GENETIC ARCHITECTURE OF SPOTTED STEM BORER RESISTANCE IN SORGHUM AS INFERRED FROM QTL MAPPING AND SYNTENY WITH THE MAIZE GENOME

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CERTIFICATE

This is to certify that this thesis entitled, **"Genetic architecture of spotted stem borer resistance in sorghum as inferred from QTL mapping and synteny with the maize genome"** submitted in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy (Agriculture) in the subject of Plant Breeding and Genetics to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research work carried out by **Mr. VINAYAN, M.T.** under my supervision and guidance and that no part of this thesis has been submitted for any other degree, diploma or other similar titles or prizes and that the work has not been published in scientific or popular journal or magazine.

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ABSTRACT

Abstract

Genetic architecture of spotted stem borer resistance in sorghum as inferred from QTL mapping and synteny with the maize genome

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The present investigation was carried out to characterize the genetic architecture of spotted stem borer resistance and related agronomic and morphological traits in sorghum. The experimental material for this study consisted of 266 RILs derived from a cross between susceptible parent ICSV 745 and resistant parent PB 15520. These RILs were evaluated for phenotypic traits during the 2007 and 2008 rainy seasons under artificial infestation of stem borer, and were genotyped with 90 polymorphic SSR markers for linkage map construction and QTL analysis.

The RILs exhibited wide variation for the observed traits across both of the screening environments. The mean performance of RILs for most of the spotted stem borer resistance except stem tunneling, neared the mid-parental value. Among the observed agronomic and morphological traits, except for testa (presence vs absence) and agronomic performance score, the means of RIL population were skewed towards that of one of the parents. High G×E interaction effects was observed in the RIL population progenies for all the resistance component, agronomic and morphological

traits except plant color score and seedling basal pigmentation score, which are largely controlled by single major genes. Across-environment, heritability estimates for the resistance component traits were low to moderate, while for most of the agronomic and morphological traits they were high. From the frequency distributions polygenic inheritance was inferred for most of the observed spotted stem borer resistance and agronomic traits. Presence of transgressive segregation for all the observed resistance component traits except recovery resistance score and all observed agronomic traits — seedling vigor, plant height, time to 50% flowering and number of nodes suggesting that favorable and unfavorable alleles for these traits are dispersed between the two RIL parental lines. A significant and positive association was observed among all the observed resistance component traits, except stem tunneling. Significant association was also observed between various agronomic and morphological traits with resistance component traits such as plant height with stem tunneling, deadheart incidence with time to 50% flowering, plant color with leaf damage score and overall resistance score and recovery resistance score with both plant height and number of nodes.

The basic linkage map constructed for this population with 266 RILs spanned 1289.4 cM representing all 10 sorghum chromosomes, with an average inter-marker distance of 14 cM across all linkage groups. This is optimum for an efficient QTL analysis provided that the markers are evenly distributed across the lengths of each of the linkage groups. Different subsets of QTLs in each environment were recorded for most of the resistance component traits, due to the major effects of screening environments on these traits. However, a cluster of stable putative QTL were detected at the distal end of SBI-07 (region between markers *Xisep0829* and *Xisep0704*), each accounting for large proportions of the observed phenotypic variation for these traits.

Among the morphological traits, major QTL explaining more than 20% of the total observed phenotypic variation were detected for seedling basal pigmentation score and plant color score on SBI-06 and for testa (presence vs absence) score, mesocarp thickness score and leaf angle on SBI-04. Six putative QTLs have been detected for time to 50% flowering and three for plant height. Three of the identified QTLs for time to 50% flowering are congruent with three of the six major sorghum maturity genes and two of the plant height QTLs correspond to two of the major dwarfing loci of sorghum.

In-silico comparison of genomic regions associated with stem borer resistance in maize and sorghum indicated that the genomic regions on the distal ends of SBI-07 (between markers *XSbAGB02* and *Xisep0829*), SBI-04 (between markers *Xtxp327* and *Xisp10229*) and on SBI-02 (between markers *Xisep0747* and *Xtxp025*) are orthologous between sorghum and maize.

Based on the phenotypic observations and QTLs detected for the resistance component traits, RILs 135, 108, 24, 93, 212, 47, 239, 35, 19, 69, 253, 250, 196 and 185 were found to harbor favorable alleles for most of the detected QTLs for spotted stem borer resistance. These RILs would hence be the good candidates for use as donors in marker-assisted breeding for stem borer resistance as many of them are agronomically elite than the original resistance donor parent PB 15520.

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INTRODUCTION

CHAPTER I

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench], the tropical plant belonging to the family *Poaceae*, tribe *Andropoganeae* and genus *Sorghum*, is the fifth most important cereal crop globally (Dicko *et al.,* 2006). The high adaptability of this crop to harsh environments, tolerance to drought and its capacity to produce reliable yield inspite of low input levels makes it the most appropriate cereal crop of the semi-arid regions of the world, which are home to more than 60% of the world's poorest population (Paterson *et al.,* 2009; Paterson, 2008). Sorghum is grown in more than 80 countries across the world with the largest area under cultivation in Africa (21.0 million ha), followed by Asia (10.0 million ha), the Americas (5.0 million ha) and Australia (0.6 million ha) (FAS, 2008). In India with its large population and fragile balance in the production-demand equation of food grains, sorghum plays a crucial role in national food security.

Prior to the year 1960, sorghum was cultivated in India under subsistence farming conditions, which was low in capital but rich in labor and relied on the use of traditional local dual-purpose varieties with some resistance to local insect pest and diseases, but having low grain yield potential. Attempts to increase the production of sorghum with the introduction of new high yielding varieties and hybrids since the year 1966, was largely unsuccessful because of the susceptibility of these cultivars to various biotic and abiotic stresses (Young and Teetes, 1977; Ajayi, 1989; Sharma *et al.,* 1993; Prem Kishore, 2001). The annual losses in sorghum due to biotic stresses are estimated at around $1 billion (Sharma, 2006) and infestation by insect pests has been reported to be the major component of biotic stress contributing to these immense losses.

Stem borers constitute the most widely distributed and serious group of insect pests on sorghum globally. Among these the spotted stem borer [*Chilo partellus* (Swinhoe)] is the most predominant species of Asia and Africa (Agrawal *et al.*, 1990). This pest can infest sorghum at any growth stage and is reported to have more than one generation per year. The spotted stem borer is also a major pest of tropical maize and pearl millet and has a wide host range, which includes several wild relatives of sorghum (Kumar, 1997; Haile and Hofsvang, 2001). Due to its high competitive ability, polyphagous nature and cryptic feeding behaviour, management of this pest has been very difficult (Kfir, 1997; Marulasiddesha *et al.*, 2007). Several management practices such as the use of pesticides, natural enemies, burning of crop residues, intercropping *etc.* have been reported to be effective in controlling this pest. However, use of these strategies would invariably increase the cost of cultivation of sorghum, which is not a feasible option for the resource poor farmers of the semi-arid tropics. Furthermore, the use of chemical pesticides could be harmful to both the environment and human health. Hence, the exploitation of host plant resistance is the only viable option both in terms of economic and environmental sustainability for controlling spotted stem borer in sorghum.

Large numbers of sorghum gemplasm lines have been identified showing considerable resistance to spotted stem borer. Most of these identified sources of resistance belong to the Durra group of sorghum (Prem Kishore, 2005). However, due to the poor agronomic performance of these lines, they were not introduced directly for cultivation and instead were used in various resistance breeding programmes. Breeding programmes to develop cultivars resistant to spotted stem borer were first initiated in India in 1966, and various conventional breeding procedures such as pedigree breeding, population improvement and heterosis breeding have been utilized

for the exploitation of resistance sources found in the germplasm (Singh and Rana, 1989). Although there have been some notable successes through conventional breeding approaches in improving plant resistance to spotted stem borer, the need for screening large number of lines and estimation of the damage parameters at different growth stages of the crop for selecting resistant plant types in the breeding process is not only laborious but also costly and time consuming. Further, the quantitative nature of resistance trait, low heritability and strong influence of environment (G × E) often complicates the selection process in the conventional breeding programmes.

Recently, the development of molecular markers and QTL analysis have offered plant breeders with a more efficient approach for studying quantitatively inherited traits and dissecting the polygenes into individual Mendelian factors for use in various breeding programmes through marker-assisted selection (MAS) (Paterson *et al.,* 1991). MAS is particularly important in resistance breeding programmes, because it would allow the selection for resistance without the need to use a resistance screen thereby saving time, resources and energy (Caetano-Anoles and Gresshoff, 1997).

The success of QTL analysis and MAS in other systems, and the availability of reasonably dense molecular marker systems in sorghum suggest that the use of molecular markers to map loci (QTLs) associated with spotted stem borer resistance traits, and to mobilize them through MAS in resistance breeding programmes would be effective. The high influence of environment on the spotted stem borer resistance traits is expected to hamper the accurate estimation of genotypic variances and location of QTLs. Hence, it is imperative to evaluate plants of the mapping population for these traits in replicated multiple environment trials, which can be accomplished primarily by using doubled haploid line (DH) or recombinant inbred line (RIL)

populations. Development of DH populations in sorghum is difficult due to the recalcitrant nature of the crop to regeneration, however the development of RIL populations is relatively easy in sorghum due to its high tolerance to inbreeding, compared to other often cross-pollinated crops. Being a set of random and homozygous products of several meiotic recombinations, a RIL population is not only useful for efficient phenotyping and accurate estimation of various genetic parameters but also greatly assists in the development of a high-resolution map with accurate map distances using both dominant or co-dominant marker systems with equal efficiency.

The significant progress made in the identification of various molecular marker technologies in sorghum including RFLPs, AFLPs, RAPDs, SSRs and most recently the DArT™ marker system has greatly facilitated the development of various medium and high-density sorghum linkage maps (Bhattramakki *et al.,* 2000; Menz *et al.,* 2002; Bowers *et al.,* 2003; Mace *et al.,* 2008, 2009) for comparative and QTL mapping studies. Among the different marker systems, at present SSRs are the most attractive markers for genotyping in sorghum because they are abundant, co-dominant, highly informative and display high levels of polymorphism. SSR markers are also immensely valuable in QTL mapping studies because the amenability of SSRs for automation greatly facilitates reliable and cost effective genotyping of large numbers of lines of a mapping population.

While several QTLs have been mapped in sorghum associated with agronomic traits (Ritter *et al.,* 2008; Brown *et al.,* 2006; Feltus *et al.,* 2006; Hart *et al.,* 2001; Rami *et al.,* 1998), abiotic stress tolerance (Kebede *et al.,* 2001; Sanchez *et al.,* 2002; Haussmann *et al.,* 2004; Feltus *et al.,* 2006), disease resistance (Boora *et al.,* 1998; Singh *et al.,* 2006; Klein *et al.,* 2001a; Reddy *et al.,* 2008) and insect resistance (Tao *et al.,* 2003; Nagaraj *et al.,* 2005; Wu and Huang, 2008) for utilization in crop

improvement programmes, the major determinants of sorghum spotted stem borer resistance have been sparingly subjected to QTL analysis. However, encouraging reports are available on mapping of putative QTLs for resistance to stalk borers in maize (the closest cultivated relative of sorghum) and their successful utilization in various resistance breeding programmes.

QTL analysis for spotted stem borer resistance in sorghum would not only enhance the opportunity of applying marker-assisted selection strategies in sorghum resistance breeding programmes but also help improve our understanding of the genetic and physiochemical mechanisms of plant defenses by permitting comparison of the QTLs for different resistance component traits. Further, such investigation would also help in determining the common genomic regions that contribute to resistance to stem borers in both sorghum and maize, and such loci could then be selected as good candidates for allele mining and linkage disequilibrium mapping studies in both of these species.

From the aforesaid views the present study was carried out with the following objectives:

1. To construct a genetic linkage map of sorghum using a RIL mapping population and a set of microsatellite markers.

2. To determine the number and chromosomal location of loci controlling spotted stem borer resistance through QTL analysis and validate the detected QTLs using sorghum-maize synteny.

3. To genetically map QTLs for various important agronomic and morphological traits that, differentiate the two parents of the mapping population.

4. To investigate the inheritance of spotted stem borer resistance traits and determine their association with other agronomic and morphological traits.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Sorghum [*Sorghum bicolor* (L.) Moench] is the most important source of food and nutrition to millions of people in the semi-arid regions of the world (House, 1996). Besides its use for direct human consumption and a source of feed grain and fodder, sorghum is emerging as the most important source of cellulosic biofuel (Wagoner, 1990; Hu *et al.,* 2003), which has compelling advantage over the grain-based form (Farrell *et al.,* 2006). Despite its growing importance, improvement of sorghum in terms of production has lagged behind that of major cereals, primarily due to its inherent susceptibility to various insect pests. Nearly 150 insect species infest this crop; however, less than 12 of them cause economically significant losses (Seshu Reddy and Davies, 1979; Jotwani *et al.,* 1980; Sharma *et al.,* 1993). The most destructive insect pests of this crop are the stem borers (Ingram, 1958; Ajayi, 1989; Kfir *et al.,* 2002) and they cause an economic loss of up to $ 334 million annually (Sharma, 2006). At least six species of lepidopteron stem borers infest sorghum in Africa (Haile and Hofsvang, 2002; Kfir *et al.,* 2002). In India this crop is infested mainly by two stem borer species, the pink borer (*Sesamia inferens*) and the spotted stem borer (*Chilo partellus* (Swinhoe)). The spotted stem borer is the most predominant species and can cause a colossal loss to both the yield and quality of sorghum produce (Sharma, 1993). Spotted stem borer resistance is a major concern in sorghum improvement programmes in Asia and Africa and breeding for resistance to this pest has been the subject of several previous studies.

The present study was aimed at utilizing recently developed molecular markers to map genomic regions associated with resistance to this pest, for utilization in spotted stem borer resistance breeding programmes. The literature pertaining to distribution, biology and control measures of this pest, with emphasis on the utility of

conventional and molecular approaches for breeding cultivars resistant to spotted stem borer, are reviewed here.

2.1. Spotted stem borer: an important yield reducer

The spotted stem borer is distributed across many countries in Africa and Asia such as India and Pakistan (Carl, 1962), Afghanistan, Botswana, Malawi and Sudan (Jepson, 1954), Bangladesh, China, Iraq, Japan, Nepal, Sri Lanka, Thailand and Uganda (Ingram, 1958), Kenya (Wheatley, 1961) and Indonesia (Young, 1970). This pest invaded the African continent from Asia, sometime before 1930 (Tams, 1932) and since then has emerged as one of the major pests of sorghum in South and Eastern Africa (Nye, 1960; Ingram, 1983; Seshu Reddy, 1983; Harris, 1990; Kfir, 1992, 1997; Kfir *et al.,* 2002). Overholt *et al.* (2000) predicted that climatic conditions of various regions of Southwestern and Western Africa are suitable for the growth and reproduction of *Chilo partellus* and hence this pest could soon distribute itself into these new regions, where at present they are not known to occur.

