density across the pairs of screening environments indicate that $G \propto F$ interaction for these traits is less important

A subset of the mapping population consisting of 93 RH s $(1_{1/2})$ was used for genotyping with 44 SSR markers A linkage map was constructed with 23 polymorphic marker loci, based on which composite interval mapping (CIM) was performed using PlabQTL for the data sets from each of the three screening environments Genetic regions with a LOD score greater than a threshhold of 2 5 were identified as putative QTLs for the traits under study. The analysis overall identified 8 QTLs for shoot fly resistance components based on single environment analyses One major QTL for glossiness located on linkage group J was identified Minor QTIs for seedling vigour I (one), seedling vigour (two) seedling height I (three) seedling height II (one) and grain yield (one) were also identified One major QTL for glossiness (Xixp94-Xixp65) was detected on LG J, with phenotypic variance explained ranging from 34 3 to 46 5% in the three screening environments High expression by the QTL for glossiness was observed in the postrainy season across the two testing locations Further the largest consistent effect for glossiness due to the QTL on LG J co-mapped with region associated with deadhearts (%) under high shoot fly pressure The two traits also showed high levels of association with each other in this environment This OTL may be useful target for marker-assisted selection for shoot fly resistance in sorghum

QTL analysis across two-environment combinations and across all three environments also detected QTL for glossiness with consistently high phenotypic variance explained Significant Q x E interaction was observed for seedling vigour and seedling height However non-significant Q x E interaction was observed for the trait glossiness. The regions identified for seedling vigour were co-mapped with regions for seedling height QTI's detected for seedling vigour and seedling height based on single environment and across environment analysis were positioned on linkage groups A, C and H

The extreme parental phenotypes for glossiness and nonappearance of transgressive segregants with intensity of glossiness higher than glossy resistant parent indicate the allele association in the parents. For trichome density, though the two parents bear extreme phenotypes the occurrence of transgressive segregants indicate the preponderance of alleles in association.

and few loci in dispersion in the parents. The appearance of transgressive segregants, and contribution of alleles observed based on QTL analysis (single and across environment analysis) for the traits seedling height and seedling vigour indicate the allele dispersion in the parents as well as in RILs.

From breeding point of view, the environment in which high variability observed for the target traits is considered appropriate for selection purpose. Selection environments in the present study were categorised based on prediction gain estimates for resistance, and observation on deadhearts (%) in resistant parent deviated from susceptible parent and susceptible control. The environment E2 was categorised as more favorable selection environment than E1 and E3 where high prediction genetic gain estimates for deadhearts, and high deviations for deadhearts (%) in resistant parent from susceptible parent and susceptible control were recorded.

The present study has assumed the feasibility of identifying QTLs for shoot fly resistance. Further experiments may be conducted using the whole set of RILs and additional markers to identify and locate other QTLs with consistent effects across environments. Ultimately high levels of resistance useful to farmers are likely to be only achieved by pyramiding multiple resistance loci into single agronomic elite genotypes.

Parental performance and RIL mean performance for glossiness were consistent across the environments suggest that reliable evaluation of this trait is possible. The moderate to high heritability estimates for shoot fly resistance component traits indicates that improvement relative to the RIL population mean should be possible for these traits by phenotypic selection of lines in this population. Single trait-selection based on mean performance apparently will be useful in accumulating favorable genes for the trait under selection. However, a form of multiple trait selection with the aid of markers linked to different component traits will be useful for improved level of resistance. The present study thus indicates that shoot fly resistance could be improved through selection and breeding if emphasis is given to highly heritable component traits such as glossiness and trichome density.

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Appendices

Appendix I. Details on pedigree of RILs (F_7) of cross BTx623 X IS 18551

(contd.,)

			(00
Entry	Origin	Pedigree	En
1	25701	(BTX 623 x IS 18551)-1-1-1-1	4
2	25702	(BTX 623 x IS 18551)-2-1-1-1-1	4
3	25703	(BTX 623 x IS 18551)-3-1-1-1-1	4
4	25704	(BTX 623 x IS 18551)-4-1-1-1	4
5	25705	(BTX 623 x IS 18551)-5-1-1-1-1	4
6	25706	(BTX 623 x IS 18551)-6-1-1-1-1	4
7	25707	(BTX 623 x IS 18551)-7-1-1-1-1	4
8	25708	(BTX 623 x IS 18551)-8-1-1-1	5
9	25709	(BTX 623 x IS 18551)-9-1-1-1-1	5
10	25710	(BTX 623 x IS 18551)-10-1-1-1-1	5
11	25711	(BTX 623 x IS 18551)-11-1-1-1	5
12	25712	(BTX 623 x IS 18551)-13-1-1-1-1	5
13	25713	(BTX 623 x IS 18551)-15-1-1-1-1	5
14	25714	(BTX 623 x IS 18551)-16-1-1-1-1	5
15	25715	(BTX 623 x IS 18551)-17-1-1-1-1	5
16	25716	(BTX 623 x IS 18551)-18-1-1-1-1	5
17	25717	(BTX 623 x IS 18551)-19-1-1-1-1	5
18	25718	(BTX 623 x IS 18551)-20-1-1-1-1	e
19	25719	(BTX 623 x IS 18551)-21-1-1-1	e
20	25720	(BTX 623 x IS 18551)-23-1-1-1-1	e
21	25721	(BTX 623 x IS 18551)-25-1-1-1-1	e
22	25722	(BTX 623 x IS 18551)-26-1-1-1-1	6
23	25723	(BTX 623 x IS 18551)-27-1-1-1	(
24	991524	(BTX 623 x IS 18551)-28-1-1-1	(
25	25724	(BTX 623 x IS 18551)-30-1-1-1-1	(
26	25725	(BTX 623 x IS 18551)-31-1-1-1	
27	25726	(BTX 623 x IS 18551)-33-1-1-1-1	
28	25727	(BTX 623 x IS 18551)-34-1-1-1-1	
29	25728	(BTX 623 x IS 18551)-35-1-1-1-1	
30	25729	(BTX 623 x IS 18551)-36-1-1-1-1	
31	25730	(BTX 623 x IS 18551)-37-1-1-1-1	
32	25731	(BTX 623 x IS 18551)-39-1-1-1-1	
33	25732	(BTX 623 x IS 18551)-40-1-1-1-1	
.34	25733	(BTX 623 x IS 18551)-41-1-1-1	
35	25734	(BTX 623 x IS 18551)-42-1-1-1-1	
36	25735	(BTX 623 x IS 18551)-44-1-1-1-1	
37	25736	(BTX 623 x IS 18551)-45-1-1-1-1	
38	25737	(BTX 623 x IS 18551)-46-1-1-1	
39	25738		
40	25739		
41	25740	· · · · · · · · · · · · · · · · · · ·	
42	25741	(BTX 623 x IS 18551)-52-1-1-1-1	
		(contd)	

Entry	Origin	Pedigree
43	25742	(BTX 623 x IS 18551)-54-1-1-1-1
44	25743	(BTX 623 x IS 18551)-55-1-1-1-1
45	25744	(BTX 623 x IS 18551)-56-1-1-1-1
46	25745	(BTX 623 x IS 18551)-58-1-1-1-1
47	25746	(BTX 623 x IS 18551)-59-1-1-1-1
48	25747	(BTX 623 x IS 18551)-60-1-1-1-1
49	25748	(BTX 623 x IS 18551)-61-1-1-1
50	25749	(BTX 623 x IS 18551)-62-1-1-1-1
51	25750	(BTX 623 x IS 18551)-64-1-1-1-1
52	25751	(BTX 623 x IS 18551)-65-1-1-1-1
53	25752	(BTX 623 x IS 18551)-66-1-1-1-1
54	25753	(BTX 623 x IS 18551)-67-1-1-1-1
55	25754	(BTX 623 x IS 18551)-68-1-1-1-1
56	25755	(BTX 623 x IS 18551)-69-1-1-1-1
57	25756	(BTX 623 x IS 18551)-70-1-1-1-1
58	25757	(BTX 623 x IS 18551)-72-1-1-1-1
59	25758	(BTX 623 x IS 18551)-73-1-1-1-1
60	25759	(BTX 623 x IS 18551)-74-1-1-1-1
61	25760	(BTX 623 x IS 18551)-77-1-1-1-1
62	25761	(BTX 623 x IS 18551)-78-1-1-1-1
63	25762	(BTX 623 x IS 18551)-79-1-1-1-1
64	25763	(BTX 623 x IS 18551)-80-1-1-1-1
65	25764	(BTX 623 x IS 18551)-81-1-1-1
66	25765	(BTX 623 x IS 18551)-82-1-1-1-1
67	25766	(BTX 623 x IS 18551)-84-1-1-1-1
68	25767	(BTX 623 x IS 18551)-86-1-1-1-1
69	25768	(BTX 623 x IS 18551)-87-1-1-1-1
70	25769	(BTX 623 x IS 18551)-89-1-1-1-1
71	25770	(BTX 623 x IS 18551)-90-1-1-1-1
72	25771	(BTX 623 x IS 18551)-91-1-1-1
73	25772	(BTX 623 x IS 18551)-93-1-1-1-1
74	25773	(BTX 623 x IS 18551)-94-1-1-1-1
75	25774	(BTX 623 x IS 18551)-95-1-1-1-1
76	25775	(BTX 623 x IS 18551)-97-1-1-1-1
77	25776	
78	25777	•
79	25778	
80	25779	•
81	25780	
82	25781	
83	25782	•
84	25783	
		. (contd)

Entry	Origin	Pedigree
85	25784	(BTX 623 x IS 18551)-108-1-1-1-1
86	25785	(BTX 623 x IS 18551)-109-1-1-1-1
87	25786	(BTX 623 x IS 18551)-112-1-1-1-1
88	25787	(BTX 623 x IS 18551)-114-1-1-1-1
89	25788	(BTX 623 x IS 18551)-115-1-1-1-1
90	25789	(BTX 623 x IS 18551)-117-1-1-1-1
91	257 9 0	(BTX 623 x IS 18551)-118-1-1-1-1
92	25791	(BTX 623 x IS 18551)-119-1-1-1-1
93	25792	(BTX 623 x IS 18551)-121-1-1-1
94	25793	(BTX 623 x IS 18551)-122-1-1-1-1
95	25794	(BTX 623 x IS 18551)-123-1-1-1-1
96	25795	(BTX 623 x IS 18551)-125-1-1-1-1
97	25796	(BTX 623 x IS 18551)-126-1-1-1
98	25797	(BTX 623 x IS 18551)-128-1-1-1-1
99	25798	(BTX 623 x IS 18551)-129-1-1-1-1
100	25799	(BTX 623 x IS 18551)-130-1-1-1-1
101	25800	(BTX 623 x IS 18551)-132-1-1-1-1
10 2	25801	(BTX 623 x IS 18551)-134-1-1-1-1
103	25802	(BTX 623 x IS 18551)-135-1-1-1
104	25803	(BTX 623 x IS 18551)-136-1-1-1
105	25804	(BTX 623 x IS 18551)-137-1-1-1
106	25805	(BTX 623 x IS 18551)-139-1-1-1-1
107	25806	(BTX 623 x IS 18551)-140-1-1-1-1
108	25807	(BTX 623 x IS 18551)-143-1-1-1
109	25808	(BTX 623 x IS 18551)-144-1-1-1
110	25809	(BTX 623 x IS 18551)-145-1-1-1
111	25810	(BTX 623 x IS 18551)-146-1-1-1
112	25811	(BTX 623 x IS 18551)-148-1-1-1
113	25812	(BTX 623 x IS 18551)-149-1-1-1
114	25813	(BTX 623 x IS 18551)-150-1-1-1
115	25814	x
116	25815	· ·
117	25816	•
118	25817	
119	25818	•
120	25819	•
121	25820	· · ·
122	25821	•
123	25822	•
124	25824	
125	25825	
126	25826	
		(contd)

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Entry	Origin	Pedigree
127	25827	(BTX 623 x IS 18551)-169-1-1-1-1
128	25828	(BTX 623 x IS 18551)-170-1-1-1-1
129	25829	(BTX 623 x IS 18551)-172-1-1-1-1
130	25830	(BTX 623 x IS 18551)-173-1-1-1-1
131	25831	(BTX 623 x IS 18551)-174-1-1-1
132	25832	(BTX 623 x IS 18551)-175-1-1-1
133	25833	(BTX 623 x IS 18551)-177-1-1-1
134	25834	(BTX 623 x IS 18551)-178-1-1-1
135	25835	(BTX 623 x IS 18551)-180-1-1-1-1
136	25836	(BTX 623 x IS 18551)-181-1-1-1
137	25837	(BTX 623 x IS 18551)-182-1-1-1-1
138	25838	(BTX 623 x IS 18551)-184-1-1-1-1
139	25839	(BTX 623 x IS 18551)-185-1-1-1-1
140	25840	(BTX 623 x IS 18551)-186-1-1-1-1
141	25841	(BTX 623 x IS 18551)-187-1-1-1-1
142	25842	(BTX 623 x IS 18551)-189-1-1-1-1
143	25843	(BTX 623 x IS 18551)-191-1-1-1-1
144	25844	(BTX 623 x IS 18551)-192-1-1-1-1
145	25845	(BTX 623 x IS 18551)-193-1-1-1-1
146	25846	(BTX 623 x IS 18551)-194-1-1-1-1
147	25847	(BTX 623 x IS 18551)-195-1-1-1-1
148	25848	(BTX 623 x IS 18551)-196-1-1-1-1
149	25849	(BTX 623 x IS 18551)-197-1-1-1-1
150	25850	(BTX 623 x IS 18551)-198-1-1-1-1
151	25851	(BTX 623 x IS 18551)-199-1-1-1-1
152	25852	(BTX 623 x IS 18551)-201-1-1-1-1
153	25853	(BTX 623 x IS 18551)-203-1-1-1-1
154	25854	(BTX 623 x IS 18551)-204-1-1-1-1
155	25855	(BTX 623 x IS 18551)-205-1-1-1-1
156	25856	(BTX 623 x IS 18551)-206-1-1-1
157	25857	(BTX 623 x IS 18551)-207-1-1-1
158	25858	(BTX 623 x IS 18551)-208-1-1-1-1
159	25859	(BTX 623 x IS 18551)-209-1-1-1-1
160	25860	(BTX 623 x IS 18551)-210-1-1-1
161	25861	(BTX 623 x IS 18551)-211-1-1-1
162	25862	(BTX 623 x IS 18551)-212-1-1-1
163	25863	(BTX 623 x IS 18551)-213-1-1-1
164	25864	
165	25865	
166	25866	6 (BTX 623 x IS 18551)-218-1-1-1
167	25867	
168	25868	BTX 623 x IS 18551)-221-1-1-1
		(contd)

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Entry C	Drigin	Pedigree
169 2	5869	(BTX 623 x IS 18551)-221-1-2-1-1
170 2	5870	(BTX 623 x IS 18551)-222-1-1-1-1
171 2	5871	(BTX 623 x IS 18551)-222-1-2-1-1
172 2	5872	(BTX 623 x IS 18551)-223-1-1-1-1
173 2	5873	(BTX 623 x IS 18551)-223-1-2-1-1
174 2	5874	(BTX 623 x IS 18551)-224-1-1-1-1
175 2	5875	(BTX 623 x IS 18551)-224-1-2-1-1
176 2	5876	(BTX 623 x IS 18551)-226-1-1-1-1
177 2	5877	(BTX 623 x IS 18551)-226-1-2-1-1
178 9	91679	(BTX 623 x IS 18551)-227-1-1-1
179 2	25878	(BTX 623 x IS 18551)-227-1-2-1-1
180 2	5879	(BTX 623 x IS 18551)-228-1-1-1-1
181 2	25880	(BTX 623 x IS 18551)-228-1-2-1-1
182 2	5881	(BTX 623 x IS 18551)-229-1-1-1-1
183 2	25882	(BTX 623 x IS 18551)-229-1-2-1-1
184 2	25883	(BTX 623 x IS 18551)-231-1-1-1
185 2	25884	(BTX 623 x IS 18551)-231-1-2-1-1
186 2	5885	(BTX 623 x IS 18551)-232-1-1-1-1
187 2	25886	(BTX 623 x IS 18551)-232-1-2-1-1
188 2	25887	(BTX 623 x IS 18551)-233-1-1-1-1
189 2	5888	(BTX 623 x IS 18551)-233-1-2-1-1
190 2	5889	(BTX 623 x IS 18551)-234-1-1-1-1
191 2	5890	(BTX 623 x IS 18551)-234-1-2-1-1
192 2	25891	(BTX 623 x IS 18551)-237-1-1-1-1
193 2	25892	(BTX 623 x IS 18551)-237-1-2-1-1
	25893	(BTX 623 x IS 18551)-238-1-1-1-1
195 2	25894	(BTX 623 x IS 18551)-238-1-2-1-1
196 2	258 9 5	(BTX 623 x IS 18551)-239-1-1-1
197 2	25896	(BTX 623 x IS 18551)-239-1-2-1-1
	25897	(BTX 623 x IS 18551)-240-1-1-1
	25898	(BTX 623 x IS 18551)-240-1-2-1-1
	25899	(BTX 623 x IS 18551)-241-1-1-1
	25900	(BTX 623 x IS 18551)-241-1-2-1-1
,	25901	(BTX 623 x IS 18551)-242-1-1-1
	25902	(BTX 623 x IS 18551)-242-1-2-1-1
	25903	(BTX 623 x IS 18551)-244-1-1-1-1
	25904	(BTX 623 x IS 18551)-244-1-2-1-1
	25905	(BTX 623 x IS 18551)-245-1-1-1
	25906	(BTX 623 x IS 18551)-245-1-2-1-1
	25907	(BTX 623 x IS 18551)-247-1-1-1
209 9	91710	(BTX 623 x IS 18551)-247-1-2-1
210	25908	(BTX 623 x IS 18551)-248-1-1-1-1

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Entry	Origin	Pedigree
211	25909	(BTX 623 x IS 18551)-248-1-2-1-1
212	25910	(BTX 623 x IS 18551)-249-1-1-1-1
213	25911	(BTX 623 x IS 18551)-249-1-2-1-1
214	25912	(BTX 623 x IS 18551)-250-1-1-1-1
215	25913	(BTX 623 x IS 18551)-250-1-2-1-1
216	25914	(BTX 623 x IS 18551)-251-1-1-1
217	25915	(BTX 623 x IS 18551)-251-1-2-1-1
218	25916	(BTX 623 x IS 18551)-252-1-1-1-1
219	25917	(BTX 623 x IS 18551)-252-1-2-1-1
220	25918	(BTX 623 x IS 18551)-253-1-1-1-1
221	25919	(BTX 623 x IS 18551)-253-1-2-1-1
222	25920	(BTX 623 x IS 18551)-255-1-1-1-1
223	25921	(BTX 623 x IS 18551)-255-1-2-1-1
224	25922	(BTX 623 x IS 18551)-256-1-1-1-1
225	25923	(BTX 623 x IS 18551)-256-1-2-1-1
226	25924	(BTX 623 x IS 18551)-258-1-1-1-1
227	25925	(BTX 623 x IS 18551)-258-1-2-1-1
228	25926	(BTX 623 x IS 18551)-260-1-1-1-1
229	25927	(BTX 623 x IS 18551)-260-1-2-1-1
230	25928	(BTX 623 x IS 18551)-261-1-1-1
231	25929	(BTX 623 x IS 18551)-261-1-2-1-1
232	25930	(BTX 623 x IS 18551)-262-1-1-1-1
233	25931	(BTX 623 x IS 18551)-262-1-2-1-1
234	25932	(BTX 623 x IS 18551)-263-1-1-1-1
235	25933	(BTX 623 x IS 18551)-263-1-2-1-1
236	25934	(BTX 623 x IS 18551)-265-1-1-1-1
237	25935	(BTX 623 x IS 18551)-265-1-2-1-1
238	25936	(BTX 623 x IS 18551)-266-1-1-1-1
239	25937	(BTX 623 x IS 18551)-266-1-2-1-1
240	25938	(BTX 623 x IS 18551)-267-1-1-1-1
241	25939	(BTX 623 x IS 18551)-267-1-2-1-1
242	25940	(BTX 623 x IS 18551)-268-1-1-1-1
243	25941	(BTX 623 x IS 18551)-268-1-2-1-1
244	25942	(BTX 623 x IS 18551)-270-1-1-1-1
245	25943	(BTX 623 x IS 18551)-270-1-2-1-1
246	25944	(BTX 623 x IS 18551)-272-1-1-1-1
247	25945	(BTX 623 x IS 18551)-272-1-2-1-1
248	25946	(BTX 623 x IS 18551)-273-1-1-1-1
249	25947	(BTX 623 x IS 18551)-273-1-2-1-1
250	25948	(BTX 623 x IS 18551)-274-1-1-1-1
251	25949	(BTX 623 x IS 18551)-274-1-2-1-1
252	25950	(BTX 623 x IS 18551)-276-1-2-1-1

Appendix II. Preparation of stock solutions

CTAB (Cetyl Trimethyl Ammonium Bromide) (2%) buffer

CTAB	20 g
1 M Tris	200 ml
5 M NaCl	280 ml
0.5 M EDTA	40 ml
Na ₂ SO ₃	2.5 g
Distilled water	460 ml

Add mercaptoethanol (0.1%) fresh while using CTAB (2%) solution.