Spotted stem borer can cause grain yield losses up to 88% (Seshu Reddy, 1988) and forage yield losses up to 40% in sorghum (Verma and Singh, 2004). During severe infestations in early stages of crop growth, re-sowing can become inevitable (Trehan and Butani, 1949; Pradhan and Prasad, 1955). The infestation by the spotted stem borer also reduces the quality of sweet stalks and syrup due to the characteristic red color in the juice of sorghum stalks caused by the production of anthocyanins by plants as a reaction to the infestation by the pest (Rebe *et al.,* 2004).

2.2. Biology of spotted stem borer

The females of spotted stem borer lay up to 500 eggs in batches mostly on the basal surface of leaves. These hatch in 5 to 6 days. The first instar larvae moves to the leaf

whorl and feed on folded tender leaves resulting in leaf scarification and “shot holes” before reaching the growing point. Feeding by the larvae causes the death of the central shoot of the plant. This symptom is termed as “dead heart” and is typically observed on crops 20-40 days old. Sometimes “dead heart” symptoms can also occur in later stages of crop growth. The plant usually responds by producing new side tillers (Singh and Rana, 1989).

The older larvae move to the base of the plant and bore into the shoot causing stem tunneling. This greatly restricts the flow of vital nutrients to the growing tips of the plant, reducing the yield of both grain and fodder (Prem Kishore, 2000). Under favorable conditions the larval period is completed in 3 to 4 weeks; however, at low temperature the larvae usually undergo diapause (Sharma *et al.,* 1996). Kfir (1991) reported that in lab while simulating the environmental conditions, the larval diapause of *Chilo partellus* lasts for shorter periods and the emergence of adults lasts twice as long as *Busseola fusca* (another important sorghum stem borer of Africa).

Spotted stem borer not only infests sorghum but is also a major pest of other crop plants like tropical maize and pearl millet (Haile and Hofsvang, 2002). Several wild relatives of cultivated sorghum such as *Sorghum halepense, S. sudanense* and *S. verticiliflorum* are also known to serve as reservoirs of this pest. In a single season several overlapping generations of this pest are known to occur. In Northern parts of India, 3-4 generations occur on the annual sorghum crop with a larval diapause during the winter season, while in southern parts of India, where sorghum crop is grown throughout the year, 8-10 overlapping generations are known to occur (Singh and Rana, 1989), and in Uganda where no larval resting period has been reported, about 11 generations occur annually (Young, 1970). The polyphagous nature of *Chilo partellus* and the high number of generations per year are cited as the major reasons for the high

colonizing and competitive ability of this pest (Kfir, 1997; Ofomata *et al.,* 1999).

2.3. Management strategies to control spotted stem borer

The control of spotted stem borer is very difficult due to the nocturnal habits of the adult moths and the cryptic feeding behaviour of the larvae (Prem Kishore, 2000, 2001; Marulasiddesha *et al.,* 2007). Jotwani (1972) reported that grain yield loss due to spotted stem borer infestation could be reduced by up to 83%, by using appropriate management strategies. The different pest management strategies recommended for control are reviewed here along with their advantages and limitations.

2.3.1. Cultural control

The oldest and the most traditional method of controlling the borers are the use of cultural practices. These are considered as the first line of defense against insect pests (Dent, 1991). Wild host plants and crop residues are a major source of refuge for spotted stem borer larvae and pupae and hence provide an important “carry on” component of the pest population to the next cropping season. Destruction of wild host plants and complete burning (Harris, 1962; Ingram *et al.,* 1973; Ajayi, 1978; Unnithan and Seshu Reddy, 1989) or partial burning (Adesiyun and Ajeyi, 1980) of crop residue after harvest has been recommended to destroy the diapausing larvae residing in stalks and stem of sorghum. However, in many African farms, crop residue is the only organic matter or nutrient added to the soil by many farmers and burning sorghum stalks could drastically affect yield especially in farms where the organic content of soil is already very low (Van den berg *et al.,* 1998). In India, sorghum stalks are a major source of fodder for the livestock. Taley and Thakare (1980) suggested that chopping the stalks into small pieces before storing it for use as fodder could kill the diapausing larvae and substantially reduce or prevent the carry over of

the pest population to the next cropping season.

Intercropping and mixed cropping systems have also been reported to reduce the incidence of spotted stem borers (Kfir *et al.,* 2002). Pats *et al.* (1997) suggested the intercropping of cowpea with sorghum and maize to control this pest. Intercropping of legumes with sorghum (Prem Kishore, 2000) and agrisilvipastural system, where sorghum is grown with fast growing poplar trees (Babu *et al.,* 2001) has also been recommended as viable strategies to control spotted stem borer infestation.

Another important cultural practice followed, is the protection of main crop with field borders of trap crops. Napier grass and Sudan grass are effective in controlling spotted stem borers in sorghum fields (Khan *et al.,* 1997, 2000). While Napier grass has an effective antibiotic mechanism towards spotted stem borer larvae (Hutter, 1996), Sudan grass acts as a reservoir of many natural enemies of this pest (Khan *et al.,* 1997). The use of both intercrop (as a repellant of spotted stem borer) and trap crop (as an attractant of natural enemies of the pest) can also be very effective in controlling spotted stem borer (push-pull strategy) (Khan *et al.,* 2000). Kfir *et al.* (2002) suggested the use of Silverleaf desmodium and Molasses grass as effective intercrops and Napier and Sudan grass as effective trap crops for controlling this pest. However, for this strategy to be successful, the initial predator density in the field must be very high (Landis and Van der Werf, 1997; Ndemah *et al.,* 2002). Koji *et al.* (2007) recommended leaving strips of trap crop after the harvest of the main crop to support the predator population during the fallow season and hence increase their density in the next cropping season.

Lower rates of infestation by spotted stem borer has also been observed in sorghum fields with late sown crops (Rahman, 1944; Ahmed and Young, 1969; Van

Hamburg, 1979; Mote, 1986), low fertilizers (Starks *et al.,* 1971; Ajayi, 1990; Van den berg and Van Rensberg, 1991) and large spacing between plants (Ampong-Nyarko *et al.,* 1994).

Though all these strategies can be effective in controlling the pest population, the use of these methods could drastically affect the yield of sorghum (Nwanze *et al.,* 1995; Kfir *et al.,* 2002). Further these methods are not very feasible in the fields of resource poor farmers, who depend mainly on the climatic conditions for any decision regarding the package of practice followed for cultivation of the crop (Van den berg *et al.,* 1998).

2.3.2. Biological control

Biological control is an important component of insect management particularly in developing countries. This system is cheap, effective, non disruptive of the ecosystem and relatively permanent (Seshu Reddy, 1984). Parasitoids are a major constituent of biological control and numerous parasitoids have been reported to control stem borers. Mohyuddin and Greathead (1970) recommended the use of larval parsitoids *Cotesia flavipes*, *Apantelis chilonis* and *Bracon chinensis* to control spotted stem borer. Due to the known effectiveness of larval endoparasitoid *Cotesia flavipes* to various *Crambid* stem borers, primarily those in the genera *Chilo* and *Diatraea*, it has been introduced into more than 40 countries across the world (Polaszek and Walker, 1991). In the year 1993, this parasitoid was released in Kenya and has since established itself in this area to control the spotted stem borer population (Overhoult *et al.,* 1994). Zhou *et al.* (2001) studied the impact of this introduced parasitoid on spotted stem borer population in Kenya and reported that there was a reduction of up to 1.6 larvae per sorghum plant, which is equivalent to a 32-55% decrease of spotted stem borer density. Further, as there is no evidence of it having reached equilibrium

with the pest, the population of *Cotesia* is suggested to still rise and provide greater control of spotted stem borer in the future (Kfir *et al.,* 2002). The success of *Cotesia* has been attributed to its gregarious reproduction and ability to attack more than one host. Kfir *et al.* (2002) reported that parasitoids that exploit more than one hosts could establish well in new area compared to parasitoids with narrow host range due to constant availability of hosts. Another important factor responsible for the parasitoid establishment is the climatic factor (Duale and Nwanze, 1999). This could be one of the major reasons for the failure of control of spotted stem borer by this parasitoid in the temperate climates of South Africa (Skorosszewski and Van Hamburg, 1987; Kfir, 1994). Ingram (1983) reported that the egg parasitoid *Trichogramma* sp. could parasitize up to 60% of eggs of spotted stem borer, implicating its efficiency in controlling spotted stem borer population in field. However being an ecto-parasitoid there is a need for repeated monitoring and release in the field during the susceptible stage of the crop.

Predators like spiders (Sharma and Sarup, 1979), nematodes and pathogens like fungus *Fusarium aleyrodis* (Sinha and Prasad, 1975) can control spotted stem borer. However none of them have been reported to keep the population of spotted stem borers below the economic injury level (Odindo, 1990; Scovgard and Pats, 1996).

Use of sex pheromones is another important biological method of controlling the pest population. Sex pheromones are chemical messengers secreted by one sex of insects, usually females to attract the members of the opposite sex. This chemical can be used to trap the male insects in field using insect traps to effectively reduce the pest population. This is a relatively inexpensive method of insect control with negligible effect on plants and animals (Durant *et al.,* 1986). Spraying the field with sex

pheromones has also been recommended to control insect pest (Matthews, 1983). Synthetic sex pheromones for spotted stem borer are commercially available (Campion and Nesbitt, 1983); however, more research is required on trap designs to effectively use this method in field (Kfir *et al.,* 2002). Durant *et al.* (1986) suggested that though this method holds promise in small farms, its effectiveness has to be assessed in large on-farm trials.

2.3.3. Pesticidal control

Use of biological or cultural control measures has not been very effective in reducing the population of spotted stem borer. However, chemical pesticides have been found to effectively control the spotted stem borer larvae. Taneja and Nwanze (1989) reported that 57-100% of loss in grain yield could be avoided using chemical pesticides. Prem Kishore (2000) recommended the use of Endosulphan (4%), Carbaryl (5%) and Lindane (3%) to control spotted stem borers. Jotwani (1982) also reported the use of these insecticides to control the spotted stem borer population. Palta and Chauhan (2004) tested the efficiency of three insecticides and application methods in controlling this pest population and found that whorl application of Carbofuran (3G) was most effective. However, extensive use of chemical pesticides pose a threat to farming particularly in developing countries, because of their high cost, unrestricted availability, lack of adequate protection gear for user, absence of safety warnings, excessive and wasteful use leading to environment pollution, development of pest resistance and residues in food (Seshu Reddy, 1984).

Prem Kishore (2000) recommended the use of various eco-friendly strategies to control spotted stem borers such as the use of plant extracts of *Azardirachta indica*, *Annona squamosa*, *Acrorus calamus*, *Catharanthus roseus* and *Blumea eriantha*. Aqueous extract of Neem seed kernel (NSKE) has also been reported to be effective

in controlling *Chilo partellus* (Spurthi and Shekharappa, 2007)

Jose *et al.* (2008) tested the efficiency of various pesticides in controlling spotted stem borer in sweet sorghum and found that, among the biopesticides *Baccillus thuringensis* was most effective followed by NSKE in controlling the leaf damage by spotted stem borer. However, among all the pesticides, chemical pesticides Carbofuron 3G and Endosulphon 35EC were most effective in reducing the leaf damage in sorghum. Spraying of biopesticide mixed with chemical pesticides is an effective way to control spotted stem borer with least damage to environment. Sharma and Odak (1996) tested the efficiency of *Bacillus thuringensis* in combination with other insecticides and found that whorl application of *Bacillus thuringensis* mixed with Endosulphon 35EC gave maximum protection from spotted stem borers by causing a larval mortality of this pest by up to 91%. However, the production of biopesticides on large scale is not feasible and very difficult due to various issues related to their stability. Further, the efficiency of this system is highly influenced by various environmental factors.

2.3.4. Host plant resistance

The most valuable and practical solution to insect pests is the cultivation of resistant varieties, because effectiveness of all the other control measures is extremely restricted under severe infestations and further, the use of all other methods could cause an immense increase in the cost of cultivation of a crop. Importantly the deployment of insect resistant cultivars would drastically reduce the usage of chemical pesticides, which in turn would have a direct impact on human health by reducing environmental pollution and the levels of chemical residues in food and food products (Sharma *et al.,* 2006).

Plant resistance is particularly important to control spotted stem borers

because they infest the sorghum crop at all growth stages and also have more than one generation per cropping season. Hence, use of other control measures may not be a viable option in controlling this pest. The host plant resistance approach to spotted stem borer management in sorghum has very good potential both in terms of environmental sustainability and acceptance by small farmers (Nwanze *et al.,* 1995). Further, this approach is compatible with the other pest management strategies used for spotted stem borer control in sorghum.

2.4. Host plant resistant breeding strategies

Host plant resistance improvement in crop plants can play a major role in minimizing the extent of losses due to insect pests. Agrawal *et al.* (1990) opined that the effectiveness of any host plant resistant breeding programme largely depends on efficient screening techniques, reliable criteria for selection, identification of stable sources of resistance, knowledge on mechanisms of resistance, inheritance of the resistance and selection of appropriate breeding procedures for utilization of the identified resistance sources and screening techniques. Literatures pertaining to spotted stem borer resistance improvement strategies in sorghum are reviewed here.

2.4.1. Screening techniques

Development of pest resistant varieties begins with screening of large numbers of genotypes to identify sources of resistance. For the identification of sources of spotted stem borer resistance in sorghum, field screening under natural infestation is carried out in areas considered as “hot spots” for the pest or in other areas such that the susceptible stage of the crop coincides with the peak activity period of the insects. Higher infestation by spotted stem borer has been reported in the Kharif (rainy) than in Rabi (post rainy) season (Trehan and Butani, 1949; Firke and Kadam, 1978;

Dhumal, 1987; Singh and Rana, 1989). In India, most of the studies involving screening under natural infestation of sorghum have been carried out at Hisar, as it is considered the “hotspot” of the spotted stem borer population (Sharma *et al.,* 1983). Severe infestation by the spotted stem borer is recorded in this region on sorghum planted during first fortnight of July. Screening of germplasm under natural infestation is also carried out in other parts of India such as Delhi, Udaipur, Indore, Kanpur, Dharwad, Akola etc.

One of the major drawbacks of natural screening technique, which strictly restricts the reliability of the results, is the uneven infestation and distribution of spotted stem borer larvae in the field. In order to build up the pest population for an even infestation in the field Singh and Rana (1989) suggested, spreading the field with stalks and stubbles of sorghum containing diapausing larvae followed by irrigation for assisting in breaking the diapause and emergence of adult moths in the field. Uniform distribution of the pest can also be achieved through artificial infestation of plants with spotted stem borer larvae reared artificially on natural (Singh *et al.,* 1983) or synthetic diets in laboratories (Chatterji *et al.,* 1968; Dang *et al.,* 1970; Lakshminarayan and Soto, 1971; Seshu Reddy and Davies, 1979; Taneja and Leuschner, 1985). The most common methods of artificial infestation involve fixing of egg mass strips at black head stage on the abaxial surface of the top leaves (Dicke *et al.,* 1963), dropping them in leaf whorls (Jotwani, 1978) or mechanically dispersing the neonate larvae on each plant using bazooka applicator (Mihm *et al.,* 1978). An important factor in artificial screening for spotted stem borer is the growth stage of the crop at the time of infestation. A desired result in terms of reduction in plant growth and yield and increase in the incidence of “dead heart” symptoms was observed when plants were artificially infested with neonate larvae at 15 days after germination

(Dabrowski and Kidiavai, 1983; Taneja and Leuschner 1985), while at 20 days after germination it resulted in foliar damage and stem tunneling (Starks and Doggett, 1970).

Preliminary screening of large numbers of germplasm through artificial infestation is both labor and cost intensive. Pradhan (1971) recommended a three stage screening methodology for spotted stem borer resistance screening of germplasm. The first stage involves general screening carried out in single row plots under natural infestation. In the second stage, promising lines showing levels of infestation are selected from the first screening process and again evaluated in a multi-row replicated trial under natural infestation. In the third and final stage the resistance of these lines are confirmed in replicated trials under artificial infestation.

2.4.2. Selection criterion

Various damage variables have been suggested for selecting spotted stem borer resistant lines during the screening process, but ambiguity still exists concerning these parameters because of the differences in the feeding sites in relation to the growth stages of the plants infested by larvae. The most commonly used parameters for determining the sources of spotted stem borer resistance are leaf injury, dead heart incidence and stem tunneling.

Many studies have attempted to determine the most efficient parameter based on their association with yield loss. Leaf feeding is the first and the most pronounced symptom of damage by spotted stem borer in sorghum. A clear relationship between leaf injury and yield loss is observed under severe infestations of spotted stem borer (Brar 1972; Jotwani 1978; Alghali 1986; Prem Kishore, 1991b). However, Singh *et al.* (1983) failed to obtain any linear relationship between these two traits. These contradictory results may have been obtained because leaf-feeding scores vary over

time and depends mainly on the growth stage at which the plant was infested with the larvae. Plants usually recover from the leaf feeding damage by spotted stem borers under moderate infestation (especially in early stages of crop growth) by producing new leaves (Agrawal and Taneja, 1989).

"Dead heart" incidence was reported to be the most important trait in determining losses in grain yield (Singh *et al.,* 1968; Taneja and Leuschner, 1985). This trait was also found to be stable across many seasons in some high yielding temperate × tropical crosses and germplasm accessions (Taneja and Leuschner, 1985). Most researchers strongly argue that screening of germplasm must be based on dead heart incidence. This trait was the primary selection criterion for identifying sources of resistance for spotted stem borer while screening sorghum germplasm under All India Coordinated Sorghum Improvement Programme (AICSIP) up to the year 1968 (Singh *et al.,* 1968). However, under certain conditions such as moderate infestation in early stages of crop growth and severe infestation at later stages of crop growth, susceptible plants failed to produce dead heart symptoms (Prem Kishore, 1990). Hence, the primary criterion for selection later shifted to leaf feeding and tunneling (Taneja and Leuschner, 1985).

Prem Kishore (1991b) reported a significant negative association between stem tunneling and grain yield; the multiple regression analysis indicated a reduction of 0.59 units of grain weight with an increase of one unit of tunnel length in various sorghum germplasm. However, no significant association was observed between these traits in various other studies (Singh *et al.,* 1983; Pathak and Olela, 1983; Taneja and Leuschner, 1985). Agrawal and Taneja (1989) opined that this parameter could be associated with loss in grain yield especially under severe infestation at later stages of the crop growth, when the tunneling damage could cause breakage of stem or

peduncle and/or interfere with the nutrient supplies by destroying the vascular system of the plants.

Improved understanding of the association between these three selection parameters could also provide insight into the genetic mechanisms of these traits and could help in determining the most efficient criterion for selection. A positive association was observed between foliar damage and dead heart incidence (Sharma *et al.,* 2007), and foliar damage and stem tunneling (Jotwani, 1978) in some sorghum varieties. However, Rana and Murty (1971) reported a negative association between foliar damage and stem tunneling and no association has been observed between dead heart incidence and stem tunneling in various other studies (Singh *et al.,* 1983; Rana *et al.,* 1985). These studies suggest independent nature of these three measures of resistance, indicating a clear difference with respect to most of the genetic loci governing these parameters. Prem Kishore (1991a) opined that all the three parameters should be considered for determining the sources of resistance, as certain genotypes that show resistance in the early stages of crop growth may show susceptibility when infested at later stages of crop growth.

The timing and the exact location of attack are critical factors in determining the selection parameters for screening of germplasm towards their reaction to spotted stem borer (Davies and Seshu Reddy, 1980). Foliar damage should be assessed twice at the 3rd and 6th week after crop emergence under natural conditions, or at 7 days after artificial infestation. The degree of foliar damage is usually rated on a scale of 1-9 (Guthrie *et al.,* 1960; Dabrowski and Kidiavai, 1983). The lower number represents very little or no feeding and higher number indicates intense feeding (Starks and Doggett, 1970). “Dead heart” incidence counts must be estimated at 15 days after artificial infestation or on the 4th and 6th week of crop growth under natural infestation

(Singh and Rana, 1989; Prem Kishore, 1990). Tunneling must be measured at the time of maturity of the crop in both natural and artificial infestation (Starks and Doggett, 1970).

2.4.3. Sources of resistance

Presence of resistance to spotted stem borer in sorghum was first reported by Trehan and Butani (1949). Pant *et al.* (1961) observed that a few cultivars of sorghum were less preferred for feeding by spotted stem borers than others, confirming the presence of resistance in the sorghum germplasm. Various sources of resistance to spotted stem borer in sorghum have been identified in different studies; some of the promising lines are listed in Table 1. Systematic screening of world sorghum germplasm against spotted stem borer was initiated first in the year 1962 in India under AICSIP (Singh *et al.,* 1968; Jotwani, 1978; Prem Kishore, 1984; Prem Kishore *et al.,* 1988).

Under this programme a general screening of accessions under natural infestation was carried out at Delhi during the years 1964–1969. A total of 8557 lines were screened from which 1375 lines were selected for further testing. These lines were retested at Delhi, Udaipur and Pune under natural infestation; and of them 244 lines were selected for confirmation under artificial infestation at Delhi, Udaipur, Indore and Kanpur. This resulted in the identification of 104 resistant lines of which 49 were promising (Agrawal and Taneja, 1989). At ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), the screening process for spotted stem borer resistance began in the year 1979, using artificial infestation (Seshu Reddy and Davies, 1979). Initial screening of 16,000 germplasm accessions of sorghum at ICRISAT through 1985 resulted in the identification of 72 promising resistant genotypes. Most of these were of Indian origin; however, 8 of the accessions were

from Nigeria, 7 from USA, 5 from Sudan, 2 from Uganda and 1 each from East Germany, Ethiopia, Pakistan, YAR and Zimbabwe (Taneja and Leuschner, 1985).

Stability of resistance in the identified accessions across different locations or seasons is important for their efficient use in resistance breeding programmes. Only a few of the accessions showing resistance in India showed the same reaction to spotted stem borers in Kenya (Saxena, 1986; Pathak, 1990). Two lines IS 1044 and IS 1151 (Seshu Reddy, 1983) and IS 1044, IS 2205 and IS 12308 (Saxena, 1986) showed resistance in both Kenya and India. Rana *et al.* (1985) identified 13 germplasm lines showing resistance to spotted stem borer across 7 locations. The promising ones are IS 5538, IS 18551 and IS 18584. Three accessions IS 18584, IS 18577 and IS 2205 showed spotted stem borer resistance at both New Delhi and Hisar (Patel *et al.,* 1995). Taneja and Leuschner (1985) identified 68 IS accessions showing resistance to spotted stem borer across more than 5 seasons.

Development of varieties resistant to multiple insect pests is extremely essential in crops like sorghum as it is host to many different insect species, hence it is essential to identify sources that show cross resistance to more than one insect species. Seshu Reddy (1985) identified 18 germplasm accession showing resistance to the borer complex of *Chilo partellus*, *Busseola fusca*, *Eldana saccharina* and *Sesamia calamistis*. Agrawal and House (1982) identified three cultivars PS 21171, PS 21217 and PS 21318 exhibiting resistance to three major pests in sorghum the spotted stem borer, shoot fly and shoot bug. Resistance to shoot fly and spotted stem borer has also been identified in cultivars E 501, E 303, E 601, SPV-19 (Prem Kishore and Jotwani, 1982), PS 14913, PS 13827 and PB 8104-1 (Agrawal and House, 1982); E 201 (Prem Kishore, 1986); SPV 1015 (PGS-1) (Prem Kishore, 1992); IS 2205, PB 15438, ICSV 700 and MASV-33/93 (Singh and Shankar, 2000); SPV 1518, SPV

1572 and SPH 1148 and SPH 1280 (Prem Kishore, 2000); KC1, PGN-1, PGN-20 and PGN-64 (Prem Kishore, 2001); DS1 to DS 6 (Prem Kishore, 2005); and IS 18551 and FCR 15 (Sharma *et al.,* 2007).

The most common and important sources of resistance identified for the use in resistance breeding programmes are BP 53 (IS 1055), Aispuri, M 35-1, IS 4906, Karad local, IS 5837 and IS 1037. Most of these identified sources belong to the Durra group of sorghums with Indian origin followed by Caudatum, Conspicuum, Caffrorum, Dochna, Roxburgii, Cerenum and Nervosum-Kaoliang (Sharma, 1993). The tall, low yield, poor harvest index, late maturing, high lodging and photosensitive nature of the identified resistant sorghum lines (which are mostly of tropical origin), hinders their direct introduction and release for cultivation. Hence, these sources of resistance have been utilized in various breeding programmes for combining the resistance trait with other desirable agronomic features.

2.4.4. Mechanism of resistance

Resistance to insect pests by the host plant is mainly governed by three mechanisms — non-preference, antibiosis and tolerance (Painter, 1958). The plant is considered not preferred by the insect pest, if choice is available or if it repels the insect pest from oviposition, feeding and shelter (Blum, 1972). Gravid female moths of spotted stem borer preferred to lay more eggs on susceptible varieties than on resistant ones (Lal and Pant, 1980; Dabrowski and Kidiavai, 1983; Singh and Rana, 1984, 1989). However under no choice situations, as is the case of monoculture, this mechanism may not be a very effective line of defense.

Antibiosis results from the antibiotic effects of the hosts on the insect pests and is manifested by decreased size or weight, decreased lifespan, decreased fecundity and reproduction and increased mortality. The antibiotic effect of the hosts

is due to either toxic agents or lack of obligatory dietary factors. DIMBOA (2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)) in certain temperate cultivars of maize has an antibiotic effect on European corn borer. Presence of antibiosis effects on spotted stem borer larval survival and growth (Pant *et al.,* 1961; Kalode and Pant, 1966, 1967; Jotwani, 1978; Lal and Sukhani, 1982; Singh and Rana, 1984) and pupal development (Lal and Sukhani, 1982; Alghali and Saxena, 1988; Singh and Verma, 1988a; Taneja and Woodhead, 1989) have been reported in many spotted stem borer resistant sorghum cultivars. This mechanism is suggested to be the primary factor responsible for resistance to this pest in sorghum (Jotwani, 1978; Pathak and Olela, 1983; Singh and Rana, 1984; Sharma, 1993).

Tolerance enables the plant to produce reasonable yield in spite of its being infested heavily by an insect pest. Kalode and Pant (1966) were the first to notice a high degree of tolerance in a sorghum genotype M 35-1. Subsequently, many cultivars of sorghum have been reported to possess tolerance of spotted stem borer (Jotwani *et al.,* 1974; Jotwani, 1978; Dabrowski and Kidiavai, 1983; Singh and Rana, 1984). Gerloff and Ortman (1971) suggested that plant tolerance is markedly affected by various agronomic characteristics and environmental factors, which are very difficult to evaluate. Sharma and Nwanze (1997) suggested that recovery resistance score is the most effective index of tolerance to spotted stem borer in sorghum.

2.4.5. Traits associated with spotted stem borer resistance

Success of spotted stem borer larvae in infesting various genotypes varies with cultivars. Various factors appear to be responsible for this tendency including environmental effects (Bernays *et al.,* 1983), physical characteristics and chemical constituents of the plants (Woodhead and Taneja, 1987). However, no comprehensive study has been carried out to determine the bases of resistance to spotted stem borer in

sorghum. A few of the traits suggested to be associated with resistance to spotted stem borer in sorghum are detailed here.

2.4.5.1. Morphological factors

Morphological traits such as leaf angle (Kumar and Bhatnagar, 1962; Taneja and Woodhead, 1989), tightness of leaf sheath and midrib, diameter of leaf whorl and internodal length (Woodhead and Taneja, 1987; Taneja and Woodhead, 1989; Prem kishore, 1991a) all influence the dispersal of neonate larvae of spotted stem borer. Early panicle initiation and rapid internode elongation (Taneja and Woodhead, 1989; Sharma *et al.,* 2007) have also been reported to be associated with resistance to spotted stem borer. The length of tunnel formed by spotted stem borer larvae on sorghum stem was positively associated with plant height and nodes per plant but negatively associated with peduncle length (Singh and Rana, 1984).

Plant height, stem thickness and number of leaves were negatively associated with dead heart incidence and genotypes showing faster initiation of panicle were found to be resistant to stem borer (Khurana and Verma, 1985). Dwarfness, pithy stems and early flowering (Kumar and Bhatnagar, 1962) and glossiness (Sharma *et al.,* 2007) were found to be associated with resistance to dead heart incidence. Another important trait found to be associated with resistance to dead heart incidence is cuticular wax. Chapman *et al.* (1983) suggested that cuticular wax and ligule hairs help in trapping the young larvae, thus reducing their success in climbing and rate of establishment. Bernays *et al.* (1983) reported that larvae climbed faster on stems in certain genotypes after the removal of cuticular wax. This suggests the presence of certain feeding deterrents on the surface wax of stems that hinders the climbing of the larvae (Roome and Padgham, 1977; Woodhead *et al.,* 1980; Bernays *et al.,* 1985).