RNase (10 mg/ml)

Dissolve RNase in water, place in a tube in a boiling water bath for 10 minutes. Allow this to cool on a bench and store at -20° C.

Chloroform: isoamyl alcohol (24: 1)

Chloroform	240 ml
Isoamyl alcohol	10 ml

Store in dark at room temperature. Make up and dispense the solution in a fumed cupboard.

Ethanol (70%)

Absolute alcohol 70 ml Distilled water 30 ml

NaCl (5 M)

Dissolve 292.2 g NaCl in 750 ml water. Make up to 1 litre with water, filter and autoclave.

Phenol/chloroform

Mix equal volumes of the buffered phenol and chloroform: isoamyl alchohol (24: 1). Store at 4 °C.

Sodium acetate (2.5 M, pH 5.2)

Dissolve 340.2 g Sodium acetate in 500 ml water. Adjust pH to 5.2 with glacial acetic acid and make volume up to 1 litre and autoclave.

Tris HCl (1 M, pH 8.0)

Dissolve 121.1 g Tris in 800 ml of water. Adjust pH to 8.0 with conc. HCl. Make volume up to 1 litre and autoclave.

EDTA (0.5 M, pH 8.0)

Dissolve 186.1 g Na₂ EDTA.2H₂O in 800 ml water. Adjust pH to 8.0 with sodium hydroxide pellets. Make up volume to 1 litre and autoclave.

T₁₀E₁ buffer

1 M Tris HCl pH 8.010 ml1 M EDTA pH 8.01 mlAnd make up to 1 litre with sterile distilled water.

T₅₀E₁₀ buffer

1 M Tris Cl pH 8.050 ml0.5 M EDTA pH 8.020 mlMake volume up to 1 litre with sterile distilled water.

10X Tris-Borate Buffer (TBE) (per litre)

Tris base	108 g ·
Boric acid	55 g
EDTA (0.5 M) pH 8.0	40 ml
dH ₂ O	Up to 1000 ml

Mix well and store at 4 °C.

6X Gel loading buffer (0.25% Bromophenol blue, 40% sucrose) (10 ml)

Sucrose	4 g
Bromophenol Blue	2.5 ml
dH ₂ O	Up to 10 ml

Store at 4 °C.

Ethidium bromide (10 mg/ml)

Dissolve 100 mg ethidium bromide in 10 ml of distilled water, wrap tube in aluminium foil and store at 4 °C. *Caution*: Ethidium bromide is extremely mutagenic

Acrylamide/biacrylamide 29: 1 (w/w)

Acrylamide29 gBisacrylamide1 gWater (deionised distilled)Up to 100 ml

Store at 4 °C for ≤ 1 month

10% (w/v) Ammonium per sulphate

Ammonium per sulphate	1 g
Water (deionised distilled)	10 ml

Make fresh stock every week and store at 4 °C.

TEMED (N,N,N',N'-tetramethyl ethylene diamine) Store at 4 °C.

Loading buffer for nondenaturing PAGE, 5X

EDTA (0.5 M, pH 8)	10 ml
NaCl (5 M)	1 ml
Glycerol	50 ml
Distilled water	39 ml

Add orange dye powder until the colour is sufficiently dark.

Binding silane buffer

Binding silane	1.5 μl
Acetic acid	5 ml
Ethanol	993.5 ml

Store at 4 °C

100 base pairs ladder (50 ng/ml)

100 bp ladder (stock conc. 1µg/µl)	50 µl
Blue (6X dye)	165 µl
$T_{10}E_1$ buffer	785 µl

							Ann term	Ann temo Ann temo
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Sl.no	Sl.no Locus	Ľ	P1 (bp) P2 (bp)	Pl (bp) P2 (bp) Sequence of torward primer	Sequence of reverse primer	(r) (r)	(K) (C)
	Kaf2-e	7	270	260	TCGGCGAGCATCITACA	TACGTAGGCGGTTGGATT	52.8	53.7
•	Xgapl	Ċ	270	272	TCCTGTTTGACAAGCGCTTATA	AAACATCATACGAGCTCATCAATG	•60	•60
~	Xgap236	Ċ,	180	181	GCCAAGAGAAACACAAACAA	AGCAATGTATTTAGGCAACACA	*57	*57
_	Xgap342	ы	350	340	TITICCTCTTTCAGATAACCGTA	CCCACCAAGGGCATC	•60	•60
	XSbKAFGKI	۰	270	265	GCTTTCGGCGAGCATCTTACAA	GCGGTTGGATTCGCCATG	60.3	58.2
	Xtxp1	8	210	190	TTG GCT TTT GTG GAG CTG	ACC CAG CAG CAC TAC ACT AC	53.7	59.4
	Xtxp3	8	220	185	AGC AGG CGT TTA TGG AAG	ATC CTC ATA CTG CAG GAC C	53.7	56.7
	Xtxp6	I	110	85	ACT GGA TCC GTC AGA TC	TCT AAG GAG GTT GCC AC	52.8	52.8
-	Xtxp7	8	250	255	ACA TCT ACT ACC CTC TCA CC	ACA CAT CGA GAC CAG TTG	57.3	53.7
0	Xtxp8	8	150	130	ATA TGG AAG GAA GAA GCC GG	AAC ACA ACA TGC ACG CAT G	57.3	54.5
-	Xtxp9	U	160	120	AATAGCACCGCCGCGCG	CATTGTGGAGTCCCTGATAC	60	57.3
2	Xtxp10	í.	145	155	ATA CTA TCA AGA GGG GAG C	AGT ACT AGC CAC ACG TCA C	54.5	56.7
ß	Xtxp12	۵	205	195	AGA TCT GGC GGC AAC G	AGT CAC CCA TCG ATC ATC	54.3	53.7
4	Xtxp15	r	225	230	CAC AAA CAC TAG TGC CIT ATC	CAT AGA CAC CTA GGC CAT C	55.9	56.7
S	Xtxp18	Н	225	205	ACT GTC TAG AAC AAG CTG CG	TTG CTC TAG CTA GGC ATT TC	57.3	55.3
ė	Xtxp21	Q	165	160	GAG CTG CCA TAG ATT TGG TCG	ACC TCG TCC CAC CIT TGT TG	59.8	59.4
5	Xtxp24	A	250	250	TTG TGT AGT CCA TCC GAT GC	TTC TAA GCC CAC CGA AGT TG	57.3	57.3
×	Xtxp25	8	140	170	CCA TTG AGC TTC TGC TAT CTC	CAT TTG TCA CCA CTA GAA CCC	57.9	57.9
6	Xtxp31	U	200	200	TGC GAG GCT GCC CTA CTA G	TGG ACG TAC CTA TTG GTG C	61	56.7
20	Xtxp32	¥	125	130	AGA AAT TCA CCA TGC TGC AG	ACC TCA CAG GCC ATG TCG	55.3	58.2
51	Xtxp33	ပ	353	360	GAG CTA CAC AGG GTT CAA C	CCT AGC TAT TCC TTG GTT G	56.7	54.5
ដ	Xtxp34	U	360	345	TGG TTC GTA TCC TTC TCT ACA G	CAT ATA CCT CCT CGT CGC TC	58.4	59.4
ຕ	Xtxp36	E	190	190	ATG GGA CGG AAA TGC AGG AG	TTA TGC CTG CCA GCA ACT TG	59.4	57.3
54	Xtxp37	A.	194	173	AAC CTA AGA GGC CTA TIT AAC C	ACG GCG ACT ATG TAA CTC ATA G	56.5	58.4
25	Xtxp40	ш	137	140	CAG CAA CTT GCA CTT GTC	GGG AGC AAT TTG GCA CTA G	53.7	56.7
							(contd)	

Appendix III. Details on parental polymorphism between BTx623 and IS18551 shown by SSR primers and characterstics of primer sets

							Ann ten	Ann temp Ann temp
Sl.no	Sl.no Locus	DJ		p) P2 (bp	P1 (bp) P2 (bp) Sequence of forward primer	Sequence of reverse primer	(F) (°)	(R) (°C)
36	Xtxp41	٩		300	TCT GGC CAT GAC TTA TCA C	AAA TGG CGT AGA CTC CCT TG	54.5	57.3
27	Xtxp43	¥	160	185	AGT CAC AGC ACA CTG CTT GTC	AAT TTA CCT GGC GCT CTG C	59.8	56.7
28	Xtxp46	¥	255	255	GGGCAATCTTGATGGCGACAT	AGGTGTGGCTCGGGGGAGAAC	59.8	63.5
29	Xtxp47	Η	300	300	CAATGGCTTGCACATGTCCTA	GGTGCGAGCTAGTTAAGTGGG	57.9	61.8
30	Xtxp50	æ	330	340	TGATGTTGTTACCCTTCTGG	AGCCTATGTATGTGTTCGTCC	55.3	57.9
31	Xtxp55	8	225	230	TCATGGCATGGGACTATTG	AAGGTTGGCGTAGAAATGTGT	54.5	55.9
32	Xtxp56	8	440	390	TGTCTTCGTAGTTGCGTGTTG	CCGAAGGAGTGCTTTGGAC	57.9	58.8
33	Xtxp57	I	240	230	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	59.8	56.7
34	Xtxp58	¥	180	185	CAAAGTGCCCGGTTAAGACCT	TTCCCTTGCTGTTGCTTGTG	59.8	57.3
35	Xtxp59	U	198	200	GAAATCCACGATAGGGTAAGG	GACCCAGAATAGAAGAGAGG	57.9	57.3
36	Xtxp61	¥	140	140	GATGCCCATGCCTTGC	CCCACTAAACTAAAGCGGGACA	54.3	57.9
37	Xtxp65	7	135	142	CACGTCGTCACCAACCAA	GTTAAACGAAAGGGAAATGGC	56	55.9
38	Xtxp69	C	195	230	ACACGCATGGTTTGACTG	TTGATAATCTGACGCAACTG	53.7	53.2
39	Xtxp75	¥	185	170	CGATGCCTCGAAAAAAAACG	CCGATCAGAGCGTGGCAGG	55.9	63.1
40	Xtxp80	•	310	330	GCTGCACTGTCCTCCCACAA	CAGCAGGCGATATGGATGAGC	61.4	61.8
41	Xtxq88	۲	145	120	CGTGAATCAGCGAGTGTTGG	TGCGTAATGTTCCTGCTC	59.4	53.7
42	Xtxp94	2	230	215	TTTCACAGTCTGCTCTCTG	AGGAGAGTTGTTCGTTA	54.5	47.9
43	Xtxp96	B	190	185	GCTGATGTCATGTTCCCTCAC	CATTCGTGGACTCTGTCGG	59.8	58.8
44	Xtxp98	١	200	200	GCCAGCTTATCGGAAACAAG	GTGTCCACTAGAGCGGCTATG	57.3	61.8
45	Xtxp100	8	145	145	CCGGCCGGCCAACCAACCAC	TGCCCCAACGCTCACGCTCCC	67.6	67.6
46	Xtxp114	ပ	245	245	CGTCTTCTACCGCGTCCT	CATAATCCCACTCAACAATCC	58.2	55.9
47	Xtxp141	U	170	160	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	52.4	47.9
48	Xtxp145	I	225	232	GTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	53.7	50.6
49	Xtxp145	¥	175	175	AGCCTTGCATGATGTTCC	GCTATGCTTGGTGTGGG		
50	Xtxp159	ы	173	173	ACCCAAAGCCCAAATCAG	GGGGGAGAAACGGTGAG	53.7	57.6 D
51	Xtxp162	•	220	214	CCCGATATCTCTCCTGAC	CCCCAGTTTAGCCAAGT	56	52.8
							(contd	

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							Ann teng	Ann temp Ann temp
il.no	Sl.no Locus	5 <u>0</u>	P1 (bp)	P2 (bp)	P1 (bp) P2 (bp) Sequence of forward primer	Sequence of reverse primer	(F) (°C)	(R) (°C)
52	Xtxp168	щ	180	180	AGTCAAAACCGCCACAT	GAGAAGGGAGAGAGAGAAA	50.4	56
53	Xtxp177	Q	163	167	GCCGGTTGTGACTTG	TTAAAGCGATGGGTGTAG	50.6	51.4
4	Xtxqp183	U	190	180	AAGTTGTAATGGGGCTATTG	TTAAGAGGTGGGATATTGGT	53.2	53.2
Ś	Xtxp205	U	205	210	CCTGCCGTGTCTTCC	TATATGCATGCCGTAGATTT	53.3	51.2
56	Xtxp207	8	190	194	ACACATCTACTACCTCTCACCCT	TGATAGACTTGTGAGCAGCTCC	62.7	60.3
r	Xtxp210	Η	190	210	CGCTTTTTCTGAAAATATTAAGGAC	GATGAGCGATGGAGGAGAG	55.9	58.8
58	Xtxp211	8	210	232	TCAACGGCCAATGATTTCTAAC	AGGTTGCGAATAAAAGGTAATGTG	56.5	57.6
6	Xtxp218	U	215	210	CCGGAAAACCTGCTACTG	ACGCCGGAAGGAGAAG	56	54.3
8	Xtxp225	7	162	162	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	51.2	52.4
61	Xtxp228	U	255	240	ACAGGTTGGCGATGTTTCTCT	TTCTTTTTCGAATTCATTCCTTTT	57.9	52.5
62	Xtxp229	¥	173	175	TGCCCAAGAGGATAAAAGGT	AGCGACGGCACATCAAT	55.3	52.8
8	Xtxp230	ίH.	192	198	GCTACCGCTGCTGCTCT	AGGGGGCATCCAAGAAAT	57.6	53.7
2	Xtxp231	U	185	187	GGAAATCCAGGATAGGGT	AGGCAAAGGGTCATCA	57.9	49.2
65	Xtxp248	¥	245	220	GGGTGTCCAATGTTGTCTGC	GGCCGTTACTGTCCCTTACTCA	59.4	62.1
66	Xtxp258	۶.	252	195	CACCAAGTGTCGCGAACTGAA	GCTTAGTGTGAGCGCTGACCAG	59.8	2
67	Xtxp262	r	185	181	TGCCTGCCCGACCTG	TTGCTGTCCCGCTTTCC	56	56
68	Xtxp265	I	228	210	GTCTACAGGCGTGCAAATAAAA	TTACCATGCTACCCCTAAAAGT66	56.5	61
69	Xtxp274	I	360	329	GAAATTACAATGCTACCCCTAAAAGT	ACTCTACTCCTTCCGTCCACAT	58.5	60.3
70	Xtxp279	¥	290	287	ATTCTGACTTAACCCACCCCTAAA	AGCTCATCAATGTCCCAAACC	59.3	57.9
1	Xtxp283	8	250	249	CGCCCGAACTCTTCTTAAATCT	ATTATGCCCTAACTGCCTTTGA	58.4	56.5
72	Xtxp284	¥	255	258	CCAGATTGGCTGATGCATACACACT	AAGGGTAATTTATGCACTCCAAGGTAGGAC	C 63	65.4
73	Xtxp285	J	265	248	ATTIGATICITCITGCITTGCCTTGT	TTGTCATTTCCCCCTTCTTTCTTTT	58.5	58.1
74	Xtxp286	B	200	230	AGCAGCAGCAGCAACAG	GCGTGGTCTTTGTGGTTC	55.2	56
75	Xtxp289	ί¥.	285	260	AAGTGGGGTGAAGAGATA	CTGCCTTTCCGACTC	51.4	50.6
76	Xtxp292	Η	325	340	CATTTGCGAAGTTACAACATTGCT	CATTCCTGACTGCCCTCTCC	57.6	61.4
77	Xtxp294	Η	330	338	GCTGGGGCTCGAGGGTTTTCATT	AGCTTCCCAAGGACAACTAGCAAGGACA	64.2	9.99

	···· /						Ann temp	Ann temp Ann temp
Slno	SI no Locus	DT) P2 (bp	P1 (bp) P2 (bp) Sequence of forward primer	Sequence of reverse primer	(F) (⁰ C)	(R) (⁰ C)
78	Xtxp295	ш		178	AAATCATGCATCCATGTTCGTCTTC	CTCCCGCTACAAGAGTACATTCATAGCTTA 59.7	59.7	65.4
6	Xtxp296	8	172	180	CAGAAATAACATATAATGATGGGGTGAA	ATGCTGTTATGATTTAGAGCCTGTAGAGTT	59.3	62.7
0	Xtxp298	æ	195	188	GCATGTGTCAGATGATCTGGTGA	GCTGTTAGCTTCTTCTAATCGTCGGT	9.09	63.2
81	Xtxp302	<	190	245	TAGGTTCTGGACCACTTTTTCTTTTTGTGTT	GAATCAACTATGTGCTTGCATTGTGCT	62.7	61.9
82	Xtxp304	B	196	193	ACATAAAGCCCCTCTTC	CTTTCACACCCTTTATTCA	51.4	50.2
83	Xtxp312	멸	188	175	CAGGAAAATACGATCCGTGCCAAGT	GTGAACTATTCGGAAGAAGTTTGGAGGAAA 63	V 63	64
84	Xtxp316	<	350	445	CCAGCTTCACTTACGAGGAGATG	ATGCCCGTTTTCTAATTCTTCTACT	62.4	58.1
85	Xtxp317	Ι	160	152	CCTCCTTTTCCTCCTCCCC	TCAGAATCCTAGCCACCGTTG	63.7	59.8
86	Xtxp319	×	155	138	TAGACATCTGAATTAAGGAGC	CATGCCCTGAAAGAGA	54	52.8
	X1xm320				TAAACTAGACCATATACTGCCATGAT			
87	(PhyB)	•	292	283	AA	GTGCAAATAAGGGCTAGAGTGTT	59.3	58.9
88	Xtoq321	H	198	188	TAACCCAAGCCTGAGCATAAGA	CCCATTCACACATGAGACGAG	58.4	59.8
89	Xtxp327	۵	153	149	ACCACTGCTCACGCTCAC	GCGGTGTACAGCTTCGTC	58.2	58.2
8	Xtxqp329		160	150	ACGACGACGAGGTGG	TTCAACAAAGGAAAGGATTC	59.7	54.8
16	Xtxp331	Ċ		220	AACGGTTATTAGAGAGGGAGA	AGTATAATAACATTTTGACACCCA	55.9	54.2
92	Xtxp336	с С	170	170	CAGCGAGCACCGACGAC	CCACCCAACCTGACCCTTCT	60.0	61.4
93	Xtxp340	•	205	200	AGAACTGTGCATGTATTCGTCA	AGAAACTCCAATTATCATCCATCA	56.5	55.9
. 4	xtxp348	8	360	350	CGACATCAGCGTTGTCTTTCTA	AGAAACTCCAATTATCATCATCA	58.4	59.3
95	Xtxp354	Н	165	165	TGGGCAGGGTATCTAACTGA	AGAAACTCCAATTATCATCATCA	57.3	54.5
8	Xtxp357	•	298	288	CGCAGAAATACGATTG	AGAAACTCCAATTATCATCATCA	46.6	55.3
ð •	* Optimum annealing	ling						

Optuine aureance LG : linkage group P1: Btx623 P2: IS18551 bp: base pairs F: forward primer R: reverse primer P

(contd...)