2.4.5.2. Bio-chemical factors

Presence of certain chemicals in plants such as high silica content (Narwal, 1973), low sugar content (Swarup and Chughale, 1962; Torto *et al.,* 1990), high levels of amino acids (Khurana and Verma, 1982, 1983), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignins (Khurana and Verma, 1982, 1983) are reported to be associated with spotted stem borer resistance.

Though various mechanisms and factors contribute to resistance, plant resistance to these insects is the collective effect of all the heritable characters by which a plant may reduce the probability of its successful utilization as a host by the spotted stem borer larvae. Hence all the above factors play a definitive role in determining the resistance to spotted stem borer in sorghum.

2.4.6. Inheritance of spotted stem borer resistance

Improved understanding of the genetic nature of resistance to the spotted stem borer could be an important factor in formulating a more effective breeding strategy. Resistance to spotted stem borer is suggested to be polygenic and partially dominant over susceptibility (Rana and Murty, 1971; Kulkarni and Murty, 1981; Pathak and Olela, 1983; Pathak, 1983, 1990; Rana *et al.,* 1984). Pathak (1985) reported partial dominance of susceptibility over resistance to spotted stem borer in tolerant × resistant and susceptible × tolerant sorghum crosses and complete dominance of resistance to spotted stem borer over susceptibility in resistant × susceptible sorghum crosses.

Additive and additive × additive gene action plays a major role in governing resistance to foliar damage (Rana and Murty, 1971; Hagi, 1984; Nour and Ali, 1998; Sharma *et al.,* 2007). Predominance of additive gene action has also been reported for dead heart incidence (Pathak and Olela, 1983; Nour and Ali, 1998; Sharma *et al.,*

2007). However, the role of both additive and dominance gene action in controlling this trait has been emphasized in various other studies (Hagi, 1984; Kulkarni and Murty, 1981; Pathak, 1990). Rana *et al.* (1985) reported the importance of non-additive gene effects in controlling this trait.

Nour and Ali (1998) suggested the role of additive gene action in controlling resistance to stem tunneling; while, the role of both additive and non-additive gene action was emphasized for this trait by Rana and Murty (1971). Hagi (1984) reported the importance of both additive and dominance gene action in controlling this trait under natural infestation and the predominance of epistasis in artificial infestation. Agrawal *et al.* (1990) also suggested the importance of epistasis in governing resistance to stem borer. The differences in gene action obtained between natural and artificial infestation indicates a differential expression of resistance mechanisms under these two conditions. This is expected as the non-preference resistance mechanisms can not be expressed under conditions of artificial infestation. From all these studies it could be inferred that resistance to leaf feeding is predominantly controlled by additive gene action, while both additive and non-additive (particularly dominance) gene effects are important for resistance to dead heart incidence. Stem tunneling resistance appears to be predominantly controlled by additive gene action in most cases, while in few cases non-additive gene action has also been reported.

2.4.7. Breeding approaches

Breeding programmes for resistance to spotted stem borer were first initiated in India in the year 1966 (Pradhan, 1971). The initial material for this breeding programme was obtained from large numbers of crosses between susceptible dwarf exotic temperate varieties and the identified tropical resistance sources. From these segregating materials, agronomically desirable lines combining moderate levels of

resistance with agronomic eliteness were selected and forwarded for varietal development by pedigree method and between - progeny selection (Tripathi *et al.,* 1976; Rana *et al.,* 1985). Continuous selection among the derivatives of these crosses over several generations is reported to have improved resistance to leaf feeding, dead heart incidence and stem tunneling by spotted stem borer (Prem Kishore, 2001). A few of the promising derivatives of continuous selection from various crosses are DU 291, U 37, U 218, U 35, DU 19, P 37, P 82, D 90, E 201, E 202 *etc.* (Agrawal and Taneja, 1989). However, most of these derivatives showed initial promise but later on lost the desired level of resistance. Promising varieties released following pedigree selection among the derivatives of temperate × tropical crosses under AICSIP are CSV 2, CSV 3, CSV 5, CSV 6, CSV 26 and SPV 9 (Agrawal *et al.,* 1990). A few of the promising improved lines bred at ICRISAT using pedigree method, showing considerable resistance to spotted stem borer are ICSV 700, ICSV 701, ICSV 825, ICSV 826, ICSV 827, ICSV 828 and ICSV 829. However, the low levels of resistance, and lack of persistence in stability of resistance over the years and low inheritance of resistance resulted in the lack luster performance of most of these varieties and failure of adoption within 2 to 3 years after their release (Prem Kishore, 2001). Development of varieties with both high resistance and high yield is very difficult as both the traits are polygenic in nature. Hence, with the objective of pooling genes for resistance and yield, continuous selection for both yield and resistance among derivatives of multiple crosses, and among progeny of high yielding intermediate derivatives of resistant × susceptible crosses and resistant sources has been recommended (Prem Kishore, 2005). Some of the varieties released following this method are P-217, P-219, P-297, P-467, P-471 and P-500. These varieties also shown resistance to spotted stem borer under both natural and artificial infestations.

To exploit heterosis for resistance to spotted stem borer large number of hybrids have also been developed, however, only CSH 9 and CSH 12 R have been successful. Starks and Doggett (1970) suggested that the most effective method of developing cultivars processing resistance to spotted stem borer must involve population improvement by recurrent selection. They suggested that the method should involve infestation of all plants of a composite or S_1 lines of a composite followed by recurrent selection. Pathak (1990) also suggested the use of S_1, S_2 and half-sib recurrent selection for improving resistance to spotted stem borer because of the predominance of additive gene action for most of the resistance component traits.

At ICRISAT while pedigree breeding is employed as a short-term approach, population breeding is used as a long-term approach for improving stem borer resistance in plants. A shoot pest resistant population (ICP 118) of sorghum has been developed at ICRISAT using male sterility genes *ms3* and *ms7*, with an objective of strengthening the sources of resistance. A total of 175 lines have been fed into this population including more than 85 stem borer resistant sources and their derivatives. Though six cycles of random mating followed by mass selection have been futile in improving the resistance component traits of this population, (Agrawal *et al.,* 1990), the use of S_2 recurrent has been found to be very successful. Some of the most promising progenies derived from this shoot pest population are PB 12342, PB 12346, PB 12380, PB 12387 and PB 12413. Some of these derived lines also show resistance to other major insect pests of sorghum such as shoot fly, midge etc.

Despite optimism about the continued improvement of sorghum through conventional breeding, sufficient levels of resistance that are stable across environments have not been achieved in varieties resistant to spotted stem borer till date, primarily due to the quantitative nature and poor heritability of the resistance

traits. Furthermore, the high influence of environment on these traits (G × E) hinders reliable phenotypic selection during the breeding process. This situation is exacerbated when a resistance component trait is tightly linked to undesirable genes, as very few individuals segregating favorably for these two traits could then be obtained for selection in the breeding population. As spotted stem borer infests all stages of crop growth and the resistance traits are often not distinguishable at seedling stage, it is necessary to grow the plant population up to the adult stage, hence increasing the time and cost required for development of resistant cultivars. In addition, it is difficult to undertake pyramiding of the large number of resistance genes, since phenotypic selection for additional genes/loci in presence of existing resistance gene could be very difficult. Therefore, it is imperative to develop tools and techniques that could overcome most of these drawbacks and accelerate the breeding process for the development of spotted stem borer resistant cultivars.

2.5. Molecular approaches for resistance breeding

The development of molecular techniques for genetic analysis has led to a great increase in the knowledge of genetics and understanding of the structure and behavior of genomes of various crop species. These technologies offer the possibility of making conventional breeding procedures more efficient by adopting a wide range of novel approaches (Ortiz, 1998; Huang *et al.,* 2002). The different approaches for improvement of resistance to insect pests in sorghum crop are reviewed here.

2.5.1. Transgenics

Transgenic technology offers breeders with access to a wide array of novel genes, which can be inserted into any cultivar for expression of the desired traits. This technology has been widely used for developing insect resistant varieties in various

crops (Sharma *et al.,* 2002), but is advancing slowly in sorghum. Very few reports on transgenic sorghum are available at present, which may probably be due to the crop being recalcitrant to regeneration (Emani *et al.* 2002). For conferring resistance to spotted stem borer the gene (*Cry 1Ac*) isolated from bacteria *Bacillus thuringenesis* responsible for the production of *δ*-endotoxin, a crystal protein that is antibiotic to the larvae of the insect pests, has been successfully introduced into a sorghum cultivar at ICRISAT (Girija Shankar *et al.,* 2005). However, very low expression of the protein was observed in the T_1 generation of the plants, leading to only partial tolerance to the spotted stem borer neonate larvae. Transgene silencing has been suggested as the major reason for the low expression of this introduced gene.

2.5.2. DNA markers as tools for resistant breeding

Another important application of molecular biology is the use of molecular markers for identifying and tracking the genes of interest (Tanksley *et al.,* 1989; Tanksley and McCouch, 1997) and chromosomal regions responsible for variation of quantitative traits that are referred to as quantitative trait loci (QTLs) (Paterson *et al.,* 1988; Tanksley, 1993). The potential value of genetic markers as selection aides has been known for over 85 years. However, it was not until the advent of DNA marker technology in the 1980s, that a large number of genetic markers could be generated to adequately follow the inheritance of important traits in some of the major crops. Molecular markers are the differences observed on the DNA nucleotide sequences of chromosomes of different individuals. These differences are referred to as DNA polymorphism, and they arise as a result of insertion, deletion, duplication and substitution of nucleotides (Yencho *et al.,* 2000). Molecular markers are not environmentally regulated and are unaffected by the conditions under which the plants are grown and are detectable (at least in theory) at all stages of crop growth.

Discovery of molecular markers has increased the efficiency of plant breeding procedure by changing the criterion of selection from phenotype to gene/s using molecular markers (marker-assisted selection/breeding). This technology has revolutionized the process of crop improvement particularly, the development of resistant cultivars by eliminating or reducing the need for field trials and making it possible to select for favorable alleles at the resistance loci indirectly with the help of linked markers (Mazur and Tingey, 1995).

This methodology has potential for improving the efficiency of spotted stem borer resistance breeding programmes in sorghum, because the polygenic nature of resistance component traits and strong influence of environment on their expression, combined with independent inheritance of resistance effecting during the different crop growth stages has made conventional breeding difficult. In addition, the high amenability of sorghum to inbreeding, its diploid nature and small genome size almost 1/3rd that of maize (Laurie and Bennett, 1985; Michaelson *et al.,* 1991), along with high levels of DNA polymorphism observed between species and manageable levels of DNA polymorphism within *Sorghum bicolor* itself (Paterson, 2008), all suggest the high suitability of sorghum crop to molecular marker analysis.

The success in identification of genomic regions associated with quantitative traits mainly depends upon five factors i) an efficient DNA marker system, ii) an appropriate mapping population iii) a genetic map with adequate number of reasonably uniformly spaced polymorphic markers iv) an effective QTL mapping procedure and v) an effective phenotypic protocol that can be used on a large number of progeny of the mapping population. Various literature pertaining to the first four factors along with few examples of QTLs identified in sorghum and QTLs identified particularly for stalk borer resistance in maize have been reviewed here.

2.5.2.1. Types of molecular markers

2.5.2.1.1. Restriction fragment length polymorphism (RFLP)

The best-known molecular markers are restriction fragment length polymorphism (RFLP) markers (Botstein *et al.,* 1980). These markers detect difference between individuals in the size of DNA fragments produced by cleaving DNA with the same restriction endonuclease. The development of RFLP markers revolutionized plant genome mapping, offering a new source of virtually unlimited numbers of co-dominant markers providing extensive genome coverage (Beckmann and Soller, 1983). This approach involves digesting DNA with restriction enzymes, separating the resultant DNA fragments by gel electrophoresis, blotting the fragments onto a filter and hybridizing probes to the separated fragments (Castagna *et al.,* 1994). Probes are obtained by either synthesizing specific regions of the genome using cloned DNA, or by testing clones from a random DNA library and selecting those that are single or low in copy. If two individuals differ for a particular restriction site that affects the size of the DNA fragment homologous to the probe, then the band revealed by the probe will appear at different locations in their respective autoradiographs. Variation in restriction sites can therefore be detected as restriction fragment length polymorphisms. Similarly, insertions or deletions between restriction sites will also generate fragment length polymorphisms (Beckmann and Soller, 1986).

This marker system was first used for the construction of linkage maps of maize and tomato (Helentjaris *et al.,* 1986) and since then it has found greatest application in mapping. RFLP markers have been used for the construction of linkage maps in sorghum using maize DNA probes (Hulbert *et al.,* 1990; Binelli *et al.,* 1992; Whitkus *et al.,* 1992) and specific sorghum probes (Berhan *et al.,* 1993; Chittenden *et al.,* 1994; Ragab *et al.,* 1994; Xu *et al.,* 2000). RFLP genetic maps have also been

used to identify QTLs for plant height and maturity (Lin *et al.,* 1995) and various agronomic, grain quality and productivity traits (Rami *et al.,* 1998) in sorghum.

However, genetic analysis using RFLP requires large amounts of DNA and the realization of a high-density genetic map is a long and time-consuming process (Rafalski and Tingey, 1993). Karp and Edwards (1997) added that this technique is also not suitable for some plant systems, where DNA extraction is problematic because of the presence of polyphenols or polysaccharides that complex with the DNA.

2.5.2.1.2. Amplified fragment length polymorphism (AFLP)

The amplified fragment length polymorphism (AFLP) technique developed by Zabeau and Vos (1993) is an alternative to RFLP. Abundant AFLP markers have been found in many plant species (Shan *et al.,* 1999) confirming their use in plant genetic studies The technique is based on selective PCR amplification of restriction fragments generated by specific restriction enzymes and oligonucleotide adapters of a few nucleotide bases. The PCR primers consist of a core sequence (part of the adapter), a restriction enzyme specific sequence and a number of selective nucleotides. Usually three selective nucleotides for each of the two primers are used for regular plant genomes, but for species with small or large genomes this number can be adjusted accordingly. The AFLP technique simultaneously generates fragments from many genomic sites (typically 50-100 fragments per reaction) that are separated by gel electrophoresis and generally yield highly informative fingerprinting profiles (Vos *et al.,* 1995).

Detection of AFLP markers requires less DNA and has a higher multiplex ratio than RFLP markers. DNA of any origin or complexity can be used with the number of restriction fragments detected in a complex genome being virtually

unlimited. Every AFLP reaction is useful, with large numbers of polymorphic bands being common (Mackill *et al.,* 1996). Additionally, the majority of AFLP fragment correspond to unique positions on the genome, and can be used as markers in both genetic and physical maps. AFLPs have the capacity to identify large numbers of loci, increasing their ability to detect polymorphism (Thomas *et al.,* 1995).