Appendix IV. Percentage of parental alleles and heterozygous loci in 93 RILs of cross BTX623 x IS 18551

(contd)

10551				(conta)			
Entry	Percentage o P1 parental alleles	f Percentage of P2 parental alleles	Percentage of heterozygous alleles	Entry	Percentage of P1 parental alleles	Percentage of P2 parental alleles	Percentage of heterozygous alleles
1	26.2	73.8	0.0	48	52.9	47.1	0.0
2	53.5	46.5	0.0	49	54.5	45.5	0.0
3	53.5	46.5	0.0	50	31.8	65.9	2.3
4	47.7	50.0	2.3	51	39.5	60.5	0.0
5	52.5	47.5	0.0	52	54.8	42.9	2.4
6	60.5	37.2	2.3	53	47.7	50.0	2.3
7	41.9	58.1	0.0	54	43.2	54.5	2.3
8	45.2	54.8	0.0	55	43.2	54.5	2.3
9	43.6	53.8	2.6	56	53.5	46.5	0.0
10	51.2	48.8	0.0	57	50.0	50.0	0.0
11	54.5	45.5	0.0	58	46.5	53.5	0.0
12	52.4	47.6	0.0	59	56.1	43.9	0.0
13	42.9	57.1	0.0	60	46.5	48.8	4.7
14	43.2	56.8	0.0	61	47.7	52.3	0.0
15	32.6	67.4	0.0	62	52.3	47.7	0.0
16	61.4	38.6	0.0	63	61.9	38.1	0.0
17	43.2	56.8	0.0	64	50.0	47.7	2.3
18	44.2	55.8	0.0	65	33.3	66.7	0.0
19	46.3	53.7	0.0	66	56.8	43.2	0.0
20	21.4	76.2	2.4	67	45.5	52.3	2.3
21	43.2	56.8	0.0	68	51.2	46.3	2.4
22	43.2	56.8	0.0	69	38.1	61.9	0.0
23	31.0	66.7	2.4	70	57.1	42.9	0.0
24	51.2	48.8	0.0	71	47.7	50.0	2.3
25	50.0	50.0	0.0	72	54.5	45.5	0.0
26	40.9	59.1	0.0	73	40.9	59.1	0.0
27	57.1	42.9	0.0	74	58.1	41.9	0.0
28	48.6	51.4	0.0	75	68.2	31.8	0.0
29	43.2	56.8	0.0	76	33.3	64.3	2.4
30	27.9	72.1	0.0	77	68.2	31.8	0.0
31	58.1	41.9	0.0	78	40.9	59.1	0.0
32	48.8	51.2	0.0	79	53.5	46.5	0.0
33	55.8	44.2	0.0	80	43.2	52.3	4.5
34	72.1	25.6	2.3	81	59.1	40.9	0.0
35	47.7	52.3	0.0	82	55.8	44.2	0.0
36	40.9	59.1	. 0.0	83	50.0	50.0	0.0
37	34.9	65.1	0.0	84	40.5	57.1	2.4
38	• 40.9	59.1	0.0	85	69.8	30.2	0.0
39	38.5	61.5	́ 0.0	86	57.5	40.0	2.5
40	32.6	67.4	0.0	87	44.2	55.8	0.0
41	36.8	63.2	0.0	88	46.5	53.5	0.0
42	50.0	50.0	0.0	89	45.2	52.4	2.4
43	47.7	52.3	0.0	90	31.8	65.9	2.3
44	39.5	60.5	0.0	91	54.8	42.9	2.4
45	43.2	56.8	0.0	92	82.5	15.0	2.5
46	36.4	63.6	0.0	93	60.0	40.0	0.0
40	47.6	52.4	0.0	P1: BTx6			

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TIAO	пло дил	UNO dun win	TIAO dnn MIN I	UNO dun win
	6.5 54.5	66.6 6.5 54.5	66.6 6.5 54.5	2.2 66.6 6.5 54.5
8.8 50.2	8.8	72.4 8.8	8.8	72.4 8.8
3.4 52.9	3.4	28.4 3.4	3.4	28.4 3.4
1.0 52.6	1.0	4.3 1.0	1.0	4.3 1.0
1.3 65.0	1.3	4.7 1.3	1.3	4.7 1.3
6.1 54.9	6.1	36.8 6.1	36.8 6.1	3.1 36.8 6.1
1.4 53.4	1.4	41.1 1.4	41.1 1.4	2.4 41.1 1.4
5.2 58.8	5.2	47.1 5.2	47.1 5.2	2.4 47.1 5.2
4.8 62.5	4.8	41.0 4.8	41.0 4.8	2.4 41.0 4.8
11.9 56.5	11.9	58.6 11.9	58.6 11.9	2.5 58.6 11.9
1.1 50.2	1.1	13.1 1.1	13.1 1.1	3.2 13.1 1.1
3.1 53.9	3.1	36.9 3.1	36.9 3.1	2.0 36.9 3.1
0.3 57.8	0.3	36.6 0.3	36.6 0.3	2.7 36.6 0.3
0.9 54.5	0.9	28.9 0.9	28.9 0.9	2.6 28.9 0.9
1.1 52.0	1.1	4.4 1.1	4.4 1.1	2.2 4.4 1.1
4.4 53.1	4.4	55.8 4.4	4.4	55.8 4.4
0.6 51.8	0.6	61.2 0.6	0.6	61.2 0.6
1.2 58.3	1.2	60.7 1.2	1.2	60.7 1.2
11.7 60.2	11.7	43.4 11.7	11.7	43.4 11.7
14.5 48.1	14.5	77.0 14.5	14.5	77.0 14.5
1.0 51.3	1.0	. 30.7 1.0	1.0	. 30.7 1.0
8.9 59.8	8.9	67.4 8.9	8.9	67.4 8.9
10.1 54.0	10.1	61.9 10.1	10.1	61.9 10.1
1.4 46.6	1.4	3.0 1.4	1.4	3.0 1.4
0.8 57.6	0.8	5.2 0.8	5.2 0.8	2.7 5.2 0.8
1.7 52.6	1.7	13.2 1.7	13.2 1.7	2.0 13.2 1.7
•	10.2	43.6 10.2	43.6 10.2	2.7 43.6 10.2
11.4 52.5	11.4	85.8 11.4	85.8 11.4	2.8 85.8 11.4
2.9 63.1	2.9	19.8 2.9	2.9	2.4 19.8 2.9
0.5 53.7	0.5	28.7 0.5	28.7 0.5	3.1 28.7 0.5
12.0 46.0				
	12.0	44.1 12.0	12.0	44.1 12.0

, mm	g]	vigI	vigII	trlw	trup	ovil	ovill	IdhI	IIHb	shtl	shtII	drywt	pslg	daf	pht	PY
33	4.7	2.5	2.7	45.0	10.5	55.9	75.9	60.8	71.4	23.6	42.9	3.4	3.0	85.4	244.0	12.1
34	3.0	2.6	3.3	81.3	17.4	56.5	77.2	58.3	72.5	23.5	43.4	3.0	2.5	78.3	136.4	20.1
35	4.6	2.2	2.3	50.9	0.0	58.0	81.5	67.2	75.4	24.6	42.2	3.2	2.5	73.2	170.1	18.8
36	3.2	2.4	2.6	62.2	7.4	59.1	77.5	53.0	72.2	25.7	43.9	3.4	3.0	78.1	294.2	9.6
37	2.0	1.8	2.1	44.4	7.5	48.5	71.9	43.2	60.2	23.6	45.3	3.3	2.8	73.1	279.2	13.2
38	4.2	2.3	2.6	58.6	17.3	49.9	71.0	52.7	68.1	23.7	43.8	2.9	2.4	84.5	243.5	12.6
39	3.4	2.2	2.1	51.3	10.1	53.9	78.2	54.2	65.6	25.4	43.1	3.3	3.2	80.4	252.9	13.3
40	2.4	2.6	2.6	44.3	5.3	52.5	80.3	59.7	71.6	24.2	42.1	2.9	2.3	70.1	196.2	16.9
41	4.0	1.8	1.9	62.7	16.8	49.7	73.8	53.5	71.8	27.8	45.3	3.6	3.5	73.0	255.0	13.4
42	3.7	2.1	1.9	43.8	5.1	52.1	74.5	50.4	64.4	27.4	45.8	3.1	2.6	79.6	288.4	6.6
43	4.5	2.4	2.8	57.9	19.5	52.7	74.8	67.5	79.3	23.6	42.4	2.8	2.4	84.1	241.3	13.2
44	4.2	2.1	2.3	55.2	5.0	57.4	72.2	60.8	70.8	25.7	45.3	3.0	2.6	80.9	260.6	21.8
45	4.9	2.7	2.7	3.3	1.4	59.6	76.7	64.1	76.5	24.9	44.1	3.2	2.7	80.1	257.0	14.8
46	3.4	2.0	2.3	56.7	2.6	59.9	79.1	66.4	75.6	25.2	46.6	3.3	3.2	74.1	279.5	14.4
47	4.7	2.5	2.4	76.4	17.7	45.3	75.0	51.6	66.1	23.5	42.5	2.9	2.4	77.0	197.6	17.0
48	4.3	2.3	2.6	23.5	4.7	55.4	80.0	64.5	78.5	25.2	41.3	3.0	2.4	72.6	240.1	22.7
49	2.3	3.3	3.0	11.8	4.8	60.09	78.4	63.3	80.5	24.5	41.I	3.1	2.5	86.2	172.8	14.9
50	3.9	2.2	2.0	49.9	7.4	51.4	70.7	51.6	62.1	27.4	47.6	3.4	2.9	80.3	238.4	8.8
51	2.6	2.4	2.1	42.6	6.3	48.0	72.7	44.4	55.3	24.0	43.9	3.1	2.9	74.6	218.0	11.8
52	4.3	2.1	2.1	47.4	11.3	54.1	72.1	59.4	69.8	26.7	45.0	3.1	2.6	74.2	250.8	17.8
53	2.8	3.1	2.3	83.0	12.3	49.5	69.5	43.0	54.1	24.1	42.9	2.9	2.6	72.6	233.8	12.7
54	2.5	2.4	2.6	36.5	4.5	57.6	76.3	61.0	68.2	24.5	41.7	3.3	2.7	86.1	241.6	14.4
55	2.1	2.3	2.4	43.6	4.4	48.5	75.5	48.6	64.7	24.5	43.1	2.9	2.4	73.4	239.5	13.5
56	3.8	1.8	2.4	42.4	4,4	55.8	76.1	69.5	80.1	26.9	45.1	3.0	2.6	76.0	292.9	9.6
57	4.5	2.2	2.5	42.7	5.1	54.1	75.4	63.4	70.8	25.4	45.0	2.9	2.4	77.0	249.7	7.3
58	4.7	2.3	3.3	22.7	3.8	57.7	80.2	72.4	85.0	24.6	42.7	3.0	2.5	91.8	269.5	7.1
59	4.0	1.9	2.8	21.9	7.6	64.6	76.7	64.8	80.2	26.6	44.4	3.1	2.6	79.8	286.9	15.3
60	3.1	2.2	2.6	65.4	2.7	63.4	73.3	62.3	76.2	25.8	43.2	3.1	2.8	74.5	275.9	15.0
61	2.2	2.6	2.5	72.9	8.7	52.2	70.2	43.2	56.7	23.2	42.1	3.0	2.3	75.8	241.9	17.6
62	3.6	1.9	1.9	40.6	3.5	42.0	73.3	52.5	66.4	26.0	43.4	3.0	2.5	81.5	253.2	10.0
63	4.7	2.2	3.3	44.3	0.9	59.3	77.5	71.1	79.9	24.1	42.3	3.4	2.8	75.1	151.0	18.3
2	3.2	2.4	2.8	89.6	5.3	58.8	78.6	61.9	70.4	25.4	43.4	3.2	2.6	75.8	208.4	18.0

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٩ ٩	13.5	22.3	12.3	17.8	25.1	20.6	8.4	20.5	7.8	8.8	20.6	18.5	18.0	15.9	11.7	19.0	18.3	13.7	18.8	14.6	26.2	9.7	12.2	9.2	10.1	80.00	12.1	21.8	15.1	11.6	17.1	13.4	21.8	;
pint	253.7	237.2	247.4	231.1	255.1	153.4	279.8	250.7	229.9	209.9	217.2	146.1	236.5	259.7	258.3	178.1	214.8	186.0	236.1	150.0	205.1	241.4	281.2	294.0	251.8	223.1	242.4	168.3	178.6	258.8	206.7	199.1	156.8	Contd
dat	72.7	81.3	74.3	69.69	75.5	73.4	75.5	79.9	80.4	84.1	73.8	72.2	75.7	84.3	80.6	74.4	75.6	73.5	74.2	74.3	81.7	76.6	78.2	1.77	74.4	87.5	77.3	74.2	75.6	72.1	77.2	69.6	70.6	
psig	2.7	2.4	2.6	2.6	2.7	2.3	3.1	2.1	2.8	2.4	2:5	2.5	2.6	2.6	2.6	2.7	2.4	2.4	3.0	2.5	2.5	2.7	2.5	3.6	3.3	2.5	2.5	2.2	2.2	2.6	3.0	2.6	3.2	
drywt	3.4	2.9	2.9	3.1	3.1	2.8	3.2	2.5	3.3	2.6	. 2.7	3.0	3.3	3.2	3.1	3.1	3.0	3.0	3.7	3.0	3.0	3.2	2.9	3.8	3.6	3.1	3.3	2.7	2.8	3.1	3.4	3.3	3.4	
shtll	42.2	44.0	43.9	43.5	44.2	40.0	45.5	42.2	43.8	42.6	42.8	43.2	42.7	43.1	44.5	43.4	42.1	42.5	43.9	42.6	44.4	43.9	44.1	47.6	45.3	43.1	45.0	40.0	42.5	44.1	42.9	45.0	43.6	
shtl	24.0	24.2	24.7	24.2	25.6	23.9	28.2	23.6	24.9	26.7	22.9	25.5	24.9	24.8	26.7	24.2	24.6	23.6	25.3	25.2	24.1	24.3	26.6	28.6	27.7	22.5	24.3	22.3	22.6	25.0	24.0	1 2 2	264	1.02
IIdb	77.8	57.7	69.69	70.5	61.8	68.8	67.4	80.3	85.4	57.0	72.6	61.4	65.5	81.8	66.2	75.2	75.6	59.3	75.1	67.6	73.0	68.9	54.8	76.0	59.7	86.1	76.6	775	60.09	20 6	0.71	U. / 0	1.11	/0.0
Idb	6.99	44.8	60.3	59.0	51.2	62.2	58.7	62.6	62.6	47.3	59.8	52.8	60.2	62.9	44.8	58.4	63.8	49.3	66.2	58.3	54.2	57.0	49.3	62.7	51.0	66.1	62.3	5 8 5		7.70	7.90	0.70	60.2	65.5
ovill	76.3	72.4	75.4	78.3	T.TT	74.7	74.7	80.9	81.8	74.9	77.1	78.2	77.0	77.5	76.4	79.3	81.5	69.5	75.3	73.0	77.8	74.0	72.2	4 LL	75.0	80.0	78.7	2.01		t	7.8/	6.28 2.12	74.3	71.5
livo	55.3	55.1	57.9	60.1	52.7	50.5	52.0	49.4	69.8	47.5	54.8	52.8	52.5	54.8	54.5	56.3	59.1	49.8	63.1	50.0	60.6	61.1	52.0	62 1	52.2	56.0	60.7	7.70	0.40	C.CC	7.42	64.2	59.1	53.6
trup	1.1	10.3	2.4	1.4	4.5	4.9	8.0	2.6	1.3	6.3	2.9	1.2	4.8	0.9	3.6	0.7	1.1	7.7	3.0	2.2	12.4	0.8	1.6	0.6	8 9	0.6	010		7.7	4. 4	0.0	1.0	1.2	14.3
trlw	3.7	83.7	35.9	51.0	34.3	71.9	68.5	53.4	3.2	27.0	54.9	45.4	56.8	3.0	11.1	41.6	43.0	42.8	36.9	48.7	45.0	4.2	66.9	1.5	36.1	3.0	16.0	101	1 .0	C.00	2.9	2.3	8.8	68.7
vigII	2.5	2.6	2.8	2.6	2.3	3.1	2.0	2.5	3.4	2.7	2.6	2.6	2.2	3.0	2.0	2.4	2.9	2.4	3.2	2.6	2.7	2.4	8	, r , r			i 0	0 4 1 7		4.7	2.6	3.1	2.4	2.3
vigI	2.0	2.6	2.1	2.3	2.3	2.6	2.0	23	23	2.7	2.4	2.7	2.2	2.4	2.3	2.3	2.3	2.3	2.1	2.3		40			0 J) F	 	7 7	7.8	7.7	2.3	2.4	1.9	2.4
gl	2.5	3.7	4.5	4.7	3.1	4.4	4.5	2.3	4.2	2.5	40	3.2	2.8	44	2.8	0 0	0.6	4	4 5	40	4 4	1.1	i c	0 1		0.1	• •	4 · 4	4.6	4.9	1.9	4.7	3.4	3.9
Entry	66	67	68	69	70	11	1	: 2	74	. Y	76	2. [-	78	20	80	5 13		5	5	2 2 2	200	00 00	.0	x x	89 00	83	16	92	93	94	95	96	97	98

yld	16.6	5.6	3 .3	5.9	e	ŋ	6.9	4.1	0.0	4	0.	5		2	4.7	1.	0.0	3.4	e	5.	4	80	5	16.3	0.7	9.6	Ξ	9.	6.9	2.1	6.9	18.2	4.
plht	243.	245.	251.	204.	235.	236.	280.	186.	233.	246.	238.	245.(245.	231.	210.	263.	250.	148.	282.	166.	217.	264.	293.(216.5	240.	311.	213.	246.	228.0	260.4	207.	149.5	209.5
daf	84.1	85.4	74.4	74.2	79.5	1.77	80.1	76.6	71.9	82.0	12.7	74.1	72.6	78.6	69.69	76.1	74.4	74.1	84.2	73.9	77.4	73.7	85.2	76.6	74.1	78.6	9.77	72.8	74.2	73.5	71.8	71.2	74.0
pslg	2.4	2.2	2.4	2.3	2.8	2.4	2.4	2.4	2.7	2.5	2.2	2.6	2.7	2.6	2.4	2.7	2.7	2.5	2.5	2.4	2.8	2.4	2.7	2.3	2.4	2.5	2.5	3.0	2.5	2.6	2.6	2.4	2.2
drywt	2.9	2.7	3.3	2.8	3.2	3.1	2.9	3.0	3.1	3.1	2.8	2.9	3.1	3.2	3.1	3.0	3.4	3.0	3.1	2.8	3.1	2.8	3.1	2.9	2.9	3.0	3.0	3.3	2.9	3.5	3.4	2.7	2.9
shtll	41.2	41.9	42.0	43.6	42.9	43.7	42.0	41.3	42.8	43.3	43.0	43.5	44.3	44.0	43.5	45.6	43.0	43.4	40.4	42.7	44.8	43.8	46.1	43.0	41.7	43.6	43.6	47.6	42.7	45.2	43.3	41.8	43.7
shtl	24.2	24.8	22.8	24.4	25.9	22.5	23.6	23.9	25.1	26.2	24.2	25.1	24.2	25.2	25.9	27.1	24.9	24.2	24.6	23.0	25.9	23.2	28.0	24.9	23.6	26.1	23.3	25.3	23.3	25.5	23.4	24.4	24.7
IIqb	6.69	67.8	81.9	75.2	80.2	73.6	17.1	82.6	59.4	69.2	58.2	63.4	64.6	77.2	72.1	53.6	80.9	84.2	76.3	59.1	61.5	59.4	73.3	62.9	66.1	66.7	61.7	69.4	79.9	84.0	72.6	78.2	76.0
Ihb	53.2	58.7	62.3	54.0	70.8	65.2	6.99	63.4	50.8	55.8	54.2	56.0	52.1	50.5	60.4	44.9	67.5	67.9	61.4	44.4	57.3	59.1	58.0	55.2	56.2	53.2	51.4	56.3	68.4	73.7	65.2	65.8	6.99
ovill	72.8	74.5	71.7	74.0	79.6	76.3	75.5	73.0	70.9	73.5	72.4	75.2	72.0	74.0	77.0	69.7	73.6	74.2	72.9	73.5	76.6	6.93	72.3	68.5	76.1	77.8	74.4	68.5	78.8	76.7	74.4	77.3	75.9
ovil	50.4	51.6	54.1	52.1	57.4	45.6	53.4	48.2	59.8	48.2	41.3	44.1	51.8	57.9	52.1	44.9	62.8	63.4	58.0	49.5	52.5	41.5	61.8	50.1	49.1	54.4	46.5	50.2	56.2	68.7	59.9	52.6	61.9
trup	6.6	3.0	1.4	1.3	1.4	1.6	13.5	2.3	8.6	8.7	4.9	8.6	0.4	10.7	0.4	14.3	1.4	1.1	2.8	12.6	2.9	14.5	1.0	11.3	4.4	2.8	15.2	13.1	0.6	0.5	13.2	0.9	0.9
triw	37.2	12.0	19.2	36.4	37.6	25.9	36.2	30.3	59.8	43.7	64.5	60.5	59.9	51.3	47.9	62.8	39.8	2.7	20.9	55.4	24.2	76.4	1.6	51.2	41.5	19.5	52.3	45.3	51.1	33.0	79.7	71.0	14.3
vigII	2.1	2.5	3.0	3.4	2.4	2.7	2.5	2.9	2.7	2.5	2.2	2.1	2.4	2.7	2.2	1.8	2.2	3.0	2.6	3.2	2.0	2.1	2.4	2.1	2.2	2.3	2.6	2.2	2.8	2.7	3.1	2.1	2.9
vigI	2.3	2.4	2.4	2.4	2.1	2.4	2.2	2.3	2.0	2.3	2.3	2.2	2.1	2.2	2.2	2.6	2.1	2.3	2.2	2.4	2.2	2.6	1.6	2.1	2.1	2.0	2.6	2.2	2.5	2.0	2.4	2.4	1.9
gl	4.0	2.5	4.6	4.1	3.0	4.1	2.6	4.4	2.2	3.8	2.3	1.9	1.9	4.0	2.6	1.4	3.8	5.0	4.3	4.6	1.9	3.5	2.4	2.7	3.0	4.3	4.1	4.5	6 .4	4.4	4.6	2.8	4.6
Entry	66	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131

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yld		-							11.2																21				18.3	11.9	-	18.6	13
plht	218.7	240.2	228.5	266.8	238.9	156.5	241.8	240.6	198.8	247.7	245.1	236.8	164.1	219.2	274.8	243.7	183.8	259.6	290.5	272.0	225.5	193.8	261.6	227.9	242.1	209.7	260.3	175.7	283.1	255.0	260.1	174.0	0 247 0
daf	70.9	75.4	75.6	80.9	T.T	78.4	81.2	72.5	74.8	75.0	72.7	71.5	80.6	82.0	85.7	78.8	72.9	80.2	81.5	78.6	74.4	81.4	74.8	74.0	75.9	72.9	76.8	72.6	88.8	80.6	9.77	70.4	010
pslg	2.7	2.5	2.5	2.9	2.6	2.2	2.5	2.9	2.7	2.8	2.6	2.6	2.8	3.0	2.4	2.5	2.3	2.4	3.0	3.5	2.5	2.5	2.8	2.3	2.6	2.6	2.8	2.3	2.6	2.8	2.2	2.9	r r
drywt	3.2	3.0	2.8	3.5	3.3	2.9	3.1	3.3	3.3	3.3	3.2	3.1	3.2	3.6	2.8	2.8	2.8	3.0	3.5	3.5	3.0	2.9	3.0	2.8	3.4	3.2	3.4	2.7	3.0	3.3	2.7	3.5	1 2
shtII	43.1	42.3	43.0	45.0	43.8	39.5	43.1	45.2	45.6	45.4	42.6	42.5	44.1	45.3	41.4	42.7	42.9	42.1	44.8	46.4	42.8	41.8	44.1	42.6	43.8	43.1	44.9	41.3	45.1	43.8	39.6	45.6	0 6 7
shtl	23.4	22.6	23.0	25.5	23.5	21.5	26.1	26.2	25.7	25.1	22.6	25.6	23.8	27.1	23.5	25.1	23.6	26.7	25.3	28.1	22.6	23.6	27.1	24.6	26.1	25.3	26.0	24.8	26.4	23.7	22.4	28.4	
IIHb	71.5	83.5	70.0	69.2	T.TT	72.7	81.2	80.7	70.8	78.6	61.2	68.0	72.9	6.09	80.4	55.5	78.7	71.4	83.5	56.9	67.9	50.2	47.3	68.4	80.2	71.3	72.6	63.5	69.7	66.5	70.7	69.5	
Idb	59.0	70.1	60.7	57.6	65.6	63.5	71.6	69.1	62.1	64.6	51.1	58.4	56.9	46.9	69.3	43.8	64.3	60.0	73.4	45.7	52.4	41.2	35.9	56.1	65.0	59.2	59.7	48.4	61.3	51.0	54.6	57.1	
ovill	71.8	75.9	78.4	73.3	78.1	T.TT	77.2	78.1	73.6	78.9	74.6	75.6	74.5	71.6	76.2	67.0	74.7	77.1	79.1	63.9	67.3	68.9	65.1	74.8	78.5	71.1	76.0	76.1	71.6	74.0	76.2	74.7	i
ovil	60.5	61.0	50.8	55.7	61.9	49.0	64.4	61.6	59.5	69.0	54.4	52.0	52.2	53.7	53.7	49.1	55.3	59.5	61.9	48.6	48.9	48.0	43.9	43.5	56.0	55.2	52.8	45.4	51.6	43.9	47.6	55.2	•
trup	6.1	1.4	13.3	2.6	0.8	0.6	1.4	0.2	1.2	1.4	2.0	5.4	13.2	8.1	0.8	0.8	1.0	0.7	1.8	1.2	4.7	3.4	5.0	1.1	5.2	5.0	3.9	0.3	2.7	0.9	1.7	1.6	•
trlw	20.8	27.6	<i>77.9</i>	39.7	9.6	3.6	33.9	21.7	2.1	47.8	59.9	39.4	74.4	70.9	8.6	51.1	42.7	5.3	7.8	62.2	67.2	28.4	65.0	44.2	30.4	59.9	47.1	35.3	30.3	2.2	3.2	8.4	0
vigII	2.0	2.6	2.7	2.6	2.5	2.6	2.9	2.8	2.7	2.8	2.7	2.7	2.8	2.2	3.0	2.7	2.9	2.2	2.7	1.8	2.9	2.0	2.2	2.5	3.0	2.8	2.6	2.5	2.2	2.4	2.7	2.3	
vigl	2.0	2.2	2.4	2.1	2.1	2.5	1.9	1.8	1.9	2.5	2.6	2.0	2.4	2.2	2.1	2.7	2.2	1.7	2.3	2.1	2.6	2.1	2.1	2.7	2.3	2.5	2.3	2.5	2.2	2.3	2.9	2.3	•
gl	3.8	3.8	4.8	4.6	4.6	4.1	4.1	4.0	4.2	4.0	3.5	4.2	4.8	2.6	4.6	2.8	4.6	4.3	4.8	2.6	2.3	3.0	3.2	3.0	4.6	2.5	4.5	3.5	2.7	2.7	4.1	3.7	
Entry	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	

Entry	gl	vigI	vigII	trlw	trup	ovil	ovill	IHb	II	shtl	shtll	drywt	pslg	daf	plht	yld
165	4.8	2.1	2.3	69.4	11.7	63.8	78.4	68.0	82.5	25.6	44.4	3.8	3.2	69.0	231.8	17.6
166	1.8	2.3	2.1	86.6	16.7	50.1	68.4	43.5	53.1	25.9	46.3	3.2	2.8	71.7	243.3	19.3
167	3.2	2.3	2.5	59.7	7.0	59.7	77.4	48.8	74.4	23.1	42.0	3.1	2.4	79.2	224.2	19.9
168	4.1	2.2	2.7	17.0	4.3	59.8	79.3	65.4	67.1	26.1	45.9	3.4	3.1	81.7	246.4	12.4
169	3.9	2.0	1.8	50.7	1.6	42.3	70.5	54.3	68.2	24.7	42.6	2.9	2.4	72.1	164.0	21.4
170	3.7	2.3	2.0	26.8	1.8	45.7	69.69	44.4	56.0	25.2	44.8	2.9	2.4	71.9	176.1	11.3
171	4.5	2.4	2.7	3.4	0.9	65.5	84.3	70.3	85.2	26.1	43.1	3.2	2.7	83.7	266.2	11.9
172	4.2	2.4	2.5	95.0	9.5	61.3	72.3	56.2	69.1	22.5	42.7	3.1	2.7	75.6	226.9	17.7
173	3.8	2.3	2.5	58.2	5.3	58.1	76.2	72.4	80.0	25.4	45.9	3.4	3.0	81.8	244.1	15.0
174	4.0	2.1	3.3	40:0	1.3	60.6	74.8	62.7	71.9	25.6	44.1	3.7	3.5	86.0	234.9	8.8
175	2.9	2.8	2.5	32.9	2.5	55.9	79.1	61.0	78.1	23.4	44.1	3.1	2.6	81.0	273.4	10.7
176	1.9	2.4	2.0	26.4	1.3	47.0	70.7	46.8	60.9	24.4	41.8	2.6	2.4	74.4	213.4	10.4
177	4.4	2.1	2.3	41.0	1.4	61.8	76.7	61.9	78.1	25.3	44.7	3.4	3.2	80.0	212.3	9.9
178	4.2	2.4	2.6	49.8	0.9	51.5	79.9	66.2	77.4	24.5	42.9	2.9	2.5	75.8	210.0	12.9
179	2.5	2.2	2.2	22.1	3.9	56.5	74.8	45.0	60.9	27.2	44.7	3.1	2.8	75.2	284.1	15.3
180	2.5	2.4	1.9	16.2	1.6	54.8	70.7	50.2	60.6	27.7	45.3	3.1	2.8	74.2	283.0	14.9
181	2.5	2.0	2.1	2.1	0.5	58.6	76.4	59.0	70.5	25.6	43.8	3.2	2.7	71.5	238.5	10.8
182	3.0	1.9	2.5	2.2	0.3	59.3	76.9	56.3	70.0	26.4	43.9	3.2	2.8	75.1	229.7	10.5
183	3.0	2.0	2.5	1.8	0.6	65.1	82.4	67.7	75.7	26.4	45.8	3.6	3.2	76.5	263.0	10.7
184	3.2	2.3	2.1	2.1	1.2	60.0	73.6	58.3	68.7	24.9	45.6	3.3	3.2	72.0	255.7	14.1
185	4.8	2.4	2.3	53.0	6.9	59.1	74.4	62.8	74.6	23.0	42.5	3.1	2.5	68.2	138.5	20.5
186	4.5	2.3	2.7	68.1	11.6	57.2	71.6	58.6	63.6	24.0	43.4	3.1	2.4	68.2	160.4	15.8
187	4.3	2.5	2.0	3.2	0.3	56.6	6.9	50.8	61.9	26.7	44.7	3.3	2.9	76.7	248.1	8.5
188	3.9	2.0	2.4	29.9	2.8	61.6	76.5	54.1	64.3	25.4	45.4	3.6	3.4	77.2	270.9	10.9
189	2.7	2.6	2.5	5.2	0.8	41.7	77.5	62.8	70.4	25.1	41.4	2.6	2.3	74.8	244.3	14.7
190	2.7	2.4	2.4	25.0	2.7	43.2	73.8	54.5	68.6	25.4	42.5	2.6	2.2	73.4	242.8	16.1
191	4.3	2.0	2.4	6.0	2.1	56.5	75.6	58.7	68.7	24.9	44.6	3.1	2.6	69.3	250.7	10.0
192	4.3	2.3	2.6	69.3	8.3	54.8	73.1	60.5	79.9	26.6	45.5	3.5	2.9	67.0	204.2	12.7
193	4.2	2.2	2.7	69.8	0.7	53.7	76.3	57.5	72.7	24.1	43.3	3.1	2.7	75.4	245.6	6.7
194	2.7	2.3	2.2	29.6	6.0	58.7	74.7	61.2	70.2	26.6	43.8	2.8	2.5	75.3	260.2	15.3
195	2.0	2.0	1.8	48.4	6.5	56.3	70.4	47.8	61.5	27.0	46.1	3.6	3.4	72.6	276.2	13.8
196	2.1	2.3	2.2	1.5	0.7	53.0	79.5	61.3	72.3	27.5	43.7	3.0	2.7	81.2	281.0	13.4
201	t	ļ	, ,	300	ſ	0.04	607	16 5		326	121	0 0	2 C	725	183 8	151

																														ć	24	4	
yld	13.9	14.1	16.9	15.3	8.1	23.6	9.5	12.0	11.4	14.5	21.4	10.8	17.4	13.7	16.2	18.8	19.9	18.8	23.7	16.9	17.8	23.9	19.6	12.7	13.9	18.5	22.0	14.5	17.7	11.2	5.9	22.0	
plht	268.8	246.2	242.8	243.6	253.9	163.0	272.5	250.0	244.0	239.5	157.5	261.8	249.3	256.5	229.1	170.5	203.8	208.6	225.9	171.8	182.2	145.1	227.0	191.9	192.0	225.4	262.3	274.8	240.0	231.5	292.1	212.4	
daf	75.4	72.2	74.1	84.4	72.3	70.5	86.1	74.7	72.0	79.7	69.0	72.8	78.1	77.4	73.9	77.5	82.8	75.6	76.3	76.9	72.6	76.8	85.5	74.5	75.8	73.6	74.8	77.0	73.2	81.1	78.8	72.9	
pslg	2.9	2.6	2.7	2.6	3.2	3.0	2.4	3.5	3.0	2.4	2.2	2.7	2.5	2.4	2.6	2.2	2.3	3.0	2.3	2.4	2.4	2.7	2.3	2.6	2.5	2.4	3.0	2.4	2.5	2.4	3.7	2.2	
drywt	3.4	3.0	3.2	3.2	3.4	3.4	2.8	3.5	3.1	2.8	2.8	3.2	2.9	2.8	3.0	2.8	3.1	3.6	2.9	2.7	2.9	3.1	2.7	3.0	3.2	3.0	3.1	3.1	3.2	2.9	3.7	2.9	
shtII	43.9	43.9	45.1	43.3	47.1	42.6	43.0	46.1	45.2	44.2	40.6	45.6	42.9	44.0	44.9	43.0	44.5	43.8	42.3	40.6	43.3	44.3	41.1	42.5	44.4	43.3	42.7	43.7	43.4	42.0	48.0	41.6	•
shtľ	25.3	23.8	23.5	24.3	28.5	24.6	24.9	27.7	28.6	25.0	23.5	26.5	22.3	23.3	26.0	22.2	24.4	25.0	22.4	23.6	24.6	24.1	23.4	24.0	25.3	23.3	24.0	24.3	26.1	21.1	29.6	26.9	
IIdb	72.4	65.1	61.0	76.6	64.6	65.4	76.2	69.0	65.3	65.2	74.7	1.17	68.1	62.7	63.1	56.5	71.2	60.0	69.8	68.0	6.99	68.3	70.1	54.1	77.1	81.4	72.8	72.3	64.3	75.3	68.4	83.9	
ЧÞ	61.0	55.3	50.8	60.2	56.1	50.4	66.0	56.8	50.5	50.1	62.1	62.0	62.4	53.0	50.2	41.7	59.6	47.4	57.2	49.9	53.4	63.1	59.6	46.3	68.3	74.6	60.1	56.6	48.9	57.4	58.8	74.1	
ovill	79.6	72.9	72.9	72.1	73.2	73.1	78.6	75.2	72.5	71.1	78.4	77.5	76.4	69.8	71.1	71.8	74.2	69.7	72.8	76.7	80.0	79.1	73.0	68.1	71.3	77.5	75.2	72.3	73.2	79.2	73.0	79.8	
ovil	56.3	49.4	50.0	57.1	53.1	53.9	50.7	58.5	57.1	53.3	57.0	60.8	47.2	46.8	50.0	46.0	59.4	49.3	52.2	46.6	57.3	56.7	50.4	46.9	54.3	57.7	55.0	55.4	50.9	44.5	58.7	58.2	
trup	6.4	5.4	3.9	8.6	1.0	7.6	1.4	4.0	3.3	11.8	2.2	2.7	6.8	12.4	9.7	17.0	7.9	1.4	11.8	1.2	1.2	9.2	13.3	5.6	3.2	3.1	1.5	0.6	1.7	1.9	-0.2	0.8	
trlw	44.8	37.3	38.6	27.7	12.6	42.2	3.3	35.9	39.1	70.7	24.5	53.8	36.6	57.2	56.2	65.6	45.6	4.6	6.69	51.8	11.2	32.4	51.7	66.3	22.3	42.0	24.1	22.4	42.9	26.1	4.0	15.6	
uigII	2.3	2.2	2.4	2.5	2.0	2.3	2.5	1.6	2.2	2.5	2.5	2.3	3.1	2.3	2.0	2.6	2.7	2.5	2.7	2.9	2.5	2.6	2.5	1.8	2.2	2.7	2.6	2.6	2.5	2.9	2.3	2.7	
lgiv	2.3	2.2	2.1	2.3	2.2	1.9	2.0	1.9	2.2	2.4	2.4	1.9	3.0	2.4	2.2	2.6	2.3	2.3	2.5	2.6	2.0	2.5	3.2	2.1	2.3	2.4	2.2	2.2	2.4	2.6	2.0	2.1	
gl	3.9	2.8	3.0	4.2	2.5	2.8	2.7	3.8	2.6	2.2	3.8	4.0	3.4	3.7	2.8	2.4	3.0	3.2	4.3	3.9	4.2	3.1	2.3	3.8	4.2	4.4	4.3	4.4	2.4	2.8	2.7	4.7	
Entry	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	