The distribution of AFLP markers within the plant genomes differs according to enzyme combinations used in the assays. Methylation insensitive enzymes include *EcoRI* and *MseI*; these generate fragments that tend to cluster in heterochromatin regions associated with the centromere and telomeres. Since expressed plant genes are generally hypomethylated, using methylation-sensitive enzymes in AFLP analysis can increase the possibility of identifying markers that are tightly linked to target genes (Boivin *et al.,* 1999). Methylation-sensitive enzyme combinations include *PstI* and *MspI* and the fragments generated by these enzymes are distributed more evenly throughout genomes (Castiglioni *et al.,* 1999; Young *et al.,* 1999).

This technique has been used to measure genetic distance between lines (Smith *et al.,* 1993), for DNA fingerprinting (Vos *et al.,* 1995) and for mapping (Becker *et al.,* 1995; Maheswaran *et al.,* 1997) in various crop plants. AFLP markers can also be integrated into RFLP-based maps to extend and provide marker coverage to chromosome regions where RFLP markers are poorly represented (Maheswaran *et al.,* 1997). Boivin *et al.* (1999) used the AFLP marker system to saturate a sorghum linkage map constructed by Doufour *et al.* (1996, 1997). Major features observed during the construction of this map were the localization of AFLP markers on sorghum linkage groups in heterochromatin-rich regions such as centromeres and telomeres and the extension of the linkage maps. Similar features of AFLP markers have also been observed in various other crops such as rice (Maheswaran *et al.,* 1997)

and barley (Becker *et al.,* 1995). Since their usage in sorghum map construction by Boivin *et al.* (1999), AFLP markers have been extensively used in sorghum linkage map construction in conjunction with various other marker systems to ensure a better coverage of the sorghum genome (Klein *et al.,* 2000; Haussmann *et al.,* 2002, 2004; Mace *et al.,* 2008)

Although the AFLP technique is powerful and reliable in identifying markers closely linked to genes of interest, it has some disadvantages for use in MAS and map-based cloning. The limitations to the large-scale, locus-specific application of AFLPs include their dominant mode of inheritance, the intensity of labor involved, and their high costs. Hence, prior to foreground selection of QTLs or genes flanked by AFLP markers, conversion of flanking or adjacent AFLP markers into sequence – specific polymerase chain reaction (PCR) markers is generally required for screening large breeding populations at low costs (Dussle *et al.,* 2002).

2.5.2.1.3. Simple sequence repeats

Microsatellites or simple sequence repeats (SSRs) are tandemly repeated motifs of 1-6 bases found in all prokaryotic and eukaryotic genomes. They are present in both plant and animal genomes and are usually characterized by a high degree of length polymorphism (Morgante and Oliver, 1993; Saghai Maroof *et al.,* 1984; Wang *et al.,* 1994; Rongwen *et al.,* 1995; Yang *et al.,* 1996; Zane *et al.,* 2002). However, the number and composition of microsatellite repeats differ in plants and animals. The frequency of repeats longer than 20 bp, has been estimated to occur every 33 kb in plants unlike mammals where it is found to occur every 6 kb (Wang *et al.,* 1994). In humans, AC or TC is a very common nucleotide repeat unit however in plants, AT is more common followed by AG or TC (Powell *et al.,* 1996). Condit and Hubbell (1991) were the first to report the presence of microsatellites or simple sequence

repeats (SSRs) in plants. However, conscientious searches for source of SSR markers on other crop plants started after Akkaya *et al.* (1992) reported length polymorphisms of SSRs in soybean.

The flanking sequences of SSRs in a genome are often unique, allowing primers to be designed that result in tagging of SSR markers on amplification, representing a single locus. The polymorphisms observed among SSRs are mainly due to slippage events during DNA replication. A majority of these allelic variations arise as a result of slip-strand mispairing (SSM). SSM involves denaturing and displacement of strands of the DNA duplex followed by mispairing of complementary bases at the site of an existing tandem repeat. When followed by replication or repair, this can lead to insertion or deletions of one or several of the short repeat units (Levinson and Gutman, 1987). Unequal crossing over can also generate tandem duplications in DNA, as well as insertion and deletion events in the sequences that flank the SSR regions (Grimaldi and Crouau-Ray, 1997).

Major attributes associated with SSRs are their abundance, co-dominant nature, high levels of variability and ease of use. These factors have made SSRs the "marker of choice" for genetic mapping, marker-assisted selection, phylogeny and population studies and for conservation, management and characterization of plant genetic resources (Lagercrantz *et al.,* 1993; Jarne and Lagoda, 1996; Goldstein and Schlöterer, 1999). Unlike other marker systems SSRs have also shown to provide highly reproducible results between laboratories (Jones *et al.,* 1997).

A linkage map of the human genome based on segregation analysis of 814 $(CA)_n$ microsatellite loci was first constructed by Weissenbach *et al.* (1992). However in plants, mapping with microsatellites markers has not reached this level of resolution so far (Weising *et al.,* 1998). Use of microsatellite variation for mapping

have been used in various crops such as in, rice (Zhao and Kochert, 1992, 1993), barley (Liu *et al.,* 1996; Dávila *et al.,* 2004; Becker and Heun, 1995), brassica (Kresovich *et al.,* 1995), soybean (Csanádi *et al.,* 2001), wheat (Roder *et al.,* 1998) and maize (Sharopova *et al.,* 2002). Kong *et al.* (2000) and Bhattramakki *et al.* (2000) suggested that the SSR markers available in sorghum are sufficiently polymorphic for extensive use in mapping and marker-assisted breeding methods.

Brown *et al.* (1996) were the first to report and characterize SSRs in sorghum and to develop SSR primer pairs using genebank SSR sequences and sequences of genomic clones in sorghum. Taramino *et al.* (1997) characterized 13 SSR markers in sorghum, 11 of them were di-nucleotide repeats and most of them had $(AG)_n$ repeat motifs. Further, 7 of these markers were integrated on to an RFLP linkage map developed by Pereira *et al.* (1994). Kong *et al.* (2000) developed 38 unique SSR primer pairs, the majority of which amplified $(AG/TC)_n$ repeat groups with an average of 22 repeats/locus and successfully integrated 31 of these markers on a framework of RFLP linkage map developed by Peng *et al.* (1999). Bhattramakki *et al.* (2000), were the first to characterize and use a large number of SSR markers for sorghum genome mapping, they isolated 313 primer sets for simple sequence repeats (SSRs) and amplified the targeted loci using 266 of these primer sets. Majority of these amplified loci belonged to $(AG/TC)_n$ and $(AC/TC)_n$ repeat groups. Of these, 116 SSR markers were integrated into a linkage map constructed using the $F_{6:8}$ recombinant inbred line population of a cross between BTx623 and IS 3620C developed by Peng *et al.* (1999). Schloss *et al.* (2002) isolated and amplified 60 SSR primer pairs derived from 69 RFLP probes, which had been mapped previously on a *Sorghum bicolor* × *Sorghum propinquum* F_2 mapping population (Chittenden *et al.,* 1994; Bowers *et al.,* 2000). A large number of the identified SSR markers contained

more than 2 repeat motifs (tri,- tetra- and hexa-nucleotide) and were equally informative as the di-nucleotide repeats implicating the efficiency of all types of SSR markers in sorghum genomic studies. SSR markers have recently being extensively used in sorghum for diversity analysis (Dean *et al.,* 1999; Grenier *et al.,* 2000; Ghebru *et al.,* 2002; Uptmoor *et al.,* 2003; Folkerstma *et al.,* 2005; Casa *et al.,* 2005), population studies (Dje *et al.,* 1999), construction of linkage maps (Klein *et al.,* 2001a; Agrama *et al.,* 2002; Haussmann *et al.,* 2002, 2004; Wu and Huang, 2007) and identification of QTLs for major agronomic and economically significant traits (Agrama *et al.,* 2002; Tao *et al.,* 2000, 2003).

Although microsatellites are always reported to be highly informative and locus specific (Powell *et al.,* 1996; Roder *et al.,* 1998; Song *et al.,* 2002), they have also several drawbacks, including the high cost and length of time required for their development. These are caused by the need to isolate them de novo for most crop species that are being examined for the first time (Zane *et al.,* 2002). Although the initial cost may be significant, once developed the cost of implementing these markers is greatly reduced. In addition, they are easily transferable between laboratories as the sequence information of primers can be distributed, allowing other research groups to generate their own primers.

2.5.2.1.4. Diversity array technology

Most of the current molecular marker technologies have many limitations, the majority of which are related to the high cost per assay and reliance on DNA sequence information. Diversity array technology (DArT™) is hybridization-based alternative to the majority of marker systems currently used and was developed to overcome these limitations (Mace *et al.,* 2008). DArT™ assays are independent of sequence information and offer high multiplexing levels enabling simultaneous typing

of several thousand loci per assay. The higher number of DArT™ markers generated in a single assay not only provides a precise estimate of genetic relationships among genotypes but also their even distribution over the genome offers real advantages for a range of molecular breeding and genomic applications. Further, as the assays are performed on a highly parallel and automated platform, the cost of data points is reduced by a great magnitude when compared to the many current gel-based or capillary-based technologies.

Being a dominant marker system, DArT™ markers generate whole genome fingerprints by scoring the presence versus absence of DNA fragments. This system was first developed in rice (Jaccoud *et al.,* 2001) and subsequently has been applied to many other plant species including barley (Wenzl *et al.,* 2006), cassava (Xia *et al.,* 2005), *Arabidopsis* (Wittenberg *et al.,* 2005), pigeonpea (Yang *et al.,* 2006) and wheat (Akbari *et al.,* 2006). In sorghum, Mace *et al.* (2008) has successfully developed and mapped 358 DArT™ markers on to 257 unique loci of a linkage map involving RIL population derived from a cross R931945-2-2 × IS 8525, demonstrating the utility of DArT markers for construction of medium density linkage maps in sorghum. The number of sorghum DArT markers have been subsequently expanded and used to develop a concensus linkage map for sorghum (Mace *et al.,* 2009)

2.5.2.2. Mapping population

An essential component for the construction of a linkage map and QTL analysis is the mapping population. Several different mapping populations can be used for the construction of linkage maps within a given species, with each population possessing its own strengths and weaknesses (McCouch and Doerge, 1995; Paterson, 1996, 2002). The mapping population sizes used for the construction of linkage map usually ranges from 50 to 250 individuals (Mohan *et al.,* 1997), but this is below the optimum

required for construction of an efficient linkage map. The commonly used populations for linkage map construction are the F_2, back cross, doubled haploid (DH), and recombinant inbred line (RIL) populations. These populations differ from each other in the time required for their generation, the purpose of the study and the amount of marker data required for an effective mapping. All mapping populations are derived by advancing the F_1 generation obtained from crossing two parents essentially differing in the trait(s) of interest. In a self-pollinated crop the parents are usually homozygous; however, in a cross-pollinated crop the mapping population can be obtained by crossing two heterozygous parents or by crossing a heterozygous parent and a haploid or a homozygous parent (Wu *et al.,* 1992). As sorghum is highly tolerant to inbreeding, the mapping populations are usually sets of RILs derived from a cross of homozygous parents. Various types of mapping populations used for construction of sorghum linkage maps have been detailed in Table 2.

2.5.2.2.1. F_2 population

F_2 mapping populations are derived from F_1 hybrids; they can be quickly developed and harbor all possible combinations of parental alleles (Lander *et al.,* 1987). This population is used to assess the specific effects of the heterozygous state at a certain locus and to estimate the digenic interactions for a trait. The interactions of higher order though present for a trait cannot be assessed using the available software packages. F_2 populations have been extensively used in sorghum for the construction of linkage maps for comparative studies and QTL analysis (Hulbert *et al.,* 1990; Binelli *et al.,* 1992; Whitkus *et al.,* 1992; Berhan *et al.,* 1993; Chittenden *et al.,* 1994; Xu *et al.,* 1994; Lin *et al.,* 1995; Pereira *et al.,* 1995; Taramino *et al.,* 1997; Ming *et al.,* 1998; Bowers *et al.,* 2000; Wu and Huang, 2007).

As each F_2 individual in the population has a different genotype no replication

or experimental design can be employed that can effectively control environmental influence. Hence, for quantitative traits having low heritability, the precision of QTL mapping using this population can be very low. To overcome this problem, evaluation of F_3 progenies derived from F_2 individuals is strongly recommended (Paterson, 1997). In this method the phenotype data is analyzed by replacing the values of F_2 individuals by their corresponding F_3 progeny means (Mather and Jinks, 1982; Zhang *et al.,* 2002, 2003). This design is called $F_{2:3}$ (F_2 derived F_3) design in plant genetics (Austin and Lee, 1996; Cockerham and Zhang, 1996; Fisch *et al.,* 1996; Jiang and Zeng, 1997; Chapman *et al.,* 2003; Zhang and Xu, 2004; Kao, 2006) and daughter or grand daughter design in animal genetics (Weller *et al.,* 1990). A major advantage of this method is that increasing the number of replicates during the phenotyping of the progeny can increase the family-based heritability of the trait. However, gains in precision are partly sacrificed using this method due to genetic heterogeneity of the F_3 families (Paterson, 1997). In addition, this population is also ephemeral similar to the F_2 mapping population.

2.5.2.2.2. Back cross (BC) population

Backcross populations are obtained by crossing the F_1 with one of the parents. This segregating population can be easily obtained and is very similar to the F_2 population in terms of its utilization, advantages and disadvantages except that information per data point (marker × individual) is only 50% of that in F_2 population, because recombination is detected in only one of the two gametes. This population can be advanced further for the development of near-isogenic lines (NILs). NILs are homozygous lines that differ for a small DNA fragment and are developed by repeated backcrossing of the segregating lines with a recurrent parent. NILs can aid in QTL analysis, but their use has been limited in crop plants because of the substantial

effort and time required for the development of appropriate genetic material. However, after identification of putative QTLs using different mapping populations, the association of a QTL with the flanking markers can be confirmed by examining the phenotype of NILs that differ for flanking marker genotype. The location of a QTL can also be narrowed to a smaller interval by evaluating a series of NILs that differ for overlapping regions of genome (Paterson *et al.,* 1990). In addition, NILs can also be used for characterizing the expression and function of specific QTLs.

Tanksley and Nelson (1996) suggested that if QTLs are identified in an advanced backcross population, one or two additional generations of marker-assisted selection of the progenies can lead to the development of NILs, this approach is ideal for combining QTL mapping experiments with subsequent studies to evaluate specific QTLs (Tanksley *et al.,* 1996). Tuinstra *et al.* (1997b) suggested the development of NILs contrasting at QTLs by selection within inbred families, where high level of homozygosity has not been achieved (heterogenous inbred family). The presence of QTLs for pre- and post-flowering drought tolerance in sorghum was confirmed by Tuinstra *et al.* (1998) by developing NILs that differed at three of the identified loci and then evaluating them in the field under pre flowering, post-flowering and non-drought conditions.