Entry	gl	vigI	llgiv	trlw	trup	ovil	ovill	Idh	IIHb	shtl	shtll	drywt	pslg	daf	piht	yld
231	4.2	2.5	2.9	49.5	1.9	58.5	81.2	62.6	72.9	21.7	42.5	2.9	2.4	72.8	234.2	19.7
232	2.6	2.2	1.4	65.2	9.8	43.6	63.7	34.4	51.9	25.0	44.2	2.8	2.5	72.6	166.3	19.4
233	3.0	2.6	2.1	60.1	4.7	43.0	67.7	46.8	61.0	24.0	43.8	2.9	2.6	72.4	238.0	16.5
234	4.0		2.5	49.2	9.0	56.0	74.3	59.3	69.69	23.9	42.4	2.7	2.4	75.3	198.5	15.8
235	2.6		2.2	51.9	4.8	48.5	75.8	53.9	70.9	25.3	44.8	3.1	2.5	72.6	258.5	16.4
236	3.7		2.5	48.2	11.6	53.8	77.2	51.5	67.3	24.2	42.8	2.9	2.4	76.6	257.9	20.3
237	2.4		2.2	10.6	2.0	56.4	71.1	56.4	67.7	25.4	44.1	3.5	2.9	73.3	228.1	13.5
238	4.0		2.0	37.3	5.5	47.6	69.5	59.5	70.1	26.9	46.2	3.6	3.6	71.0	256.7	6.3
239	2.3		2.1	1.7	1.4	45.6	75.5	50.4	63.3	23.5	42.2	3.1	2.6	81.1	246.6	6.9
240	3.5		2.6	2.4	0.5	59.2	78.4	68.9	80.2	25.7	44.8	3.1	2.8	82.1	285.0	7.9
241	4.3		3.0	18.2	1.5	64.8	83.5	75.2	85.6	25.2	45.1	3.2	2.6	82.5	296.8	11.9
242	2.4		2.6	65.7	8.2	58.1	71.6	42.7	55.3	24.3	43.5	2.9	2.4	75.7	245.1	13.8
243	2.3		2.7	64.1	7.2	50.5	76.2	36.9	50.4	24.0	43.1	2.7	2.4	73.4	210.1	20.6
-245	3.7		2.7	54.0	6.7	49.3	70.2	50.4	65.7	23.0	42.2	3.0	2.5	75.8	227.2	17.6
246	4.0		2.7	11.1	1.9	67.3	79.2	71.5	86.6	24.4	43.0	3.2	2.6	72.0	211.2	9.1
247	3.4		2.4	34.0	5.4	54.8	77.8	59.4	70.7	21.7	41.8	2.8	2.4	70.6	160.9	15.4
248	4.7		2.7	36.0	2.7	63.5	T.9T	67.5	80.2	25.3	42.6	3.0	2.5	72.8	197.5	11.3
249	4.8		3.0	42.7	1.5	66.4	78.7	70.4	78.3	25.1	45.4	3.6	2.7	71.7	182.0	15.2
250	4.1		2.5	21.7	1.2	66.4	78.1	71.3	80.5	26.6	43.2	3.3	2.7	80.2	231.6	12.1
251	4.0		2.9	4.2	0.8	62.5	74.8	71.7	82.3	23.9	44.0	3.2	2.5	76.8	236.4	9.7
252	2.5	2.1	1.9	87.9	11.6	50.9	73.2	52.1	65.2	26.7	46.0	3.4	3.2	68.8	238.1	9.7
BTx623	4.9	2.5	3.1	1.5	0.8	64.3	83.7	72.1	82.1	24.3	43.0	3.3	2.7	76.1	146.8	20.6
IS 18551	1.4	2.1	1.8	76.9	5.2	39.2	67.5	30.8	43.2	27.3	45.8	3.5	3.6	78.3	285.3	9.5
CSH 1	4.8	2.9	3.2	0.9	0.6	61.2	78.2	73.7	84.2	24.7	44.4	3.5	2.7	73.9	193.8	34.3
796 B	4 8	17	34	1.7	1.2	54.1	78.5	647	811	22.4	39.4	26	23	79.5	150.4	35.6

Entry	6	lgiv	llgiv	triw	trup	ovil	ovill	Idh	IIHb	shtI	shtII	drywt	pslg	daf	plht	yld
-	3.7	2.5	2.6	72.2	2.3	13.2	62.4	14.9	34.0	22.9	41.0	4.2	3.3	77.5	173.6	9.3
7	4.2	1.7	2.1	83.7	3.0	12.9	60.5	17.4	26.4	22.8	42.8	4.2	3.4	72.7	178.0	9.5
ŝ	2.6	2.3	2.5	48.3	2.2	21.9	72.2	25.4	43.9	23.6	43.6	4.6	4.5	75.7	181.6	15.0
4	2.8	2.4	2.4	2.9	0.6	20.5	78.4	31.6	62.4	22.6	42.1	4.4	4.1	70.0	132.9	1.1.1
s	4.7	2.0	2.1	1.6	0.3	18.1	76.0	41.9	61.9	20.0	39.2	4.3	3.8	76.6	149.9	11.4
9	2.6	2.4	1.9	50.4	6.5	11.5	58.6	11.0	26.6	23.4	43.7	4.6	4.4	69.69	131.9	16.3
7	3.9	2.0	2.0	73.2	0.6	12.8	54.1	11.5	28.0	24.2	47.1	4.6	7.3	72.2	162.8	15.2
∞	4.7	1.6	8.1	51.8	0.4	29.4	89.1	38.0	62.8	23.2	40.9	4.1	3.1	77.0	159.5	11.0
· 6	3.1	3.2	2.6	58.4	5.1	16.3	72.0	20.4	40.1	22.2	44.2	4.7	5.7	70.5	162.7	11.0
10	4.0	1.9	1.7	114.0	21.0	11.9	57.1	16.4	33.2	23.0	43.0	4.3	4.0	69.2	130.9	12.5
11	4.9	1.9	2.2	8.8	0.5	30.9	80.4	52.1	76.5	22.4	38.5	4.2	3.1	82.7	138.9	5.4
12	1.6	1.7	1.4	51.7	0.6	18.4	59.1	19.4	36.3	26.6	45.2	4.6	6.1	67.3	160.0	9.4
13	4.2	2.4	2.6	60.2	0.4	14.6	66.5	20.2	50.6	20.8	43.7	4.5	4.3	71.0	156.3	11.5
14	4.7	1.6	1.5	68.3	0.5	24.3	72.3	39.1	50.9	24.7	46.0	4.5	5.3	75.6	171.0	12.6
15	2.6	2.2	2.4	12.6	1.4	18.1	73.9	26.2	53.5	21.6	41.0	4.4	4.3	74.5	140.5	14.0
16	3.0	2.2	1.9	106.3	5.2	12.7	54.3	15.0	25.5	22.8	43.4	4.6	5.0	73.8	144.8	11.7
17	4.4	2.1	2.3	102.6	0.3	17.7	58.6	15.1	27.5	25.1	43.4	4.3	5.3	62.9	140.4	6.3
18	2.5	1.5	2.2	79.9	0.2	23.5	81.6	37.2	61.3	22.6	40.4	4.2	4.7	71.5	153.9	11.5
19	4.0	1.5	2.1	79.2	13.8	17.8	60.0	26.1	41.0	25.9	45.1	4.2	3.8	73.9	186.9	10.6
20	3.3	1.9	2.2	7.66	10.6	10.4	55.7	12.7	25.2	21.5	41.0	4.4	5.0	69.69	135.9	14.2
21	2.6	1.8	1.4	38.5	0.2	24.8	84.1	36.9	58.1	25.0	42.2	4.4	4.0	70.5	151.3	7.1
22	4.0	1.6	1.7	98.5	10.5	13.6	65.6	19.0	25.0	24.8	49.0	4.3	8.5	71.7	1.061	10.5
23	4.4	3.0	2.2	85.4	7.6	12.1	63.9	16.8	37.5	22.5	42.5	4.4	4.9	71.2	151.3	15.9
24	4.0	2.0	2.1	2.9	0.1	10.7	52.4	12.5	17.4	24.5	46.6	4.7	6.3	68.0	156.2	8.6
25	4.2	1.8	1.8	3.1	0.5	20.9	69.1	36.0	68.2	21.1	39.9	4.2	4.4	76.9	166.8	11.6
26	3.0	1.8	2.1	22.3	0.5	11.9	64.7	17.8	44.2	22.4	43.8	4.5	7.6	70.7	157.1	11.8
27	4.9	1.8	2.2	46.8	8.5	13.6	66.3	20.2	45.2	22.4	40.8	4.4	3.6	69.3	124.6	12.3
28	4.7	2.0	2.3	96.4	6.4	12.1	60.8	16.0	38.1	22.9	42.0	4.5	4.3	82.9	138.9	9.2
29	4.5	1.7	1.6	30.1	2.0	28.5	61.3	24.2	44.4	22.6	43.8	4.6	5.1	70.4	155.7	11.1
30	3.3	2.0	2.6	36.8	0.5	12.8	71.3	25.1	52.3	20.7	43.3	4.4	4.1	75.3	162.5	10.1
;			.	0 7 3	Y C	111	60.4	120	26.9	75.1	43.6	4.5	5.3	65.9	130.3	11.2

Appendix VI. Mean performance of RILs (1-252), parents (BTx623 and IS 18551) and susceptible checks (296 B and CSH 1) evaluated under screening environment E2 (rabi, Patancheru) during 2000

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- 1	81 8	lgiv	ligiv	triw	ţ	ovil	ovili	됳	Į	shtl	shtII	drywt	pslg	daf	म्	PX
	2.1	2.2	1.6	40.8	8.9	18.7	52.1	22.8	34.2	24.8	45.4	4.5	4.7	69.7	154.9	13.6
	4.2	2.6	2.1	34.5	8.2	15.7	75.5	24.5	47.3	23.3	42.1	4.4	3.9	76.0	165.1	15.7
	2.8	2.4	2.9	86.7	11.8	19.2	72.4	33.5	52.1	22.1	41.7	4.2	3.4	75.2	105.4	10.8
	4.7	1.8	1.7	73.6	0.4	20.3	67.9	27.8	55.6	25.3	42.4	4.2	4.1	71.4	131.4	11.4
	2.8	1.9	2.4	65.0	5.2	15.7	72.1	15.7	38.5	24.2	43.1	4.3	4.4	17.4	204.9	10.1
	3.1	1.8	1.7	43.3	3.3	14.7	62.6	17.1	32.8	23.9	44.5	4.5	5.5	72.9	181.3	14.6
	4.4	2.0	2.3	67.8	4.9	22.8	64.3	29.7	41.3	23.5	43.2	4.5	3.6	83.5	167.5	5.7
	3.8	1.5	1.8	83.4	4.5	9.5	57.9	14.9	26.4	23.8	46.0	4.8	7.9	77.2	184.6	13.9
	2.1	2.4	2.0	56.5	0.4	18.6	79.5	45.7	65.4	21.2	40.2	4.2	.3.1	-68.9	142.4	10.0
	4.0	1.4	1.4	74.6	15.5	15.6	67.3	15.5	24.2	28.7	51.0	4.6	9.4	70.0	176.1	12.2
	4.0	1.7	1.4	43.3	3.1	27.7	66.1	24.2	43.3	26.5	50.0	4.7	7.0	74.7	194.4	13.7
	4.4	2.4	1.7	6.69	6.8	15.2	8.69	17.3	41.0	23.9	42.9	4.3	3.7	77.8	164.9	10.4
	4.5	2.6	2.5	82.6	7.3	17.5	62.4	27.3	38.7	24.5	45.4 -	4.5	4.9	6 . <i>L</i> L	173.9	14.6
	4.2	1.6	2.0	13.0	1.6	20.9	68.1	39.5	66.7	22.2	42.2	4.3	3.5	78.3	157.2	10.1
	3.0	1.6	1.7	83.5	1.3	31.1	77.1	41.3	57.6	24.0	45.5	4.6	6.6	74.3	181.7	11.8
	4.9	1.8	1.4	73.8	12.7	9.9	61.7	11.1	27.1	23.2	46.8	4.5	4.6	73.3	145.4	12.9
	4.2	2.0	1.8	3.9	0.3	20.4	69.0	30.9	54.1	20.5	43.6	4.3	5.6	68.2	155.7	13.5
	2.1	2.8	2.2	21.1	2.3	22.5	75.6	28.1	59.4	21.9	40.2	4.3	3.8	77.5	143.6	11.9
	3.7	1.7	1.4	57.5	2.5	12.8	49.3	13.7	27.4	24.7	44.6	4.5	5.0	71.3	150.5	8.6
	1.9	2.2	2.0	49.3	3.6	9.3	55.1	9.1	24.3	21.3	44.8	4.3	7.0	67.6	138.4	8.9
	4.5	1.2	1.3	40.8	7.6	13.4	67.5	19.1	25.0	26.6	45.2	4.6	9.1	68.8	161.3	15.5
	2.1	2.6	2.5	85.2	9.7	10.0	50.3	11.6	19.0	24.0	44.0	4.3	6.0	69.0	151.7	13.1
	2.3	1.6	2.1	35.1	2.3	20.0	78.4	23.9	43.4	22.6	41.3	4.4	4.0	77.1	165.9	15.0
	2.1	1.6	2.2	35.2	1.7	19.1	60.5	24.1	34.3	23.6	44.6	4.3	4.8	70.1	156.3	14.9
	4.2	1.7	1.5	50.0	0.3	20.5	62.9	33.8	46.8	24.4	45.3	4.4	5.3	71.8	187.9	9.9
	4.7	2.2	2.0	61.9	2.9	16.5	69.1	25.3	53.1	23.6	43.3	4.4	3.9	73.9	165.9	12.4
	4.5	2.1	2.1	40.5	0.4	29.5	75.3	43.2	72.0	20.5	41.4	4.5	3.3	88.2	186.8	5.8
	4.9	1.8	1.3	29.3	7.9	27.1	69.0	38.8	51.9	25.8	49.0	4.6	5.4	76.7	208.5	13.9
	4.2	1.7	1.5	84.5	0.4	21.7	76.0	26.8	48.9	25.6	44.2	4.4	8.1	71.0	187.4	10.9
	2.8	3.3	2.7	83.7	5.3	15.9	71.1	18.8	39.8	22.0	42.5	4.4	3.1	75.8	162.6	12.3
	4.5	1.4	1.4	29.5	3.0	13.6	73.2	18.2	40.3	24.8	44.1	4.4	4.6	75.8	175.2	11.3
		01	,	0.02	Ň	9 26	525	0 7 0	\$2.0	126	411	4 3	3.6	78.0	123.9	10.5

Entry	ß	vigI	vigII	triw	trup	ovil	ovill	ĮĄ	IIHb	shtJ	shtII	drywt	pslg	daf	plht	Pł
5	3.3	2.2	2.1	115.2	3.8	15.2	68.5	20.5	42.9	23.2	40.1	4.0	2.6	75.1	149.8	11.3
65	3.1	2.2	2.7	91.2	0.9	22.7	75.5	30.4	58.7	22.7	43.9	4.4	4.7	72.7	161.8	13.0
99	2.1	1.8	2.1	3.4	0.5	29.7	65.7	24.4	43.4	22.9	44.1	4.6	7.0	66.8	156.3	8.7
67	4.0	2.2	2.1	109.8	10.1	18.1	62.4	21.8	35.9	23.4	42.2	4.3	3.0	81.1	156.1	1.7
68	4.0	2.2	2.2	42.8	0.5	19.5	55.5	. 6.61	42.2	21.7	42.1	4.2	3.6	75.5	155.9	9.4
69	4.7	1.6	1.5	68.7	0.5	23.3	71.7	33.7	46.5	24.7	43.4	4.4	3.8	70.6	159.3	12.9
70	2.6	2.0	1.8	44.6	1.0	24.0	67.5	23.2	47.1	24.8	44.0	4.5	5.8	71.6	178.6	14.9
71	4.7	2.8	2.8	63.3	4.1	20.6	60.4	22.1	49.3	22.3	40.8	4.3	3.5	72.3	127.3	10.4
72	4.5	1.4	1.4	80.6	3.6	14.3	69.4	20.6	36.6	24.1	46.0	4.6	8.1	71.5	167.9	10.1
73	3.0	2.9	2.6	44.9	0.6	20.4	78.0	25.0	58.5	22.7	41.9	4.3	3.0	75.9	164.0	12.9
74	4.2	2.0	1.6	3.2	0.5	32.5	73.1	43.6	69.69	23.8	46.2	4.7	4.6	71.8	167.6	8.6
75	2.6	2.3	2.7	26.2	2.0	13.9	0.69	16.2	31.5	23.3	43.2	4.4	4.0	17.2	170.3	14.6
76	4.7	2.0	2.0	70.6	0.7	20.2	72.4	35.5	58.2	23.4	41.7	4.3	4.7	71.2	156.3	12.5
11	2.8	3.0	3.3	79.8	0.5	11.5	71.5	22.7	43.3	22.9	41.6	4.3	3.8	70.8	121.0	11.9
78	2.6	2.0	2.0	49.0	1.2	15.9	60.5	15.1	20.8	23.6	43.0	4.6	4.4	72.1	151.8	14.0
62	4.9	2.2	1.6	9.0	0.7	21.2	64.1	28.7	46.7	24.1	42.8	4.4	3.5	83.6	171.2	9.9
80	2.1	2.2	1.5	37.5	4.2	19.4	74.5	19.8	38.3	26.5	46.9	4.4	5.7	75.8	175.5	14.4
81	3.1	1.6	1.6	38.4	0.7	29.2	74.4	27.2	43.5	25.6	43.3	4.9	6.1	68.0	138.3	13.5
82	3.0	2.9	2.1	64.3	0.6	24.5	75.6	42.1	55.9	23.6	43.0	4.4	5.7	70.5	129.6	11.7
83	4.4	2.4	2.3	41.3	2.1	13.1	54.9	22.9	29.2	22.0	44.6	4.5	4.0	70.6	141.2	9.3
84	4.7	2.3	2.1	47.4	5.3	12.0	61.9	21.9	44.9	22.5	42.3	4.4	4.4	71.0	148.7	16.0
85	4.5	2.3	2.3	56.3	0.5	19.2	67.5	26.9	43.6	25.3	46.0	4.6	4.0	70.9	118.7	10.3
86	4.7	2.1	2.0	50.0	14.0	13.8	74.9	27.4	43.6	23.3	43.0	4.8	3.9	77.5	155.7	14.4
87	4.7	1.9	1.9	3.9	0.6	15.5	64.4	33.2	49.1	24.4	43.5	4.4	4.8	71.0	156.5	12.0
88	2.8	1.9	1.5	92.6	19.5	14.2	53.1	15.5	20.5	25.2	45.8	4.6	5.6	78.4	199.9	11.3
89	3.5	1.4	1.4	2.7	0.6	20.7	69.3	27.2	50.9	24.3	49.6	4.7	9.0	70.6	190.3	15.4
90	2.5	1.8	1.5	27.6	5.0	18.4	61.2	20.0	28.3	24.9	45.4	4.5	5.4	73.1	175.6	11.9
16	4.9	2.3	2.3	2.2	0.3	29.5	81.2	49.3	78.3	23.5	42.0	4.7	3.9	80.5	152.7	10.6
92	4.9	1.8	1.6	28.4	0.5	27.4	76.9	51.0	67.4	24.7	44.6	4.5	3.6	74.0	171.0	8.5
93	4.9	3.0	2.4	60.4	0.5	30.9	79.7	33.0	69.8	22.6	41.8	4.7	3.5	78.5	107.3	11.8
94	4.7	1.9	1.8	47.0	5.7	17.2	71.1	19.5	40.3	23.4	42.8	4.3	3.6	70.7	136.8	12.6
50	76	1 8	01	14	0.5	26.6	803	41.0	68.6	21.5	41.7	4.3	4.4	72.7	183.0	10.4