2.5.2.2.3. Doubled haploids (DH) population

A DH population is a permanent population that can be maintained indefinitely for phenotyping, genotyping and across QTL analysis studies. Doubled haploids are commonly used in plant species that are amenable to anther or microspore culture (from F_1 plants), and chromosome doubling. As in this population the plant has two identical homologues, the amount of recombinational information is exactly equivalent to a backcross. However, DH individuals are completely homozygous and

can be self-pollinated to produce large numbers of progeny, which are all genetically identical. This permits replicated testing of phenotypes and also facilitates distribution of identical DH populations to many different researchers.

A major disadvantage of DH population is that it is not possible to estimate dominance effects and related types of epistasis and the rates that pollens or microspores can be successfully turned into DH plants can vary with genotypes, thus causing segregation distortion and false linkage between some marker loci. DH populations has been extensively used for mapping in rice. One of the major limitations in using sorghum for development of doubled haploid populations is its recalcitrant nature for tissue culture and regeneration (Emani *et al.,* 2002).

2.5.2.2.4. Recombinant inbred lines (RILs)

Recombinant inbred lines are homozygous or 'permanent' populations that can be obtained by traditional means i.e., by selfing or sib-mating individuals for many generations starting from the F_2 and advancing by the single seed descent (SSD) approach until almost all of the segregating loci become homozygous which could take 6 to 8 generations.

Most genetic mapping studies in plants involve the use of RIL populations owing to several advantages of their use, including the possibility of reproduction, which favors the genetic analysis of quantitative traits because experiments can be replicated over years and locations; and the use of dominant marker types with the same efficiency as the co-dominant ones (Saliba-Colombi *et al.,* 2000). RILs are expected to provide an increased power of QTL detection compared to $F_{2:3}$ derive populations because of complete homozygosity at QTLs and marker loci (Moreno-Gonzalez, 1993). In sorghum RIL populations have been extensively used for construction of linkage maps and QTL mapping studies (Tuinstra *et al.,* 1996, 1997a;

Dufour *et al.,* 1997; Tao *et al.,* 1998ab; Boivin *et al.,* 1999; Peng *et al.,* 1999; Kong *et al.,* 2000; Tao *et al.,* 2000; Xu *et al.,* 2000; Bhattramakki *et al.,* 2000; Klein *et al.,* 2001a,b; Haussmann *et al.,* 2002; Agrama *et al.,* 2002; Tao *et al.,* 2003; Mace *et al.,* 2008). These studies have found a great number of QTLs even with small effects for various agronomic traits than observed using other type, of mapping populations. RILs also provide a better resolution of marker order in linkage maps because many generations of selfing or sib mating increases the chance of recombination events as compared to those using F_2, BC or DH populations. Random mating of the F_2 individuals prior to the development of a recombinant inbred line population following single seed descent method doubles the frequency of recombination, helping in the development of a more refined map.

Development of RIL populations requires a long time and consumes large space and costs. Further, it is not possible to obtain individuals homozygous at all the segregating loci through limited generations of selfing or sib mating. Another major drawback of RIL population is the inability to estimate the specific effect of heterozygous state at a certain locus. Crossing of the RIL population with testers and assessing the phenotypes of the offspring hybrids can be done to overcome this disadvantage. However, this approach would consume further time, labor and space and even then it would not be possible to assess precisely the effect due the interaction between the heterozygous locus and different genetic backgrounds.

2.5.2.3. Linkage maps

The molecular information of a crop genome is usually presented in the framework of genetic linkage maps that are useful to locate or tag genes of interest and facilitate marker-assisted selection (MAS) and map-based cloning. Linkage maps have also been used to study the conservation of gene order among various plant species (Devos

and Gale, 1997). Construction of a genetic linkage map is based on the observed recombination between marker loci in the segregating families, e.g. F_2 population, BC progenies, DH population or RIL population of the experimental cross. Markers are assigned to linkage groups using various computer programs like MAPMAKER/EXP (Lander *et al.,* 1987), Join Map (Stam, 1993), GMENDEL (Echt *et al.,* 1992) and Map Manager QTX (Manly *et al.,* 2001).

The assigning of the markers to the linkage groups is based on the ratio of probability of two loci being linked at a recombination frequency over the probability of two loci being not linked. This ratio is called the logarithm of odds (LOD) value or score (Risch, 1992). The critical LOD score usually used for establishing a linkage group is 3.0, which indicates 1000 times more likelihood of linkage between two markers than no linkage (Stam, 1993). Higher critical LOD values will result in larger numbers of fragmented linkage groups each with small numbers of markers, whereas a low critical LOD value will result in few linkage groups each with large numbers of markers. Ideally the number of linkage group obtained must be equal to the haploid chromosome number of the species under study. Difficulties associated with obtaining an equal number of linkage groups and chromosomes are primarily due to the non-random distribution of markers on chromosome (Paterson, 1996) and unequal frequency of recombination along chromosomes (Young, 1994; Hartl and Jones, 2001).

Distance along the linkage map is measured in terms of frequency of recombination between genetic markers (Paterson, 1996). Frequency of recombination and frequency of cross over are not linearly related (Hartl and Jones, 2001; Young, 1994), hence, mapping functions are required to convert recombination fractions into centimorgans (cM). The Haldane (1919) and Kosambi (1944) mapping

functions are commonly used for converting the recombination fractions to map units. The Haldane mapping function takes into account the occurrence of multiple crossovers while the Kosambi mapping function also accounts for interference, which is the phenomenon of one cross-over inhibiting the formation of another in its neighborhood (Ott, 1985).

The accuracy of measuring genetic distance and determining marker order is directly related to the number of individuals studied in a mapping population. Mohan *et al.* (1997) suggested the use of 50-200 individuals for construction of preliminary linkage maps, however, larger population size will be required for reliable high resolution mapping studies (Collard *et al.,* 2005), Vision *et al.* (2000) suggested the use of a two-pronged strategy for obtaining a saturated linkage map. First, is the development of a reliable high confidence framework and the second, is the addition of new markers to the framework. This strategy allows many markers to be placed on a well measured map with minimum genotyping and avoids loss in map resolution, which may result from arbitrarily reducing the size of mapping population.

With the discovery and abundance of molecular markers in plants, genetic maps nearly saturated with polymorphic molecular markers can be generated for almost any species. The linkage maps constructed in sorghum for comparative and QTL analyses are detailed in Table 2. The first DNA-based genetic linkage map of sorghum was developed using 37 maize RFLP probes screened on 55 F_2 plants of a cross between Shanqui Red and M91051 cultivars (Hulbert *et al.,* 1990). This map was constructed to initiate the development of detailed genetic maps in sorghum and compare genomic regions between maize and sorghum. Eight linkage groups were identified in this study with a total map distance of around 283 map units based on recombination frequency.

Most of these early RFLP genetic maps in sorghum used probes from maize (Binelli *et al.,* 1992; Whitkus *et al.,* 1992; Berhan *et al.,* 1993; Pereira *et al.,* 1994; Pereira and Lee, 1995) primarily for comparative studies. All these studies have reported the conservation of linkage and marker order between sorghum and maize with few rearrangements in the loci. Mapping exclusively with maize genomic probes as found in most of these studies may leave certain segments of chromosomes undetected and such gaps could be obstacles in developing a detailed RFLP map, hence for a better coverage of the sorghum genome, use of sorghum DNA clones along with other marker system has been recommended (Ragab *et al.,* 1994; Xu *et al.,* 1994). Many RFLP linkage maps constructed in sorghum have used probes mainly from sorghum (Chittenden *et al.,* 1994; Ragab *et al.,* 1994; Xu *et al.,* 1994; Lin *et al.,* 1995) as well as from other cereal crops such as sugarcane, barley and rice. Chittenden *et al.* (1994) were the first to develop an RFLP map mainly using sorghum DNA probes. This was also the first sorghum study based on a mapping population developed from interspecific cross (*S. bicolor* and *S. propinquum*), while most of the other maps are based on populations derived from intraspecific crosses. This map provided evidence supporting the ancestral duplication of chromosomes in the evolution of diploid sorghum. Another long-term objective of this study was the development of a high-density sorghum linkage map.

Tuinstra *et al.* (1996) were the first to use a RIL population for linkage map construction in sorghum. This study used mainly RAPD markers, which were mapped to 17 linkage groups spanning 1580 map units based on recombination frequency. Dufour *et al.* (1997) constructed a composite map from two RIL populations mainly using RFLP anchor probes from maize and sugarcane. This map was further saturated by addition of heterologous probes and AFLP markers by Boivin *et al.* (1999). With

the discovery of microsatellites in plants and their utilization for mapping, SSR markers have also been used in the construction of sorghum linkage maps (Taramino *et al.,* 1997; Tao *et al.,* 1998a, 2000, 2003; Kong *et al.,* 2000; Bhattramakki *et al.,* 2000; Haussmann *et al.,* 2002, 2004; Agrama *et al.,* 2004). Peng *et al.* (1999) developed a linkage map using 323 RFLP probes from maize oat and barley, a comparison of this map with maize linkage map revealed rearrangement of linkage groups and homeologies between sorghum and maize genome that were not reported in earlier studies. Kong *et al.* (2000) improved this map by 33 SSR loci. This map provided the most detailed map based on intraspecific cross of sorghum, after the addition of 116 SSR markers by Bhattramakki *et al.* (2000) and 2500 AFLP markers by Menz *et al.* (2002). Another, highly detailed map based on interspecific cross combination was developed by Bowers *et al.* (2003). This map is based on 2512 STS loci (RFLP) spread over 0.4 cM interval. Mace *et al.* (2008) has developed a medium density linkage map in sorghum using the new marker system DArT^{TM} along with AFLP marker system and 2 morphological markers; this map spans 1431.6 cM based on Kosambi map function. This was recently followed by development of a high density consensus linkage map of sorghum based on DArT^{TM} and SSR markers genotyped across progeny of 4 RIL populations (Mace *et al.,* 2009).

Though rapid advancement of molecular technology has facilitated the development of large number of linkage maps in sorghum and their successful comparison with other cereal crops like maize (Hulbert *et al.,* 1990; Binelli *et al.,* 1992), sugarcane (Doufour *et al.,* 1997; Ming *et al.,* 1998), rice (Ventelon *et al.,* 2001) for synteny and evolutionary studies, the critical issue that remains is the lack of correspondence between the information obtained from different maps of sorghum. For better organization and utilization of sorghum genome it is imperative to align

and integrate the maps from different studies.

The first attempt to compare and align several previous RFLP maps of sorghum was made by Tao *et al.* (1998a). However, only half of the linkage groups could be aligned due to the lack of sufficient common loci. More recently, with the availability of large-numbers of RFLP probes, many studies have successfully aligned various RFLP maps of sorghum (Peng *et al.,* 1999; Subudhi and Nguyen, 2000). Haussmann *et al.* (2002) successfully aligned various linkage maps of sorghum (Peng *et al.,* 1999; Bennetzen *et al.,* 2001; Bhattramakki *et al.,* 2000; Kong *et al.,* 2000) using a combined linkage map of two recombinant inbred lines developed with RFLP, AFLP, SSR and RAPD markers. The successful alignment of these maps has allowed selection of a large number of markers for any region of the sorghum genome with many potential applications in plant breeding.

One of the major drawbacks in aligning various linkage maps in earlier studies was the lack of a common nomenclature in designating the different linkage groups. Kim *et al.* (2005a,b) have successfully karyotyped the sorghum chromosomes using the FISH (Flourescent In-Situ Hybridization) technique and established a size-based nomenclature for the 10 sorghum chromosomes. This system is being presently used in most of the sorghum mapping studies. Feltus *et al.* (2006) aligned the two most detailed linkage maps of sorghum available; one based on an interspecific cross (Bowers *et al.,* 2003) and the second based on an intraspecific cross (Menz *et al.,* 2002) and used this nomenclature to construct a bridge map with 106 markers common to both maps. They were also able to map various common QTLs determining traits of agronomic and economic significance. This map is suggested to be immensely useful for comparative studies and identifying common genomic regions that frequently account for phenotypic variation of traits.

2.5.2.4. Mapping of Quantitative trait loci (QTL)

In crop plants QTL mapping has been a major area of genetic study for the past two decades because most traits of agriculture and economic significance such as yield, plant maturity, insect resistance and stress tolerance are governed by polygenes. A QTL is determined based on the association of markers and the phenotypically evaluated traits.

The earliest report on the association between morphological markers and quantitative traits was made by Sax (1923). He interpreted this association as a result of genetic linkage between a single gene governing the qualitative character (seed color) and polygene(s) controlling the quantitative trait (seed weight). Thoday (1961) suggested the use of single gene markers to systematically characterize and map polygenes controlling quantitative traits. However, the lack of availability of mapped monogeneic morphological markers and their large effects on the phenotype of quantitative traits have hindered the successful identification of polygenes and their effects (Tanksley, 1993).

With the development of molecular markers, mapping populations and efficient statistical techniques, it is now possible to accurately estimate the genetic location of QTLs and their effects. A number of methods for mapping QTLs and estimating their effects have been suggested and investigated (Edwards *et al.,* 1987; Haley and Knott, 1992; Jiang and Zeng, 1995; Lander and Botstein, 1989; Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). The most commonly used methods for QTL mapping are single marker analysis, simple interval mapping, composite interval mapping and multiple interval mapping.

Single marker analysis (SMA) is the simplest method of detecting QTL. In this method phenotypic means of the marker classes are compared using F-statistics,

linear regression or non-parametric tests (Sax, 1923; Soller *et al.,* 1976; Edwards *et al.,* 1987). Linear regression is most commonly used because the coefficient of determination (R^2) from the marker explains the phenotypic variation arising from the QTL linked to the marker (Collard *et al.,* 2005). This method does not require a complete linkage map and can be performed using basic statistical software. Map Manager QTX (Manly *et al.,* 2001) and Q gene (Nelson, 1997) are some of the software packages used for simple marker analysis. However, SMA often fails to provide a reliable estimate on the number and position of QTL (Mc Millan and Robertson, 1974; Lander and Botstein, 1989). Further, the magnitudes of QTL effects obtained are often underestimated (Tanksley, 1993) because SMA provides a single value confounding QTL effects with distance of QTL from a given marker.