1																														2	49	}
ΡĶ	11.0	12.0	13.1	15.4	16.6	12.3	11.8	8.0	8.1	10.0	14.4	12.3	13.1	6.6	13.0	9.7	12.8	12.8	11.6	14.5	12.6	7.0	16.3	10.6	7.7	12.2	14.7	12.3	9.1	13.7	13.9	14.0
plht	126.3	138.2	127.8	165.7	164.1	167.6	159.5	169.7	162.3	170.3	139.3	158.0	156.3	161.2	167.7	164.4	160.3	147.8	182.2	155.2	118.9	190.8	134.5	159.5	158.5	192.5	146.2	161.8	211.4	152.2	164.8	149.1
daf	73.6	69.3	66.7	78.0	76.0	69.3	75.6	76.9	74.3	76.5	74.2	72.8	72.8	70.9	68.7	71.3	77.1	70.3	76.4	74.3	73.3	71.9	69.8	73.7	71.4	76.2	70.2	71.0	78.0	73.8	70.4	75.7
pslg	4.0	5.6	4.2	4.0	4.0	7.8	2.8	3.8	4.8	3.4	4.4	3.6	3.8	4.2	7.1	5.7	4.8	3.8	4.3	4.6	3.1	6.7	4.1	3.7	6.1	6.2	4.0	6.2	4.3	3.6	7.2	3.6
drywt	4.3	4.8	4.4	4.5	4.4	4.8	4.3	4.2	4.3	4.2	4.7	4.3	4.2	4.3	4.4	4.3	4.4	4.4	4.4	4.5	4.3	4.5	4.5	4.2	4.4	4.6	4.2	4.5	4.2	4.3	4.4	4.5
shtII	41.8	46.4	41.9	43.3	43.6	47.3	40.5	42.1	42.0	42.8	44.3	42.2	41.0	43.5	43.1	43.1	40.5	45.3	48.2	42.0	40.4	45.9	41.9	42.8	43.7	47.1	45.8	43.9	43.7	40.8	46.1	43.6
shtl	25.1	27.3	25.2	22.0	23.0	24.6	21.6	23.5	23.0	23.1	21.9	22.2	26.1	24.0	24.0	24.5	23.8	24.2	26.1	22.8	23.6	23.6	24.1	24.1	24.0	23.6	21.0	23.6	23.9	23.2	25.5	26.6
dhit	58.3	40.9	31.5	41.2	44.9	52.1	61.3	52.2	48.3	44.8	52.9	29.9	23.6	41.7	24.2	43.0	32.9	45.3	17.7	56.4	73.4	40.7	38.5	39.8	38.6	54.2	31.4	43.2	53.7	40.0	23.7	70.0
IdbI	33.5	25.1	19.7	22.9	31.4	32.1	35.8	25.8	22.6	19.5	41.1	15.8	11.7	18.3	13.9	22.7	18.0	18.1	7.6	40.0	57.5	25.2	20.3	19.1	16.9	36.7	13.0	21.4	36.0	21.3	11.5	53.7
ovill	75.9	64.7	64.2	63.2	69.0	69.0	80.4	67.8	78.8	74.2	77.5	56.3	57.2	68.2	60.2	63.0	58.5	65.8	57.8	76.0	73.3	77.8	69.0	63.0	64.9	79.8	63.5	68.2	67.6	67.5	64.7	81.5
ovil	30.9	21.7	10.9	20.4	23.7	22.7	25:7	14.7	14.1	15.9	18.8	12.1	12.7	14.7	13.8	15.9	13.9	19.4	7.9	24.1	21.7	19.6	16.7	14.5	11.5	20.8	10.5	18.2	27.1	15.3	9.8	25.1
trup	0.7	0.6	9.6	3.4	0.8	0.8	0.5	2.1	0.4	14.1	3.4	12.8	4.0	6.0	4.8	0.5	11.4	0.6	11.2	0.5	0.6	3.4	4.9	2.9	10.0	0.6	5.4	1.8	4.1	20.6	10.5	0.2
trlw	3.1	5.7	86.6	35.8	8.1	21.6	56.8	64.4	60.8	48.9	33.9	97.8	42.4	84.5	87.6	82.0	74.7	63.4	83.3	50.8	3.2	25.0	54.7	28.4	89.1	3.0	32.7	25.6	27.3	98.6	41.5	73.8
vigII	1.9	1.3	1.9	2.2	1.9	1.7	2.2	2.2	2.0	2.0	1.8	2.3	1.6	2.4	2.1	2.0	2.4	1.7	1.5	1.5	1.9	1.6	2.0	2.1	1.8	1.2	2.2	1.9	1.5	2.3	1.9	1.5
vigI	2.0	1.2	2.1	2.4	1.8	2.3	1.9	1.6	2.1	1.8	2.0	1.6	1.6	1.6	1.8	1.7	1.6	1.6	1.6	1.8	2.0	1.2	2.2	1.8	2.4	1.7	2.3	2.0	1.3	2.6	2.6	1.6
gl	4.9	3.7	3.7	3.3	2.6	4.7	4.2	2.8	4.2	2.6	4.7	2.8	4.0	2.1	2.3	1.1	4.0	1.6	1.1	3.0	4.9	4.4	4.9	1.9	3.0	1.9	3.3	2.8	4.7	3.5	4.7	4.9
Entry	8	97	98	66	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127

1																													2	25	0	
ЫY	12.4	9.9	9.4	13.9	11.0	15.9	9.7	8.8	10.1	12.6	11.8	11.7	10.9	15.7	8.7	12.0	7.9	9.4	8.2	13.2	10.4	10.7	13.4	11.8	17.0	13.4	15.1	13.5	12.0	12.4	13.0	11.8
plht	173.8	122.5	117.5	148.8	142.5	179.9	167.6	188.5	148.3	121.9	170.9	156.1	127.6	154.0	163.5	150.0	122.0	154.7	175.1	145.9	136.4	170.1	192.4	198.8	147.5	118.1	164.9	152.1	158.6	159.1	170.6	129.2
daf	72.9	73.4	67.8	73.0	66.3	75.1	76.3	79.2	71.6	68.1	78.1	73.7	67.9	72.2	70.3	67.7	7.97	80.0	83.1	75.6	70.1	76.3	79.9	77.8	70.9	69.0	72.1	75.2	75.3	71.8	76.1	68.9
pslg	4.5	2.9	4.4	4.7	5.1	3.8	4.4	4.1	4.5	4.2	5.0	3.6	5.4	4.6	4.1	5.6	2.9	4.2	3.8	5.1	4.3	6.6	5.6	7.7	5.2	4.4	5.3	4.0	4.9	4.1	4.1	5.1
drywt	4.5	4.2	4.4	4.1	4.5	4.4	4.4	4.6	4.3	4.6	4.6	4.4	4.5	4.7	4.3	4.5	4.1	4.6	4.5	4.7	4.4	4.8	4.7	4.5	4.4	4.6	4.4	4.2	5.0	4.4	4.5	4.7
shtll	47.2	40.4	40.8	43.8	44.3	41.6	42.8	45.6	40.6	43.7	42.8	44.5	42.3	43.0	43.1	44.9	40.6	46.4	42.0	45.2	41.7	48.1	45.8	47.3	41.9	42.4	47.0	43:0	46.0	42.5	44.7	43.6
shtl	24.2	21.9	22.2	23.0	26.6	24.3	20.8	23.6	21.6	23.0	23.3	24.6	24.2	23.6	21.2	25.4	22.8	24.4	22.5	23.9	23.3	23.0	25.7	25.8	21.9	22.9	24.8	20.7	21.3	22.0	21.8	24.2
IIHb	71.6	48.3	60.6	51.0	45.8	74.4	60.9	43.2	66.0	75.6	66.7	73.2	55.6	58.4	41.5	38.9	44.9	25.9	66.5	42.6	64.3	48.7	65.6	49.8	32.3	28.3	27.6	48.7	62.1	47.6	70.5	32.1
Iłb	49.0	30.7	34.0	26.8	29.5	54.4	30.8	22.2	31.3	41.3	43.7	58.3	39.1	44.2	18.7	22.4	21.2	17.3	50.0	17.8	43.6	21.7	42.6	25.2	16.9	17.7	15.1	26.9	49.1	24.5	44.4	15.7
ovill	80.8	68.7	75.1	63.6	73.4	85.8	72.6	73.1	76.6	71.0	78.8	81.7	64.2	76.0	70.6	53.3	73.7	59.5	74.7	67.0	72.5	60.7	74.4	66.3	67.6	56.9	60.4	72.8	76.3	76.2	81.7	69.3
ovil	31.9	20.3	19.4	17.8	24.6	35.6	23.5	21.3	21.7	21.8	30.3	33.5	29.9	38.9	9.7	17.4	12.1	12.9	36.4	12.4	31.1	12.1	24.3	10.0	13.3	15.2	9.0	13.5	25.9	18.5	23.2	16.3
trup	0.5	10.8	0.5	0.6	0.5	0.4	8.2	2.6	0.5	0.4	0.5	0.7	0.6	0.5	0.8	2.7	8.1	10.5	0.7	0.7	0.4	0.6	1.1	0.7	1.7	4.5	6.8	0.4	1.3	9.8	2.0	0.5
triw	59.6	105.3	96.7	18.2	10.9	45.0	78.0	31.2	19.2	2.9	47.5	46.1	12.9	50.0	74.3	44.7	62.7	87.6	3.7	67.4	52.6	2.9	11.8	60.3	92.8	41.2	99.3	77.5	40.8	78.3	62.9	68.4
vigII	1.6	2.7	2.4	1.8	1.3	2.3	2.5	1.9	2.3	2.5	1.9	1.7	2.2	2.4	3.6	1.6	1.8	2.1	2.3	2.8	1.7	1.9	2.0	1.8	2.6	2.4	2.2	3.1	2.3	2.8	2.5	1 8
vigI	1.6	2.8	2.2	2.2	1.4	2.0	2.3	2.2	2.2	2.5	2.1	1.9	1.8	2.5	3.9	1.4	2.2	2.0	2.0	3.1	1.8	1.8	1.6	1.8	2.9	2.7	2.4	2.2	2.4	3.0	2.0	0.0
gl	4.7	4.7	2.8	4.9	4.0	3.0	4.9	4.4	4.9	4.7	4.7	5.0	4.7	4.0	4.0	4.7	4.9	2.3	4.7	3.3	4.5	4.4	4.7	2.1	2.1	3.5	2.8	3.7	4.7	2.6	4.7	77
Entry	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	150

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9.6 1.5		
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I																													25	52		
bř	10.9	10.9	10.6	13.8	15.8	8.4	10.6	14.6	13.0	16.3	9.6	15.6	8.7	11.3	12.0	9.2	13.3	9.3	11.4	9.3	9.7	14.0	9.3	12.4	15.5	7.6	12.0	12.1	13.8	11.0	8.9	8.0
plht	143.0	167.1	167.5	181.6	195.6	136.5	177.7	151.3	145.3	169.0	166.5	116.9	179.7	174.2	163.0	157.6	125.8	175.5	156.4	173.3	164.7	132.8	151.7	148.1	139.3	131.5	134.1	129.6	150.8	136.7	139.1	156.6
daf	68.3	73.6	70.6	73.9	ררד	67.6	75.9	68.7	70.5	1.1.	6.69	69.0	80.4	76.6	68.8	78.3	66.2	75.3	73.7	74.0	68.8	74.4	87.9	73.8	72.0	74.8	69.1	72.2	75.5	73.6	6.69	74.3
pslg	6.3	4.8	8.4	5.9	3.8	3.9	4.4	8.0	5.5	5.7	9.8	4.4	4.8	5.9	5.2	3.7	4.7	3.9	4.0	3.3	6.4	2.6	2.8	4.3	3.5	4.0	3.7	4.0	4.7	3.7	3.8	4.2
drywt	4.6	4.5	4.6	4.6	4.4	4.3	4.5	4.6	4.2	4.7	4.9	4.4	4.4	4.4	4.4	4.4	4.8	4.5	4.2	4.2	4.3	4.4	4.3	4.5	4.4	4.5	4.2	4.5	4.5	4.3	4.4	4.4
shtII	45.9	43.1	47.3	47.9	41.9	41.7	42.3	44.3	43.1	42.9	48.2	43.6	45.5	46.9	42.6	44.2	42.5	46.6	42.1	42.5	45.5	40.2	42.9	43.1	42.6	43.3	39.7	44.6	41.3	40.1	44.7	42.9
shtl	24.7	23.5	24.7	24.1	23.6	23.0	23.6	23.8	23.5	24.2	25.4	23.9	23.9	23.2	22.5	23.4	23.9	24.7	23.4	21.0	26.4	23.1	24.7	23.7	22.4	21.7	22.5	23.4	23.3	23.0	25.3	222
IIHb	37.6	47.1	35.4	37.8	58.7	25.1	36.5	32.2	46.7	34.0	42.8	33.9	74.1	33.7	33.7	40.7	41.2	59.5	49.5	32.1	27.5	26.1	53.9	54.1	44.1	41.1	54.3	44.5	31.5	24.0	35.6	53.0
ldh	21.2	19.8	20.5	17.0	37.9	13.1	21.3	18.2	26.9	17.6	25.3	18.3	49.8	14.7	20.4	21.2	26.4	39.2	19.6	18.8	16.7	12.1	30.7	18.8	30.0	25.5	28.9	20.8	13.0	11.8	23.4	20.2
ovill	63.7	ĽЦ	73.2	64.6	80.8	53.5	68.3	69.3	73.8	51.8	65.4	63.6	83.4	65.3	68.9	62.7	65.8	74.7	64.4	59.5	58.9	62.6	72.1	76.5	60.2	71.9	68.2	9.77	51.9	49.9	62.3	68.7
ovil	13.6	24.7	28.0	12.7	22.1	10.7	14.2	15.9	15.1	16.8	17.8	16.5	38.1	15.6	18.6	14.5	17.6	24.1	11.3	12.9	20.3	10.2	15.8	17.1	23.5	13.5	17.9	16.7	13.1	6.7	13.4	C 1 C
trup	2.4	0.5	5.8	6.4	0.6	1.3	3.0	3.3	3.5	11.6	0.5	5.3	0.5	1.4	1.3	21.7	4.5	0.5	1.1	12.6	7.7	25.1	3.6	1.6	15.1	0.8	0.4	8.7	12.5	5.2	3.5	50
trlw	64.1	86.1	36.4	55.6	3.5	48.1	42.2	47.3	50.8	42.5	20.7	44.3	3.7	49.4	29.9	95.0	61.4	64.6	44.9	78.1	72.9	101.6	43.9	9.5	74.9	52.2	31.4	39.8	55.8	76.9	28.3	615
vigII	1.6	1.7	1.6	2.1	2.3	2.4	2.1	1.8	1:9	2.9	1.6	1.3	1.7	2.0	1.7	1.8	1.6	1.7	2.9	2.1	1.5	2.1	1.7	2.5	2.2	2.9	2.6	2.2	2.8	2.6	2.0	0 0
vigI	1.5	1.8	2.0	2.4	1.9	2.1	2.2	1.8	1.5	3.2	1.8	1.6	1.4	1.8	2.0	1.8	1.9	1.6	2.9	2.2	1.9	2.2	1.9	2.0	2.3	2.7	2.4	2.8	3.3	2.0	1.8	0 0
gl.	4.7	4.7	2.3	2.3	2.6	3.3	4.0	2.1	2.1	3.7	2.3	2.5	2.3	4.4	2.5	2.1	3.3	4.2	3.8	3.7	2.3	3.0	3.3	3.3	4.4	4.7	4.9	3.0	2.6	4.2	4.7	0 4
Entry	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	

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Jon	w

Entry	gl	vigI	vigII	triw	trup	oviI	ovill	dhI	dhII	shtI	shtll	drywt	pslg	daf	plht	yld
224	4.7	1.9	2.3	57.0	0.5	22.6	71.4	33.6	50.9	22.5	42.1	4.4	7.2	71.5	170.3	13.8
225	4.7	2 .3	2.3	15.1	0.6	21.0	66.7	26.3	57.4	24.2	46.2	4.5	4.3	74.5	1 74.8	11.5
226	2.8	2.6	2.3	56.6	3.9	18.4	78.3	24.7	43.3	24.0	44.3	4.6	5.7	71.5	150.3	12.1
227	3.2	3.2	3.8	75.0	2.8	14.1	75.6	27. 2	80.9	19.5	35.0	4.2	2.8	77.8	1 56.9	6.4
228	2.8	2.3	1.8	3.9	0.7	14.0	44.6	15.8	20.8	26.4	47.2	4.7	7.0	72.4	192.1	10.7
229	4.9	1.4	1.3	39.0	0.5	40.0	79.1	53.5	74.0	25.3	41.8	4.6	4.6	71.5	145.0	13.9
230	4.2	3.2	3.2	62.0	0.5	27.2	71.5	37.6	72.4	20.5	38.9	4.2	3.4	75.2	148.4	8.6
231	4.4	2.6	2.9	32.8	7.6	22.6	81.3	49.2	76.6	21.7	40.9	4.2	3.4	76.3	172.0	9.4
232	2.3	1.9	1.9	64.2	5.3	7.7	50.4	5.5	14.7	26:1	43.4	4.4	4.4	69.5	127.4	14.4
233	2.8	3.0	2.8	81.9	5.7	8.6	48.9	8.9	17.3	23.8	42.2	4.1	3.7	70.1	147.1	13.7
234	3.3	2.4	1.9	73.1	11.4	13.3	61.5	16.4	29.5	23.2	42.2	4.4	4.7	68.6	147.0	14.2
235	4.2	1.4	2.2	77.9	7.7	16.7	74.3	19.9	44.8	24.5	43.1	4.3	4.3	70.4	170.7	13. 2
236	4.4	3.0	2.7	49.9	6.8	13.2	61.4	17.1	43.3	- 22.0	43.0	4.3	3.7	74.4	174. 1	14.1
237	2.1	2.0	2.2	3.3	0.4	22.3	73. 2	30.7	65.7	22.0	38.3	4.3	3.1	74.2	159.2	12.9
238	4.0	1.8	1.2	36.8	0.8	14.9	53.4	9.4	24.3	25.2	46.4	4.8	10.0	66.5	150.5	7.5
239	2.8	2.4	2.6	3.7	0.4	16.7	75.8	37.8	62.6	21.2	42.6	4.3	3.4	77.0	164.4	13. 0
240	4.7	2.4	2.0	3.4	0.4	36.8	76.4	62.0	67.8	22.1	41.9	4.5	4.1	80.5	1 99 .1	8.3
241	4.7	2.2	1.9	30.2	0.4	25.0	77.4	38.3	73.3	22.7	43.9	4.4	3.7	82.4	217.4	8.8
242	2.1	2.6	2.4	78.6	3.6	15.1	72.9	21.7	40.6	21.5	43.6	4.6	3.9	74.4	154.7	11.7
243	3.5	3.0	2.3	103.8	5.1	10.9	58.8	20.3	35.5	20.7	42.4	4.3	3.6	72.4	139.9	12.9
244	3.7	2.2	2.2	79.6	7.9	14.0	63.5	16.3	24.9	22.4	43.6	4.5	4.9	73.6	156.8	9.6
245	3.5	2.6	2.5	70.3	2.8	12.6	56.7	16.4	33.6	20.7	42.2	4.5	5.3	69.6	151.9	16.6
246	4.5	3.0	2.3	21.4	0.5	29.1	80.8	44.2	60.5	22.2	43.1	4.7	5.2	66.0	153.6	5.9
247	4.0	2.3	2.8	39.5	4.2	11.7	69.0	15.3	33.2	21.9	40.5	4.4	4.1	65.8	125.4	4.9
248	4.9	1.6	1.5	61.4	1.6	41.2	74.3	47.7	71.1	25.1	43.7	4.7	4.1	71.4	133.1	12.2
249	4.9	2.1	1.3	67.6	2.1	45.7	82.8	60.3	69.5	25.0	43.3	4.7	3.8	70.5	126.2	10.0
250	4.4	2.1	2.3	50.9	0.3	24.6	76.4	32.1	57.1	23.4	44.9	4.4	3.7	76.2	172.2	9.5
251	4.7	1.4	2.2	25.4	0.5	20.5	70.8	34.4	62.6	21.9	43.8	4.4	3.8	70.7	1 6 3.7	9.2
252	1.4	2.9	2.1	116.8	9.4	7.6	64.3	8.9	15.8	23.4	45.9	4.6	5.3	68.3	148.2	11.6
BTx623	4.9	1.8	2.2	3.9	0.5	23.8	73.1	45.6	74.1	21.9	43.7	4.4	3.6	71. 9	126.2	13.0
IS 18551	1.2	1.7	2.0	98.2	2.8	5.2	44.7	13.1	20.5	23.9	45.3	4.4	6.5	77.7	201.1	12.0
CSH 1	4.9	1.2	1.9	3.7	0.4	28.8	78.7	54.0	81.3	21.8	39.7	4.4	3.4 .	7 9 .6	139.5	12.8
296B	4.2	4.5	4.0	4.3	0.6	13.5	76.6	36.8	63.1	15.9	33.5	3.9	2.4	94.1	109.6	11.9

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yld	19.6	16.6	23.8	17.2	22.8	20.0	23.3	29.6	16.4	18.2	14.7	21.1	19.4	23.1	18.9	19.9	13.0	18.6	16.0	16.1	14.5	19.4	24.9	16.2	19.7	17.4	18.4	20.2	21.0	14.9	
plht	197.7	202	203.6	141.2	184.5	140.7	206.6	155.9	196.5	143.5	166.9	217.3	180.7	197.4	149.2	166	182.5	195	174.5	167.7	150.7	219.8	172.9	184.9	199.4	188	138.5	140.5	182.8	153.9	
daf	78.9	79.4	6.77	75.3	79.1	77.5	78.6	81.2	77.0	1.17	80.3	75.4	78.4	80.1	76.1	79.8	75.8	75.0	80.7	74.7	75.0	77.3	79.5	77.3	81.8	78.0	79.0	81.1	L.LT	82.7	
shtII	31.1	31.8	31.7	32.2	28.6	28.4	31.0	31.5	30.6	30.7	28.4	34.2	30.5	30.5	32.2	28.4	32.9	30.7	31.5	31.2	32.9	33.8	29.3	31.2	31.6	32.6	29.2	30.2	31.9	30.8	
shtľ	16.2	17.0	18.4	16.2	15.4	13.5	17.0	16.3	17.1	16.1	16.1	18.3	15.1	15.9	17.3	14.5	16.9	15.2	17.1	17.0	17.4	18.0	14.6	18.2	17.4	16.2	16.2	16.1	17.6	16.5	
IIdb	85.6	79.1	86.3	83.0	86.6	89.4	85.4	87.1	83.3	82.7	88.2	84.0	87.3	88.1	80.9	89.3	81.2	84.0	90.6	85.1	87.2	88.6	87.6	82.9	87.2	85.8	86.0	85.9	88.5	83.6	
Idb	8.69	69.7	61.9	71.3	78.7	71.6	76.8	79.6	75.0	71.2	77.2	71.7	77.8	78.8	63.1	83.6	70.0	69.2	80.0	68.8	75.3	7.67	77.8	69.5	77.0	69.8	74.0	74.9	79.5	68.0	
ovill	93.3	91.8	92.8	92.6	93.0	93.0	93.2	93.2	91.7	92.1	93.4	92.8	- 93.0	93.5	91.7	94.1	92.0	92.9	94.3	92.6	93.0	93.6	94.1	92.5	92.2	93.3	93.2	92.3	93.3	91.8	
ovil	85.3	76.1	77.6	79.5	83.2	82.5	81.5	87.1	80.2	79.4	81.1	83.4	82.0	82.1	80.4	87.2	77.6	81.9	85.4	81.2	82.5	83.1	80.9	79.1	83.9	79.1	81.5	80.5	86.6	79.3	
trup	13.1	9.2	3.1	1.8	1.6	14.5	1.2	1.0	11.7	15.2	0.9	8.2	0.8	0.3	0.7	22.5	0.6	0.5	18.4	21.9	1.7	7.7	15.5	1.1	-0.3	-0.3	11.2	14.6	2.0	1.1	
triw	67.6	71.2	31.5	5.1	4.5	54.9	36.7	19.5	54.5	68.6	14.1	57.6	43.5	40.6	1.1	89.3	78.5	35.7	70.2	80.7	35.6	63.2	69.2	5.0	1.1	11.5	37.9	66.8	18.7	42.6	
vigII	2.1	2.1	2.4	1.9	2.2	2.9	1.9	2.2	2.1	2.5	2.2	1.7	2.2	2.5	1.6	2.8	2.0	2.2	2.3	1.8	1.4	2.2	2.8	1.5	1.8	2.1	2.9	2.6	2.5	2.3	
vigI	3.4	3.2	3.2	2.9	3.2	3.2	3.1	2.5	3.1	2.9	2.9	2.4	2.6	2.7	3.3	2.9	2.8	2.6	2.9	2.6	1.9	2.2	3.9	2.6	2.2	3.1	2.9	3.3	2.7	3.5	
13	4.4	4.2	2.7	3.0	4.7	3.1	3.7	4.7	2.3	4.6	4.9	2.3	4.2	5.0	2.6	3.3	3.8	3.1	4.9	2.9	3.1	4.7	5.0	3.0	4.0	3.3	4.9	4.7	4.8	3.7	
Entry	-	7	e	4	Ś	9	7	80	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	>

5	lgi	vigII	triw	đ	ovil	ovill	Ę	IIसृ	shť	shtll	daf	plht	Pł ,
3.1		2.0	48.6	6.11	79.4	93.2	78.3	87.4	16.7	30.3	82.7	189.3	24.6
3		2.5	73.4	15.3	84.0	93.2	72.9	82.8	16.1	30.4	78.0	127	32.4
3	9	2.0	48.3	-0.1	82.2	92.6	76.7	85.3	16.7	31.7	77.5	131.7	18.1
,	4	1.8	65.1	5.9	75.5	92.5	70.3	82.1	16.8	31.4	79.3	223.2	20.8
0	9	1.8	51.2	11.5	80.5	91.1	65.0	81.3	16.9	32.3	9 .77	227.2	22.5
2	ون	1.5	56.7	14.3	80.5	93.1	76.0	84.4	16.3	32.6	82.9	186.3	13.4
2	Ľ	2.0	47.5	7.4	84.8	93.7	77.8	87.6	16.9	32.5	82.8	195.6	21.6
2	9	2.0	54.7	1.3	83.1	92.6	70.9	85.1	17.2	32.3	74.9	151.7	13.7
2	г.	1.3	64.2	22.0	83.2	93.0	72.3	80.8	18.1	32.5	77.3	221.5	22.5
ŝ	Γ.	1.9	45.9	8.3	81.0	92.5	68.4	83.1	16.1	30.2	82.0	203.6	20.8
	3.1	2.0	47.4	8.9	81.9	93.2	70.9	85.0	15.6	31.0	80.9	187.6	18.3
	6.9	2.2	69.8	7.8	80.2	92.8	76.9	86.0	17.1	30.9	81.3	207.3	21.0
	2.6	2.0	0.9	-1.0	7.97	93.0	73.6	82.9	17.1	32.0	79.8	201.4	20.1
	2.2	2.3	61.9	7.4	84.1	92.9	77.5	87.0	17.8	30.9	80.9	194.8	17.1
	3.3	2.5	57.6	13.1	82.4	93.2	73.0	82.3	18.3	30.1	79.6	162	17.9
	2.9	1.7	5.8	1.4	84.9	93.2	79.9	87.1	18.8	30.9	74.2	198.8	17.2
	3.0	1.7	21.9	7.9	74.5	90.0	55.4	77.2	18.4	31.7	83.3	158.9	16.5
	2.5	1.8	48.3	7.4	84.8	93.8	78.2	87.0	16.8	30.8	80.4	191.4	14.0
	2.2	1.2	36.0	3.8	78.3	91.6	61.3	76.5	18.1	30.0	79.4	176.6	15.0
	2.4	2.2	47.0	7.9	85.2	93.4	77.2	86.5	17.6	31.0	79.9	191.2	18.1
	2.9	1.8	87.0	20.5	75.0	92.0	63.0	78.5	19.0	32.7	76.6	181.1	18.0
	3.0	2.4	42.3	11.6	80.6	91.9	6.69	79.9	15.9	31.7	82.0	206.6	19.8
	2.6	1.8	28.9	1.3	83.8	93.2	75.1	87.1	18.1	31.5	75.5	204.9	17.8
	2.3	1.8	44.0	1.6	80.4	92.7	69.2	79.8	17.8	31.5	80.8	178.6	18.3
	3.5	2.1	42.2	3.6	84.0	94.0	80.3	87.6	16.4	29.5	80.3	185.9	18.1
	2.7	2.5	18.7	0.6	82.5	93.4	77.8	88.8	16.0	29.6	78.4	182.6	20.6
	2.6	2.4	25.5	10.2	82.9	93.7	80.0	88.4	17.1	31.1	82.1	222.7	27.1
	2.4	2.0	59.6	3.6	84.1	93.5	80.0	87.1	18.3	31.3	79.5	224.2	19.9
	2.9	2.3	76.2	13.5	80.9	92.7	70.6	84.9	16.7	31.0	79.3	167.7	22.2
	2.2	1.7	49.2	11.5	78.2	92.7	78.3	85.7	17.1	30.9	83.0	183.4	18.4
	2.8	2.5	44.0	0.1	85.4	93.8	77.6	88.1	17.4	29.4	79.6	135.8	21.9
	2.9	2.4	74.4	6.3	82.9	93.3	76.8	85.2	15.8	28.8	78.5	156	19.1
	7 0	1 0	70.4	3.0	76.3	90.4	64.6	78.3	16.6	30.5	76.4	207.5	18.3

																														2	56	5	
yld	18.5	19.8	18.2	15.5	27.4	19.5	18.0	23.3	20.8	15.7	15.3	15.2	18.9	30.2	23.2	15.2	18.7	38.0	20.0	16.5	22.7	19.9	18.5	21.5	15.2	15.7	18.8	20.4	16.6	19.3	19.8	14.6	17.0
plht	190.8	155.9	180	165.6	197.5	179.7	200	178.5	181	182.3	174.6	123.3	200.6	171	196.8	139.1	161.4	143.3	183.1	141.7	128.7	169	209.7	241.7	204.3	160.4	169.6	154	136.4	218	156.6		
daf	76.3	80.0	79.7	74.0	79.6	11.2	80.5	79.1	78.7	80.8	76.5	75.2	79.6	82.6	80.4	75.5	76.9	77.0	77.4	78.0	80.7	78.2	80.5	78.4	77.1	79.0	78.8	79.0	78.7	79.1	76.0	74.0	75.6
shtH	28.5	30.8	31.4	31.6	29.4	30.6	32.4	31.0	28.0	30.9	29.7	33.2	29.2	30.0	32.6	30.2	30.5	33.3	32.8	31.0	30.1	29.8	32.8	31.8	31.8	30.0	30.3	28.1	31.0	31.2	30.5	29.1	30.4
shtľ	16.5	15.5	16.6	16.8	18.0	15.5	17.6	16.5	16.2	17.2	16.8	17.7	17.8	16.0	19.7	17.5	17.3	16.9	16.6	16.9	15.1	15.7	18.1	17.8	19.6	15.3	16.0	14.8	16.7	17.0	16.5	17.0	17.2
IIHb	88.1	85.6	85.6	84.5	86.5	86.0	84.1	85.7	88.3	85.6	85.5	83.1	81.6	90.6	86.4	85.6	87.1	88.3	85.2	90.1	84.6	88.4	85.2	82.8	83.8	85.9	88.9	90.6	85.7	86.1	86.1	91.1	86.2
Ihb	77.8	76.6	78.6	71.0	75.9	78.9	72.7	68.2	83.5	73.4	74.0	65.1	71.9	86.4	72.5	75.8	74.9	82.8	68.8	77.6	71.4	76.3	72.0	74.5	62.2	78.7	83.2	80.5	75.3	77.8	79.0	81.3	76.9
ovill	93.9	92.6	92.9	90.9	92.9	93.2	92.4	93.2	93.8	93.2	92.8	93.4	92.8	93.5	93.4	92.8	93.8	94.0	93.4	92.6	93.1	93.3	92.0	93.0	92.1	93.5	93.6	93.6	93.5	93.4	92.5	93.7	91.9
ovil	83.2	83.3	84.6	79.4	82.0	83.2	81.1	75.0	81.8	81.5	81.1	85.2	82.5	86.4	79.0	82.5	78.7	85.1	77.3	82.9	80.3	85.4	80.3	83.4	81.2	84.2	86.3	86.0	83.9	84.5	79.8	84.7	83.9
trup	1.3	4.4	7.8	1.0	3.7	10.6	7.9	0.3	1.8	7.9	1.3	1.1	10.0	0.8	3.4	1.1	1.1	6.1	3.5	0.4	17.8	1.5	15.7	1.6	2.7	0.4	1.9	0.1	8.7	2.3	1.8	2.2	14.6
trlw	2.0	79.7	33.2	43.7	33.1	59.4	49.1	42.2	1.8	29.2	50.5	50.3	54.2	1.7	18.1	34.8	37.7	39.9	30.7	38.9	51.9	1.9	76.6	3.2	32.5	2.3	8.8	28.7	49.6	5.7	3.8	10.7	68.6
ligiv	2.3	2.1	2.3	2.4	2.3	2.6	1.5	2.0	2.7	2.0	1.9	1.6	2.1	3.0	1.2	1.6	2.2	2.0	1.6	2.0	2.6	2.4	1.6	1.6	1.3	2.4	2.1	2.6	2.4	2.0	2.9	2.1	1.8
vigI	2.4	2.9	2.9	2.7	2.9	3.1	1.8	2.9	2.9	2.9	2.3	2.8	2.6	2.5	2.6	2.4	2.8	2.8	2.8	3.5	3.5	2.8	2.6	2.6	2.0	3.3	3.1	3.5	2.8	2.2	3.3	2.6	2.8
gl	3.0	4.1	4.7	4.5	3.1	4.8	4.2	2.4	4.1	2.4	4.3	2.8	2.4	5.0	2.1	3.1	3.1	3.9	4.7	4.9	4.9	4.7	3.0	4.2	1.9	4.9	4.9	4.9	4.9	2.8	4.7	4.2	4.0
Entry	8	67	88	69	20	71	22	73	47	75	76	L L	78	62	80	81	82	83	2	85	98	87	88	68	6	16	92	93	94	95	96	97	98

vigII	trlw	trup	ovil	llivo	Ę	IIHb	shtl	shtl	daf	plht	PX :
	44.1	5.5	81.8	92.7	73.1	86.2	15.3	29.0	83.2	190.2	16.0
1.4	8.2	1.6	80.5	92.8	61.5	83.0	17.2	31.2	80.1	191.3	22.5
_	17.6	1.4	84.6	93.7	80.9	87.7	15.8	31.9	76.2	179.9	22.5
	29.1	1.3	79.0	93.7	73.8	83.6	16.5	31.4	78.5	190.6	18.9
~	35.9	3.1	81.3	93.3	78.1	89.6	16.0	31.0	6.67	171.1	14.5
1.7	35.6	4.3	T.TT	92.5	72.0	85.1	17.2	30.8	80.0	172.7	17.5
8.	53.2	20.9	79.5	92.8	69.7	84.1	16.8	30.2	80.1	198.9	23.5
	27.1	3.8	80.5	93.0	73.9	85.7	16.1	28.3	78.6	124.4	17.6
	78.0	14.5	76.1	92.0	68.0	83.1	15.2	29.7	75.5	175.5	16.2
	56.1	9.3	76.7	93.7	64.6	75.9	17.4	32.1	79.8	199.8	18.2
	74.2	4.0	77.0	92.1	66.2	80.5	17.6	32.9	7.5.8	200.9	14.3
	61.1	7.0	79.2	92.8	65.4	78.1	18.9	31.1	75.9	225.7	19.1
8	56.0	1.6	7.9.7	92.3	75.8	83.1	16.7	31.5	76.7	193.4	20.1
	45.9	7.1	82.7	94.0	80.7	87.8	17.6	31.7	83.4	173.3	17.5
	46.0	1.5	84.7	93.4	67.7	80.5	17.4	31.6	78.1	197.8	18.5
-	69.0	16.9	82.7	93.7	70.5	79.8	18.2	34.0	79.5	203.4	18.4
5.5	25.6	1.7	82.8	93.4	72.6	87.5	17.1	29.2	80.3	171.6	21.2
2.9	1.7	1.1	83.1	93.2	77.3	90.2	15.3	31.1	75.2	139.9	24.7
2.3	29.0	6.4	82.7	92.8	81.0	87.7	17.3	31.3	81.5	215.6	22.7
2.6	57.0	10.4	82.1	93.4	76.6	85.8	16.4	29.0	76.0	128.1	23.4
4.	21.7	4.2	82.7	92.8	64.1	82.8	18.9	33.0	79.1	178.9	22.1
	66.3	10.3	81.1	93.0	67.0	85.4	17.5	31.3	79.3	204.4	15.4
	2.1	0.8	83.3	93.6	72.7	81.9	17.8	33.8	78.9	213.7	18.6
	51.5	16.3	76.8	93.6	79.9	86.8	16.4	32.0	78.6	174.7	16.7
<u>80</u>	18.9	3.1	80.5	92.2	72.4	85.5	17.5	30.8	78.3	210.5	21.3
~~	41.0	6.4	82.6	93.1	77.2	89.2	16.8	32.1	80.2	228.8	20.8
2.0	59.6	11.9	76.7	92.1	60.7	79.1	16.9	31.7	78.8	164.9	18.8
9.	49.4	12.1	84.5	93.6	73.5	84.4	18.6	30.2	79.7	208.2	22.2
	42.0	2.1	81.9	92.9	80.2	88.6	15.3	30.7	76.0	170.9	18.6
2.6	22.8	1.5	85.6	92.9	82.9	87.8	14.7	28.8	78.7	190.5	24.2
~	86.0	16.9	85.4	93.3	73.6	81.8	16.3	29.9	75.2	155.1	13.5
1.5	58.9	1.4	83.0	93.9	79.8	84.9	17.4	32.3	74.0	122.2	14.3
	۲ v	2,2	84.7	92.8	814	89.6	16.8	29.6	78.9	172.7	18.9