Thoday (1961) provided the concept of simple interval mapping (SIM) and Lander and Botstein (1989) presented the mathematical treatment of this method. Instead of analyzing single markers as in SMA, the SIM makes use of linkage maps and analyzes intervals between adjacent pairs of markers along chromosomes simultaneously and to estimate the ratio of likelihood of a QTL in an interval to the likelihood of no QTL at that interval (LOD). This method is statistically more powerful than single point analysis (Liu, 1998). Software programmes like Mapmaker/QTL (Lincoln and Lander, 1990) and Q gene (Nelson, 1997) are usually used for QTL estimation by simple interval mapping. This approach is limited by its inability to test QTLs unlinked to markers and to accurately locate QTLs beyond the terminal markers of a given linkage group (Staub and Serquen, 1996). Composite interval mapping (CIM) was proposed as a solution to these limitations (Jansen and Stam, 1994; Utz and Melchinger, 1994; Zeng, 1994). In CIM the analysis is performed in the same way as performed in SIM except that the variances from other

QTLs are accounted for by including the partial regression coefficients from markers (Cofactors) in other regions of the genome. CIM is more effective at mapping QTLs compared to SMA and SIM, especially when unlinked QTLs are involved. Map Manager QTX (Manly *et al.,* 2001), QTL Cartographer (Basten *et al.,* 1994) and PlabQTL (Utz and Melchinger, 1996) have been used for CIM.

Multiple interval mapping (MIM) is another method that is considered as more powerful and robust than the other three methods of estimation. This method uses multiple marker intervals simultaneously to fit various putative QTLs directly into the model for mapping QTLs (Kao *et al.,* 1999). MIM is a very appropriate method for identification and estimation of genetic architecture parameters including number, genomic position, effects and interaction of significant QTLs and their contribution to genetic variances. QTL Cartographer (Basten *et al.,* 1994, 1997) is used for the estimation of QTLs using this method.

Regardless of the mapping approach or method followed for their estimation, detection of QTLs that are consistent over environments and generations (Lande and Thompson, 1990; Dijkhuizen, 1994) would contribute to their successful utilization in various breeding procedures.

2.5.2.4.1. QTL mapping studies in sorghum: some examples

Numerous studies have been undertaken to map genes and /or loci governing traits of agronomic and economic significance in sorghum. Major studies on mapping and identification of the genomic regions responsible for trait variation in sorghum are presented in Table 3 and a few of the studies have been reviewed here.

2.5.2.4.1.1. QTLs for agronomic traits and abiotic stress resistance

Paterson *et al.* (1995) first mapped the loci governing quantitative traits in sorghum.

This study was undertaken to map traits responsible for weediness i.e. rhizomatousness, tillering and regrowth in *S. halepense*. Since, *S. halepense* is an interspecific derivative of *Sorghum bicolor* (*S. bicolor*) and *S. propinquum* (*S. propinquum*), the mapping population of the study consisted of F_2 and BC_1 population derived from a cross between elite *S. bicolor* breeding line BTx623 and an unnamed wild accession of *S. propinquum*. This study mapped various major loci governing traits such as rhizomatousness, tillering and seedling growth on different linkage groups. One of the most important findings of this study was the mapping of a single gene locus for shattering on linkage group C (SBI-01). Of the four QTLs identified for tiller number in this study, the QTL located on LG D (SBI-06) partially overlapped the identified QTL for basal tiller number by Hart *et al.* (2001), indicating that these two regions may be orthologous.

An important quantitative trait in sorghum is plant height; this trait is reported to be largely controlled by four independent genes *Dw1*, *Dw2*, *Dw3* and *Dw4* (Quinby and Karper, 1954). Various molecular marker based studies have identified the genomic regions associated with plant height (Lin *et al.,* 1995; Pereira and Lee, 1995; Rami *et al.,* 1998; Klein *et al.,* 2001a; Hart *et al.,* 2001). Based on these studies *Dw1* and *Dw4* are reported to be associated on LG B (SBI-06) and E (SBI-08) (Pereira and Lee, 1995) where as, the gene *Dw2* and *Dw3* are located on LG E (SBI-07) and D (SBI-04), respectively (Klein *et al.,* 2001a). These identified regions were also found to be orthologous to regions on maize genome affecting plant height (Pereira and Lee, 1995). *Dw2* is reported to have pleiotropic effect on panicle length, main head yield, seed weight and leaf area (Graham and Lessman, 1966) and the *Dw3* locus is suggested to have pleiotropic effects on panicle length, kernel weight, tiller number and panicle size (Casady, 1965), while no pleiotropic effects has been observed for

the other two loci. Some other loci controlling plant height have also been reported to influence leaf characteristics of sorghum plants.

The trait maturity in sorghum is reported to be controlled by 4 genes, Ma_1 to Ma_6 in sorghum (Quinby and Karper, 1945; Rooney and Aydin, 1999). Of these Ma_1 is regulated specifically by photoperiod (Quinby, 1967) and a mutation on Ma_3 reduces the sensitivity of the plants to non-inductive day lengths suggesting its role in synthesis of phytochrome B. QTL studies have mapped these loci on different linkage groups of sorghum. Ma_1 has been mapped on LG D (SBI-06) (Lin *et al.,* 1995), Ma_3 on LG A (SBI-01) (Childs *et al.,* 1997) and Ma_4 on LG G (SBI-10) (Hart *et al.,* 2001). Some more loci have been identified affecting flowering time and are reported to be located on LG B (SBI-02) and G (SBI-09) (Lin *et al.,* 1998), LG G (SBI-01) and B (SBI-10) (Crasta *et al.,* 1999); LG F (SBI-09) (Hart *et al.,* 2001) and on LG C (SBI-06), F (SBI-04) and H (SBI-01) (Chantereau *et al.,* 2001). Few of the QTLs identified for maturity are also reported to effect plant height in sorghum (Lin *et al.,* 1995; Kapran and Axtell, 2000) indicating the association of plant height genes with maturity.

Tuinstra *et al.* (1996) identified around six regions in sthe orghum genome on LG D (SBI-07), LG F (SBI-01) and LG H (SBI-05) to be associated with yield, yield stability and seed set under pre-flowering drought stress alone implicative of their association with drought tolerance mechanism. In addition, eight genomic regions associated with yield and yield stability under irrigated conditions has also been identified, few of which showed coincidence with QTLs identified under drought stress. A similar study by Tuinstra *et al.* (1997a) identified around 13 loci responsible for post-flowering drought tolerance, two of which on LG F (SBI-01) and LG I (SBI-08) had major effects on yield and the stay-green trait under drought stress. These

regions were also found to be associated with yield under irrigated conditions indicating pleiotropic effect of these regions. Another important feature of this study was the identification of a QTL on LG E (SBI-10) associated with high rate and low duration of grain development. This region was also found to be associated positively to high seed weight and yield stability. However, one of the drawbacks of this QTL was its association with reduced yield indicating a yield penalty for this type of drought tolerance mechanism.

Tuinstra *et al.* (1998) confirmed the effects of the QTLs on LG F (SBI-01) and LG N (SBI-04), for drought tolerance by developing NILs for the identified QTLs and screening them in pre- and post-flowering drought stress. As the identified QTLs for pre- and post-flowering drought tolerance were mapped using RAPD markers, these regions could not be aligned to regions on other maps primarily due to the lack of common loci with other maps, which have used mainly SSR, RFLP or AFLP marker systems.

An important trait associated with drought tolerance in sorghum is the stay-green trait and many regions associated with this trait have been identified (Tao *et al.,* 2000). Of the identified QTLs, two QTL one on LG B (SBI-02) and one on LG I (SBI-10) were found to be consistent in multiple environments indicating their value in marker-assisted selection. Xu *et al.* (2000) identified four regions on the sorghum genome influencing the stay-green trait. Of these, QTLs on LG A (SBI-01) and LG D (SBI-04) were found to be consistent across environments. Further, it was found that regions on LG A, where the two stay-green QTLs were present, are also associated with genes responsible for key photosynthetic enzymes, heat shock protein and an absicissic acid (ABA) responsive gene indicating the importance of LG A in effecting drought tolerance and yield in sorghum.

Kebede *et al.* (2001) also identified nine QTLs for post-flowering drought stress tolerance on seven linkage groups of sorghum, of them the QTLs on LG A (SBI-03), LG G (SBI-01) and LG J (SBI-05) were consistent with those mapped in previous studies. Further, two QTLs one on LG A (SBI-03), and one on LG B (SBI-10) showed correspondence with the QTLs identified for stay-green traits on chromosome 8 and 9 of maize.

The bridge map developed by Feltus *et al.* (2006) between the two most highest density sorghum linkage maps using 106 common markers has also been used for mapping some of the common QTLs identified for different traits. This study is also important because, of the four QTL mapped for leaf senescence on this bridge map, position of two QTLs (on SBI-03 and 09) overlapped with the stay-green QTLs detected by Tao *et al.* (2000), suggesting the use of these regions for breeding for drought tolerance in sorghum.

Rami *et al.* (1998) identified various QTLs for grain quality using both SIM and CIM. This study has suggested the importance of LG F (SBI-02) for traits of grain quality such as amylose content, kernel texture and tannin content. The allele for tannin content as observed by presence or absence of testa (*B2/b2*) located on this linkage group has been reported to render resistance to various biotic factors such as grain mold, bird damage (Klein *et al.,* 2001a; Rami *et al.,* 1998) etc. This linkage group is also associated with another simply inherited morphological trait mesocarp thickness (*Z/z*) (Tao *et al.,* 1998a; Boivin *et al.,* 1999). These morphological markers may be used as a landmark for the linkage group. Some of the other important traits for which controlling regions have been identified are seed weight (Pereira *et al.,* 1995), panicle characteristics (Rami *et al.,* 1998; Hart *et al.,* 2001; Feltus *et al.,* 2006), leaf angle (Hart *et al.,* 2001), plant color (Klein *et al.,* 2001a), leaf morphology

(Feltus *et al.,* 2006), restorer genes Rf_1 (Klein *et al.,* 2001b) and Rf_4 (Wen *et al.,* 2002), cold tolerance (Ejeta *et al.,* 2000) and sugar content (Ritter *et al.,* 2008).

2.5.2.4.1.2. QTLs for biotic stress resistance

One of the important factors responsible for low production of sorghum is its inherent susceptibility to various biotic factors. Resistance to most of these factors has been quantitatively controlled and hence breeding for these traits has been very difficult. With the advent of molecular markers it has now become possible to map these regions and most efficiently exploit some of them in various resistance breeding programmes.

Oh *et al.* (1994) successfully tagged a major head smut resistance gene (*Shs*) on LG A (SBI-08), using two RFLP probes and one RAPD marker. Subsequently many authors have reported the linkage of markers with various disease resistance traits such as anthracnose (Boora *et al.,* 1998; Mehta, 2002), rust (Tao *et al.,* 1998b), downy mildew (Gowda *et al.,* 1995; Oh *et al.,* 1996), leaf blight (Boora *et al.,* 1999) and grain mold (Klein *et al.,* 2001a).

Striga is an important parasitic weed responsible for very low production in sorghum. One of the important striga resistant genes responsible for low production of a germination stimulant (*lgs*) has been successfully mapped on LG A (SBI-08) of sorghum. Ejeta *et al.* (2000) have identified six genomic regions responsible for resistance to *Striga hermonithica* and five QTLs for *Striga asiatica* through single marker analysis and interval mapping. Two of the identified QTLs were present on the same linkage group as the *lgs* gene implicating the importance of this chromosome in striga resistance. Haussmann *et al.* (2004) have also identified 9 and 11 QTLs for *Striga hermonithica* resistance on two different recombinant inbred line mapping population. Of them 5 QTLs were suggested to be excellent candidates for

MAS, as their effects had been validated across environments, years and independent RIL samples, however, they mapped the QTL for *lgs* on LG I (SBI-06).

Another major biotic constraint of sorghum is its susceptibility to various insect pests. Many reports are available on mapping of regions responsible for resistance to different insect pests in sorghum. Agrama *et al.* (2002) identified 9 genomic regions across—linkage groups associated with resistance to green bug biotypes I and K. Four SSR markers and one RAPD marker were found to be associated with biotype non-specific resistant genes. These markers could be successfully utilized in breeding programmes using MAS for the development of varieties resistant to all biotypes of green bug. Further, the QTL on LG J (SBI-05) was found to be consistent in another mapping population (Nagaraj *et al.,* 2005). Katsar *et al.* (2002) also identified nine QTLs associated with resistance to green bug biotypes I, C, E and K spread across 7 linkage groups; however, none of these QTLs conferred resistance to all four biotypes.

Sajjanar (2002) identified 27 QTLs for traits associated with resistance to shoot fly. Of these four QTLs responsible for deadheart resistance has been found tightly linked to various SSR markers, which can be used in marker-assisted selection. Deshpande (2005) carried out a similar study using RIL mapping population derived from a different parental cross combination. One of the important feature of this study was the identification of two QTLs one for trichome density on LG G (SBI-10) and other for glossiness on LG J (SBI-05) which are important traits associated with shoot fly resistance, in the same regions as identified in the previous study using a different mapping population, indicating this region to be good targets for MAS. QTLs for antixenosis and antibiosis for sorghum midge have been identified by Tao *et al.* (2003) on LG A (SBI-01), LG G (SBI-09) and LG J (SBI-07). They suggested the use

of markers linked to these QTLs for exploring new sources of midge resistance and resistance gene pyramiding.

Studies using molecular markers for identification of loci governing resistance in sorghum to spotted stem borer, have not been carried out till date. However in other cereals especially in maize, which is closely related to sorghum, such studies involving various stem borer species have been carried out widely. As much of the genomic regions of maize and sorghum are conserved (Paterson *et al.,* 2009), knowledge on these maize genomic regions responsible for resistance to various stem borers would be extremely beneficial in the present study for interpretation of the results. The QTL studies for stem borer resistance in maize have been detailed in Table 4 and reviewed here.

2.5.2.4.2. QTLs for stem borer resistance in maize

Lepidopterous stem borer larvae cause serious economic losses in maize the world over (Ortega *et al.,* 1980; Dicke and Guthrie, 1988). There are three major stalk borer species in maize, the European corn borer (ECB) (*Ostrinia nubilalis*) is a pest of maize grown in temperate regions whereas the sugarcane borer (SCB) (*Diatrea saccharalis*) and southwestern corn borer (SWCB) (*Diatrea grandiosella*) are found in tropical and subtropical maize producing areas (Bohn *et al.,* 1996). With the rapid advancement of molecular technology in maize, extensive research has been carried out for identifying regions responsible for resistance to temperate and tropical stem borers for use in marker-assisted breeding programmes. Some of these studies have also been conducted to validate certain assumptions on the genomic regions associated with resistance traits such as common genomic regions associated with the mechanisms of resistance for the three major stem borer species (Thome *et al.,* 1992) and association of the genomic regions for resistance with genes responsible for

production of DIMBOA (2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*) (Simcox and Weber, 1985), and/or protein, fibre and cell wall components (CWCs) such as phenol, ADF, NDF and lignins etc (Bergvinson *et al.,* 1996, Beeghly *et al.,* 1997; Ostander and Coors, 1997).