																														2	51	8	
yld	17.6	21.6	16.4	17.2	15.6	22.5	20.7	19.1	17.4	16.7	17.6	23.9	24.5	20.6	19.9	25.5	13.2	19.2	32.2	23.7	18.9	17.0	20.6	18.4	17.8	21.7	25.6	22.1	28.7	16.6	17.3	18.2	
plht	171.1	202.5	163.8	202.5	175	127.6	195.7	192.3	158.1	184.6	199.6	200.3	124.6	175.4	196.9	186.3	133.6	199.5	215.7	204.8	180.6	148.3	197.6	184	176.6	170	198.9	143.7	204.8	207.2	202.6	152.1	
daf	73.1	80.8	81.9	80.1	80.2	78.2	83.0	76.4	75.3	78.0	77.5	77.1	81.3	84.0	82.6	79.4	77.6	80.7	80.4	80.7	80.0	78.0	79.9	79.3	78.9	79.1	79.6	74.9	82.0	75.1	81.1	75.0	
shtll	28.7	28.5	29.7	31.8	30.1	27.1	30.3	33.5	31.0	29.8	30.4	31.9	33.4	32.6	26.6	32.6	29.0	29.6	30.1	34.6	30.5	32.5	30.8	30.0	30.8	29.6	29.9	30.7	32.6	30.9	29.6	31.9	
shtl	16.6	15.9	16.0	17.4	16.1	15.1	17.0	16.6	17.0	16.3	17.1	16.4	17.6	18.0	13.9	17.0	16.0	18.0	16.6	19.5	16.5	16.9	16.8	15.6	16.1	17.5	17.3	16.3	17.5	17.3	16.5	18.4	
IIHb	87.5	87.3	87.5	85.0	84.2	89.1	89.1	86.2	88.0	84.6	83.8	89.9	80.6	83.7	91.2	87.1	86.2	89.3	89.0	84.2	85.6	83.6	87.6	84.8	88.3	82.5	85.8	78.9	86.8	84.0	87.0	87.2	
Iub	78.5	75.1	76.5	74.1	70.2	81.2	78.8	78.0	78.9	73.5	72.0	81.7	71.7	72.6	83.0	70.3	71.6	71.3	81.2	70.0	76.1	69.7	6.67	62.7	74.9	53.1	75.5	68.9	73.6	70.7	79.1	79.9	
ovill	94.0	92.3	93.6	93.0	92.7	93.5	93.1	92.9	94.2	92.2	92.6	93.7	92.2	92.3	93.9	92.9	93.3	93.4	93.8	92.3	92.1	92.8	93.7	91.4	93.0	91.7	93.1	92.0	93.2	92.6	93.5	93.1	
ovil	87.5	83.6	84.8	84.6	80.9	85.8	84.2	82.3	85.6	80.9	80.4	86.5	82.7	81.7	86.4	83.4	85.4	86.6	86.2	81.9	78.6	78.9	87.2	<i>6.77</i>	82.3	76.3	83.2	82.0	80.5	81.0	83.0	80.6	
trup	5.3	0.9	17.3	7.5	2.2	0.6	2.5	2.5	1.5	1.1	5.6	4.7	23.7	12.3	1.8	1.8	1.7	0.0	1.6	2.2	7.8	4.6	6.6	2.2	4.7	12.8	4.2	1.6	11.7	-0.7	0.5	1.9	
trlw	18.3	29.7	80.1	48.1	10.3	3.4	24.1	24.4	3.6	45.0	60.6	43.8	85.0	83.4	5.7	-50.5	38.5	0.8	4.9	61.9	63.2	42.1	60.1	30.2	33.8	69.2	45.5	50.6	51.9	0.5	0.3	25.0	
vigII	1.6	2.0	2.2	1.8	2.2	2.5	2.4	1.7	2.6	2.2	2.2	1.8	1.8	2.0	3.0	1.9	2.3	2.0	2.6	1.5	2.0	1.5	2.2	2.1	2.6	1.5	2.2	1.9	2.0	1.5	2.2	1.9	
vigI	2.4	2.9	2.7	2.9	2.9	2.7	2.8	2.6	2.9	2.8	2.9	2.6	2.9	2.8	2.8	2.9	2.4	2.4	2.9	2.2	3.1	2.8	2.7	3.1	2.9	2.9	2.9	2.8	2.6	2.8	3.3	2.4	
g	4.0	3.7	4.8	4.5	4.7	4.7	4.8	4.5	4.5	4.1	3.7	4.5	4.9	3.0	4.7	2.8	4.7	4.6	4.9	2.7	2.8	3.0	3.0	3.3	4.9	2.1	4.7	4.0	2.8	2.6	4.7	2.8) i
Entry	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163)))

	b	1814	vigII	triw	trup	livo	llivo	IHb	IIdb	shtľ	shtll	daf	plht	yld
165	4.7	3.3	2.9	58.1	9.1	83.7	94.0	80.8	89.0	16.6	27.8	76.9	185	16.9
90	2.1	2.6	1.8	85.6	19.7	79.2	93.1	61.4	79.3	17.1	32.4	77.6	161.3	20.0
167	3.1	2.6	2.6	61.2	6.5	85.2	93.3	82.3	88.7	15.1	28.0	81.8	205.6	18.5
89	4.2	2.2	2.2	12.9	3.2	83.9	93.5	78.9	88.5	15.1	29.9	79.3	216.4	22.4
69	3.9	2.4	1.6	40.1	1.9	83.3	91.9	69.4	82.9	16.4	31.5	75.5	148.3	15.3
170	3.6	2.4	1.3	44.5	6.6	80.9	93.0	70.2	87.4	16.9	31.5	75.9	158.1	12.9
11	5.0	3.1	2.8	16.0	3.7	88.4	94.3	84.7	92.3	15.0	28.2	84.2	197.6	20.3
12	4.7	3.1	2.3	86.1	16.6	84.3	93.7	82.4	88.3	15.6	28.2	79.3	173.8	19.7
13	4.0	2.4	1.6	39.0	3.8	83.6	94.0	76.2	86.4	17.3	32.8	6.77	196.1	19.6
74	4.7	- 2.4	2.2	43.1	3.7	85.4	93.5	79.9	89.4	15.9	31.0	79.2	190.5	16.5
75	33	3.1	1.6	26.3	3.1	79.2	92.6	69.4	81.8	i8.6	31.2	76.9	178	19.2
76	2.0	2.9	1.4	17.5	1.9	77.4	92.1	68.7	79.2	18.5	31.3	75.5	198.3	14.9
17	4.4	2.6	1.9	48.8	1.7	82.3	93.3	76.7	88.3	18.6	30.1	74.1	170.6	18.7
78	4.7	2.7	2.2	29.5	0.5	84.9	94.1	84.0	90.4	15.7	29.9	76.5	166.8	16.3
62	2.5	2.4	1.4	9.6	2.7	83.8	93.4	76.5	86.8	17.9	34.2	76.7	235.9	26.3
180	2.3	2.2	1.3	17.8	3.4	79.4	92.6	64.6	79.8	19.9	31.4	76.9	209.7	24.7
181	3.0	1.9	1.5	5.8	1.9	84.5	93.3	74.8	88.2	17.0	31.3	76.8	181	19.1
182	3.0	2.2	1.7	7.1	1.7	78.5	92.0	72.3	84.3	17.2	30.8	80.2	183.5	24.1
183	3.0	2.0	2.0	6.3	1.8	85.1	93.0	80.2	89.1	16.6	29.7	75.8	207.3	21.0
184	3.0	2.7	2.4	1.2	0.5	83.3	93.6	76.5	88.3	16.5	30.3	75.4	177.9	18.5
185	4.9	2.9	2.7	55.4	13.4	82.6	93.2	77.1	84.6	15.7	29.7	78.6	104.9	13.4
186	4.9	2.8	2.4	74.0	13.0	83.5	93.3	9 .77	88.9	15.2	31.5	75.5	123	12.4
187	4.3	3.0	2.2	5.7	2.5	84.7	93.6	78.1	86.2	17.9	30.8	78.5	193.2	24.8
188	4.9	2.7	2.2	33.4	9.8	83.7	93.1	81.6	87.6	16.2	30.0	77.3	193.2	21.1
189	2.8	2.6	1.6	4.6	1.4	79.2	92.8	73.6	84.8	17.9	32.9	75.2	229	17.2
061	2.8	2.9	1.4	25.6	3.5	82.8	92.5	63.3	84.7	19.0	33.6	76.2	193.6	19.3
191	4.7	2.4	2.2	10.3	2.3	83.0	92.7	74.9	86.6	16.0	32.8	77.1	191.2	12.7
192	4.6	2.9	2.2	43.3	6.0	83.7	93.0	75.2	85.1	15.9	30.7	72.9	169.5	15.0
193	4.0	2.6	2.1	52.9	1.5	84.7	93.2	73.0	85.0	16.8	31.4	78.5	207	21.0
194	2.8	2.4	1.6	40.8	9.5	84.8	93.1	70.1	85.9	17.7	33.3	77.2	208.9	21.1
195	2.5	2.0	1.5	43.5	4.8	82.0	93.2	73.2	86.3	17.6	30.4	80.9	224.7	21.7
196	2.4	2.8	1.5	1.1	1.0	75.3	92.2	64.0	84.3	17.0	32.6	80.8	206.3	25.4
197	3.8	2.9	1.5	47.7	6.9	76.2	91.7	61.7	82.3	18.1	31.3	76.6	155.7	13.4

																															28	50	
yld	21.4	17.4	18.1	19.5	15.9	16.5	24.2	18.2	16.6	15.5	10.7	17.2	20.4	16.3	17.9	16.9	26.5	23.1	27.2	17.0	16.2	16.7	20.3	17.3	20.2	14.4	22.6	23.5	19.3	16.1	21.0	22.7	17.8
plht	196.4	179.5	168.1	175.8	198.7	134	215.3	194.3	196.7	- 192.4	127	194	189.3	209.9	207.4	151	174.3	160.1	161.8	143.8	146	126.1	180.4	171.1	152.2	174.9	212.5	223	191.4	192.8	208.8	168.3	170.2
daf	80.3	76.6	78.6	82.6	75.7	75.4	80.0	79.6	76.7	79.4	73.7	78.4	80.1	79.4	76.0	79.4	83.2	1.77	78.3	78.3	77.0	77.3	79.3	78.0	78.6	75.3	77.6	80.7	76.6	80.9	77.9	77.4	76.8
shtII	28.9	31.0	31.3	29.3	33.0	32.4	32.6	31.6	31.2	31.8	31.4	30.7	30.0	- 32.1	32.2	30.2	30.2	32.1	29.5	29.9	32.2	30.8	28.7	30.9	29.6	30.3	30.2	31.5	29.1	29.3	35.6	29.9	27.5
shtl	16.6	16.8	16.6	15.2	17.6	18.8	19.5	18.7	16.5	18.4	16.1	16.9	16.0	16.7	18.4	18.2	16.0	16.9	16.3	16.3	15.8	17.0	17.5	16.4	16.2	16.8	15.7	17.6	16.7	15.8	18.0	16.9	15.6
IIHb	86.7	82.3	85.6	86.7	86.1	81.2	85.3	87.5	85.7	80.9	85.9	87.4	80.9	86.2	84.3	78.5	89.4	82.7	85.0	70.9	89.3	89.1	81.2	87.4	87.3	89.4	88.8	88.0	83.0	83.0	84.3	8.68	83.3
Idh	77.0	63.4	70.1	74.4	80.0	64.4	68.3	75.8	66.2	57.0	74.0	79.4	70.6	75.6	71.6	59.4	83.1	68.0	75.8	59.4	82.1	76.4	66.8	76.1	79.4	79.7	79.3	80.0	69.2	62.7	76.4	82.5	74.0
ovill	93.0	92.2	92.8	93.5	93.1	93.0	92.2	93.1	92.7	92.0	92.6	93.2	92.4	92.4	91.8	92.3	93.0	92.9	93.1	92.7	93.4	92.6	91.9	93.8	93.5	92.7	93.5	92.5	93.4	91.5	92.5	93.4	92.6
ovil	83.8	73.3	77.3	83.5	81.1	82.1	82.1	82.4	7.9.7	77.8	80.7	85.2	82.5	78.8	80.6	6.77	84.3	84.3	83.5	76.2	82.1	82.1	74.2	85.7	85.1	80.2	83.4	83.2	80.5	70.0	82.8	84.2	80.8
trup	6.8	6.3	8.3	15.9	2.4	7.6	1.6	4.8	5.2	18.0	3.6	2.5	8.4	23.6	10.2	24.4	6.9	4.1	10.4	4.8	2.5	8.6	13.9	11.0	8.4	0.4	2.0	3.4	3.7	5.8	1.6	2.5	2.0
trlw	38.9	48.8	45.6	52.8	19.5	45.4	4.9	46.3	51.3	66.0	34.0	42.0	44.8	64.0	51.0	85.5	33.3	30.0	77.6	44.4	30.1	32.5	51.0	74.3	31.0	39.8	29.3	10.5	45.1	37.8	3.3	19.0	49.7
vigII	2.0	2.0	2.3	2.5	2.3	1.6	1.7	1.8	1.7	1.3	2.0	1.9	2.2	1.5	1.6	1.6	2.4	1.8	2.3	1.8	1.9	1.6	2.3	1.8	2.6	2.0	1.4	1.9	2.0	2.3	1.7	2.5	2.5
vigI	2.8	2.6	2.6	3.2	2.2	2.4	2.2	2.4	2.6	2.7	3.0	2.6	3.3	3.1	2.2	2.9	2.6	2.8	3.1	2.7	3.1	2.9	4.0	2.6	3.3	1.8	2.7	2.7	2.9	3.5	1.8	3.1	3.7
gl	4.2	2.6	2.8	4.7	3.5	3.0	2.8	4.1	2.6	3.0	3.6	4.2	4.2	4.0	3.0	3.1	3.2	2.8	4.7	4.7	4.4	3.3	2.2	5.0	4.9	4.9	4.9	4.9	3.0	3.0	3.3	5.0	4 5
Entry	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	730

(contd)														
Entry	gl	vigI	vigII	triw	trup	ovil	ovill	ldh	IIub	shtl	shtll	daf	piht	yld
231	4.7	2.4	2.3	36.1	1.2	86.4	93.6	79.4	88.2	15.7	28.7	11.1	187.9	17.7
232	2.6	2.2	1.5	83.2	13.3	80.6	93.2	76.0	85.6	18.3	33.2	75.5	139.4	21.6
233	2.3	2.7	1.6	53.3	8.9	80.2	92.5	69.1	81.9	18.0	32.0	75.6	211.2	19.9
234	4.2	3.3	2.7	61.9	13.3	86.1	93.6	80.9	88.3	14.8	31.0	77.5	158.8	15.8
235	3.5	2.0	1.4	51.8	4.4	81.0	93.4	72.2	87.7	18.5	33.9	75.6	222.1	19.9
236	4.2	3.5	2.1	48.1	10.6	76.6	93.2	75.9	83.9	16.3	30.6	76.9	228.8	21.9
237	3.0	2.4	1.6	4.1	1.2	77.3	92.9	68.0	82.2	16.4	29.6	76.3	174.2	17.7
238	4.5	2.6	2.0	59.4	8.0	87.5	94.1	81.4	88.3	16.7	29.7	77.5	196.9	18.1
239	2.6	2.4	1.7	4.9	1.8	ררד	92.1	68.5	82.0	16.3	31.0	78.6	205.2	24.3
240	4.6	2.7	2.1	2.3	1.7	83.5	93.3	74.4	86.1	16.6	31.5	79.2	248.1	28.5
241	5.0	2.4	2.3	15.6	1.6	87.9	94.2	83.5	88.8	16.3	31.6	80.0	207.1	16.8
242	2.3	2.9	1.7	76.5	9.4	79.4	92.8	70.4	86.5	16.4	31.0	6.77	164.4	20.2
243	2.6	3.3	1.8	72.0	12.5	74.8	92.1	59.5	78.2	16.4	32.0	76.2	168.4	21.1
244	4.2	3.1	2.2	55.0	7.5	80.6	92.5	78.3	84.1	16.0	32.5	78.7	196.9	18.7
245	4.0	3.6	2.5	61.6	10.6	78.1	94.1	69.7	81.8	15.1	30.7	T.T.	189.1	25.7
246	4.3	2.9	2.0	24.1	3.6	81.9	92.9	7.9.7	85.9	17.1	30.9	76.0	155.8	15.1
247	4.1	2.8	2.2	45.8	8.0	83.5	93.0	77.3	87.7	15.5	28.8	76.2	159	14.4
248	5.0	2.6	2.4	45.2	2.6	87.6	94.3	78.9	90.0	17.1	30.5	76.4	166.7	23.4
249	4.9	2.6	2.0	40.7	3.6	78.6	93.5	74.7	83.4	16.9	30.4	75.7	152.6	15.3
250	4.6	2.8	2.0	12.6	2.2	82.3	92.8	78.2	85.9	15.3	29.7	81.6	178.1	20.9
251	4.6	2.4	2.1	21.0	1.3	82.1	92.5	77.3	87.7	16.0	30.9	77.8	184.6	23.3
252	1.6	2.1	1.3	97.9	19.4	81.1	92.7	68.5	79.4	17.5	34.3	74.8	188	15.3
BTx623	4.9	3.5	2.9	2.2	1.0	83.9	93.7	81.2	87.0	15.4	28.5	79.1	132.1	25.1
IS 18551	1.1	2.7	1.2	70.8	6.8	75.4	91.4	53.2	68.7	19.5	34.8	81.2	216.5	19.5
CSH 1	5.0	3.3	2.7	4.7	1.9	80.7	92.9	76.8	84.4	16.3	31.8	72.3	145	20.7
296B	4.7	4.0	2.8	2.4	0.6	82.9	92.5	71.2	82.4	14.6	29.1	78.7	140.5	18.8

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F	X150229	╞	k	K	k	E	K	L V	8	B	B	B	•	B	B	A B		<	•	B	B
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Appendix VIII. Genotyping data for 93 RILs (derived from the cross BTx623/IS 18551) screened with 44 SSR markers

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Genetic analysis and molecular mapping of components of resistance to shoot fly (*Atherigona soccata* Rond.) in sorghum [*Sorghum bicolor* (L.) Moench]

2002

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Present investigation was initiated to study the genetic architecture of host plant resistance to shoot fly and its component traits, and to identify markers linked to QTLs controlling these traits in relation to varying levels of shoot fly infestation in sorghum. Mapping population consisting of 252 RILs of cross between BTx623 (susceptible) and IS 18551 (resistant) was subjected to phenotypic evaluation under artificial infestation in three environments. A subset of this mapping population (93 RILs) was used for genome mapping with SSR markers. The two parents differed for phenotypic characters. Variances due to genotypes and G x E interaction were significant for the traits studied. Highly significant and negative correlation coefficients were observed between the shoot fly resistance traits (oviposion% and deadhearts%) and the component traits, such as, glossiness, trichome density and seedling vigour.

Continuous distribution of RILs suggested quantitative nature of the traits studied. The resistance traits recorded moderately high degree of variability and heritability. Glossiness and trichome density recorded consistently high degree of variability, heritability and Spearman's rank correlation coefficients, in individual and across the environments. Genetic analysis revealed their control by major loci. Predicted correlated genetic gain was high for shoot fly resistance when glossiness, trichome density and seedling vigour were used as indirect selection criteria. Transgressive segregants with phenotypic values outside the parental limits were observed for most of the traits, except for high intensity of glossiness and low deadhearts

Parental polymorphism with 96 SSR primer pairs showed 80% of these to be polymorphic, and 49% detected gel-scorable polymorphism. A genetic linkage map was constructed in which 23 markers were assigned to linkage groups (LGs) A, B, C, H and J. QTL analysis for single environment identified eight QTLs. One major QTL for glossiness (*Xtxp94-Xtxp65*) was detected on LG J, with high phenotypic variance explained ranging from 34.3 to 46.5% in individual and across the three screening environments. Minor QTLs for seedling vigour (three), seedling height (four) and grain yield (one) were also identified. Further the QTL identified for glossiness on LG J co-mapped with region associated with deadhearts (%) under high shoot fly pressure. The present study has assumed the feasibility of identifying QTLs for shoot fly resistance and pave the way for the use of marker assisted selection in breeding for shoot fly resistance.