Conventional cytogenetic mapping methods like use of aneuploids and chromosomal translocation stocks have been used for mapping regions on chromosomes responsible for resistance to ECB (Ibrahim, 1954). Scott *et al.* (1966) used reciprocal interchanges in chromosomes and identified five major regions containing genes for resistance to European corn borer on short arms of chromosomes 1, 2 and 4 and on long arms of chromosomes 4 and 6. However, the number of regions identified for resistance in this study was probably an underestimate due to the inherent weaknesses of the method used. With the advent of molecular markers in maize focus shifted to the use of these technologies for identification of resistance genes or loci.

Schön *et al.* (1991) identified 4 QTLs for resistance to generation I of European corn borer (ECB I) (leaf feeding) on chromosomes 1, 4, 6 and 9 using 150 F_3 lines derived from a cross between Mo17 and H99. Seven regions contributing to variation in resistance to second generation of European corn borer (ECB II) (sheath and collar feeding and stem tunneling) on chromosomes 1 (2 QTLs), 2 (2 QTLs), 3, 7 and 10 were identified using F_3 lines of a cross between B73 and B52 (Schön *et al.,* 1993). This study was extended by Cardinal *et al.* (2001), who used the $F_{6:8}$ RIL population of the same cross and validated the effects of 2 QTLs on chromosomes 2 and 3 for resistance to ECB II. Consistency of QTLs across generations or between populations is highly essential for a successful marker-assisted breeding programme. Some of the QTLs of this study have also been mapped in a different population

segregating for resistance to ECB II, such as the QTLs on chromosomes 3 and 5 (Krakowsky *et al.,* 2002). The presence of the QTL for resistance to ECB II on chromosome 5 was also validated in another mapping population (Jampatong *et al.,* 2002). Another important finding of this study was that many of the loci for ECB II resistance were also associated with resistance to SWCB and SCB (Krakowsky *et al.,* 2004), providing a strong evidence of common genetic mechanisms for resistance to all three major species of maize stem borers.

Using F_3 families derived from three different crosses B73 × B52, B73 × DE811 and Mo17 × B52, Lee (1993) identified 16 major QTLs conferring resistance to ECB II. Lee and Veldbloom (1993) identified 2 major QTLs for ECB I on chromosomes 1 and 4, controlling 16 and 17 % of observed variation, respectively. Using 150 $F_{2:3}$ progeny of a cross between Mo17 and H99 Beavis *et al.* (1994) used topcross and F_4 families derived from 112 F_2 plants of a cross between B73 and Mo17 (the female parents of the crosses used by Schön *et al.,* 1991, 1993 for QTL analysis) and mapped three major QTLs affecting ECB II on chromosomes 7, 8 and 9. F_3 families derived from a cross between D06 and D408 were used for QTL analysis for ECB stem tunneling by Bohn *et al.* (2000). The study identified six major QTLs on chromosomes 1, 3, 5 (2 QTLs), 9 and 10 for this trait.

Jampatong *et al.* (2002) used 244 $F_{2:3}$ families of a cross B73Ht × Mo 47 and identified 9 and 7 QTLs, respectively for resistance to ECB I and ECB II; five of the identified QTLs were located at different positions on the genome indicating separate mechanisms of resistance to the two generation of ECB. A major QTL for ECB I mapped on to short arm of chromosome 4, near the region of *bx1* locus (required for the synthesis of DIMBOA), validating the role of DIMBOA for resistance to leaf feeding by temperate stem borers (Simcox and Weber, 1985). Many studies in maize

have suggested the non random distribution of insect and disease resistance genes. This study validated this concept as most of the QTLs identified were clustered around or between genomic regions where major disease resistance genes had already been mapped.

Another important concept is the association of cell wall components (CWCs) with ECB II resistance. This view had also been validated in many studies involving resistance to stem borer (Cardinal and Lee, 2005; Krakowsky *et al.,* 2004, 2005, 2006, 2007). In these studies many of the QTLs identified for ECB stalk tunneling were located at regions associated with production of various CWCs such as neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignins implicating a pleiotropic effect or tight linkage between the genes of these traits, which are also associated with variation in maize silage digestibility..

Though many studies have been carried out on resistance to temperate maize stem borers, few studies have been carried out for identification of genomic regions associated with resistance to tropical maize stem borers. The pioneering work was carried out by Bohn *et al.* (1996), who identified 10 QTLs on chromosomes 1, 2 (2 QTLs), 5 (2 QTLs), 7, 8, 9 (2 QTLs) and 10 on a mapping population of 171 F_3 families derived from a cross CML 131 × CML 67 contributing resistance to generation I of SCB (SCB I) (leaf feeding damage). The QTL on chromosome 2, first QTL on chromosome 5 and second QTL on chromosome 9 were mapped to genomic regions known to carry genes involved in cell wall biochemistry supporting the hypothesis of a major role of CWCs in the resistance to SCB.

Bohn *et al.* (1997) extended this study and used the same population for screening with first generation of southwestern corn borer (SWCB I) along with SCB I. A majority of the QTLs (7 out of 10) were found to be pleiotropic for resistance to

both the insect species. However, only 3 of these QTLs (2 on chromosome 5 and one on chromosome 9) were detected on concurrent genomic positions in another population formed from a cross between Ki3 and CML 139 (Khairallah *et al.,* 1998). Groh *et al.* (1998) further extended these studies by mapping leaf feeding damage QTLs for SWCB I and SCB I on $F_{6:8}$ and SWCB I on $F_{7:8}$ RIL populations of crosses CML 131 × CML 67 and Ki 3 × CML 139, respectively. Moderate consistency was observed among the QTLs for leaf feeding damage between SCB I and SWCB I between the RIL populations, an evidence of shared genetic bases of resistance to SCB I and SWCB I.

Sader and Weber (2002) attempted to map QTL contributing to SCB and SWCB resistance on the physical map of chromosome 9 using FISH technology. However, the interval between the markers flanking the QTL was found to encompass 70% of the physical length of chromosome indicating non-feasibility at present of isolating the genes by a map based-cloning approach. Hence, it was recommended to saturate the map further to obtain more markers closely linked to the QTL before attempting to clone the gene(s) responsible for this resistance QTL.

The inconsistency observed among the QTLs identified for resistance to the three species of stem borers across different screening environments and mapping populations as in most of the studies, limits the utility of these QTLs in marker-assisted breeding programmes. Though a few of the identified QTLs conferring resistance to the stem borers that were mapped using early generation mapping population showed significant dominance gene effects, most of them were governed by additive gene effects implicating the effectiveness of recurrent selection as a breeding method for improving resistance to stem borer.

2.5.2.4.2.1. Conclusions from stem borer resistance mapping studies

In the traditional models of quantitative genetics, simplifying assumptions are made about equality and strict additivity of gene effects (Falconer and Mackay, 1996). From the results of the QTL mapping experiments it is clear that these assumptions are not true. Further, a relatively small number of QTLs generally accounts for very large portions of phenotypic variance. Although this may be an artifact resulting from modest size of the mapping populations used in these studies, it clearly contradicts the assumption that large number of genes, each with small effects contribute to the expression of quantitative traits.

The proportion of the variation explained by each QTL and all QTLs together depends on heritability of the trait, as well as on the portion of revealed QTLs. QTLs are spread across the chromosomes; however, a few of the QTLs also seem to be clustered around some regions of chromosomes where genes controlling important traits are known to occur. Differences occur in QTL incidence (number, location and effects) between different mapping populations and/or generations and/or environments. However, comparative studies have revealed conservation of locations of some QTLs. A high agreement between QTL positions across mapping population generations is essential for a successful marker-assisted breeding strategy (MBS), because QTLs are usually identified in early generations and the flanking markers are then used for selecting lines during backcrossing or selfing.

Lack of consistency in identified QTLs across generations may be due to low power of detection of QTLs or due to biological reasons. According to Lande and Thomson (1990), low power of detection is a function of h^2 N, where h^2 refers to heritability of the trait and N refers to the mapping population sample size, a larger sample size would always increase the power of QTL mapping so long as heritability

of the observed trait can be maintained with larger number of test units.. While looking for consistency across generations (early –F_2 and late –RILs F_6 onwards), it has been reported in many studies that several QTLs would map to the same chromosome in $F_{2:3}$ population and in RIL, however their positions might differ because of the large confidence intervals of QTL positions. Hence, it is important to introgress large sections of chromosomes while using MBS based on early generation QTL mapping.

Another important factor to be considered in QTL analysis for insect resistance is the potential variability introduced with the biology of insect. The insect species, feeding habits are not only influenced by the crop but also influenced by environmental conditions, availability of alternate hosts and other biological factors. While it cannot be discerned which environment(s) best mimics the natural population, it would seem wise to pick markers linked to QTLs showing major effects and consistency across various screening environments.

Barton and Keightly (2002) opined that the actual number of loci and their effects on a quantitative trait could be very difficult to determine. QTL mapping studies can often underestimate the number of QTLs and overestimate their effects as QTLs can be undetected if they are closely linked to each other and have opposite effects because of the lack of recombinants or if the threshold limit for QTL detection is too small. They can also be overlooked when closely linked QTLs, with effects in the same direction, appear as a single QTL with a large effect. The effects of a statistically significant QTL are often overestimated particularly when the mapping population size is too small. Further, the detection of a QTL would depend on the quality of the genetic map, in particular the resolution of the map determined by the number of markers and the mapping population size.

2.5.2.4.2.2. Prospects of marker assisted selection (MAS)

The detection of relationships between genetic markers and QTLs is extremely valuable for building realistic models of phenotypic variation and response to selection in crop plants (Haley, 1991). The information derived from the map position of the traits and linked molecular markers paves way for the identification and introgression of genes into crop plants through marker-assisted selection (MAS) (Francia *et al.,* 2005). Koebner and Summers (2003) suggested that MAS would be essential in many practical breeding programmes where trait-based selection had proven to be inefficient and impractical, such as selection of single plants for a trait (or combination of traits) in situations where it is conventionally ineffective either because of environmental variation or because phenotypic-based assessment is difficult and expensive, or for traits that are under multigenic and/or recessive gene control and not readily amenable to phenotypic selection. Furthermore, MAS is most efficient, when traits show low heritability, and when a large fraction of the additive genetic variance is associated with the linked marker loci (Lande and Thompson, 1990). Collard and Mackill (2008) classified the application of MAS in plant breeding into five broad areas: i) evaluation of breeding materials, ii) marker-assisted backcrossing, iii) marker-assisted pyramiding of genes, iv) early generation marker-assisted selection and v) combined marker-assisted selection. In cereals, primarily marker-assisted back crossing (Joseph *et al.,* 2004; Toojinda *et al.,* 2005; Mackill *et al.,* 2006) and marker-assisted pyramiding (Sharma *et al.,* 2004; Jiang *et al.,* 2004; Werner *et al.,* 2005) have been successful in introgressing QTLs/genes showing resistance to various biotic and abiotic stresses.

Bohn *et al.* (2001) investigated the prospect of improving resistance to tropical stem borer (*Diatraea* sp.) in maize, based on QTLs identified in previous studies

through MAS and conventional selection procedures. They found that MAS alone was less efficient for improving this trait than phenotypic selection; however, there was a slight increase in the efficiency of improvement when MAS was combined with phenotypic selection. Willcox *et al.* (2002) used both MAS and phenotypic selection on three backcross generations of a population for improving resistance to tropical maize stem borers (*Diatraea* sp.) and found that though there was significant improvement in resistance to leaf feeding on the lines selected using both the methods compared to the susceptible parent, there was no difference in improvement between the lines selected through MAS and phenotypic selection procedures. However Flint-Garcia *et al.* (2003) observed that MAS was more efficient than conventional selection procedure in improving resistance to ECB II in maize, implicating the effectiveness of MAS in breeding for resistance to this pest in maize.

Several reasons have been cited for the elusive performance of MAS, which includes non-reliability and inaccuracy of QTL mapping (Young, 1999), insufficient linkage between marker and QTLs (Sharp *et al.,* 2001) and interaction of QTLs with background genome (Holland, 2001). Collard and Mackill (2008) suggested that for the potential of MAS to be realized, it is imperative that there be a greater integration of molecular marker technology with breeding programmes and current barriers to the use of MAS for practical breeding purposes must be well understood and appropriate solutions should be developed.

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The plant materials utilized in the present study were phenotyped at the Patancheru research station of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) research fields (Vertisol soils) and genotyped at the M.S. Swaminathan Applied Genomics Laboratory, ICRISAT-Patancheru with the broad objective of identifying genomic regions associated with resistance to spotted stem borer and various agronomic traits. The materials and methods utilized in the present study are detailed here.

3.1. Plant materials

The plant population for this study constituted of 266 $F_{9:10}$ recombinant inbred lines (RILs) developed from a cross ICSV 745 × PB 15520. The female parent ICSV 745 is susceptible to sorghum spotted stem borer and resistant to sorghum midge. In contrast, the male parent PB 15520 is resistant to spotted stem borer and susceptible to sorghum midge. The salient features and pedigree of the parental material has been presented in Table 5.

3.1.1. Development of mapping population

The set of 266 $F_{9:10}$ recombinant inbred lines used as the mapping population in the present study were developed at ICRISAT- Patancheru following "single seed descent" approach. The germplasm line ICSV 745 used as female parent in the development of the mapping population were pollinated with the pollens from the male parent PB 15520. The F_1 seeds obtained were advanced to the next generation by selfing of a single randomly selected F_1 plant. The resulting F_2 seeds were space planted and all the plants were selfed. The selfed seeds of the F_2 plants were harvested and grown separately in progeny rows. From each of the progeny row, a single plant

was selected and selfed for the development of the next generation of mapping population. The process of random selection and selfing continued up to F_9 generations. The seeds from the F_9 plants of each row were bulked to produce the 266 $F_{9:10}$ recombinant inbred line population. Each of the $F_{9:10}$ RIL would represent the single F_2 plant from which it has been derived.

3.2. Field Experiment

3.2.1. Experimental design and environment

The field experiments were conducted at ICRISAT- Patancheru research fields during the *kharif* seasons of 2007 and 2008. Each year the experiment was laid out in 28 ×10 alpha-lattice designs (Patterson and Williams, 1976) with three replications. Each of the 266 recombinant inbred lines, their parents (ICSV 745 and PB 15520; repeated 3 times per replication) and control entries (a susceptible control — ICSV 1 and a resistant control — IS 2205; repeated 4 times per replication) were planted in 2- row plots of 2m length. The rows were 60 cm apart and the plants were spaced at 15 cm within a row. All standard cultural practices were followed to raise a successful crop, except that no insecticide treatment was applied after the crop was infested with stem borer larvae.

3.2.2. Screening for spotted stem borer resistance

The natural population of spotted stem borer at ICRISAT-Patancheru research fields is low and irregular. Hence, for attaining a uniform infestation, the plant materials were artificially infested with spotted stem borer neonate larvae obtained from the Insect rearing laboratory at ICRISAT– Patancheru.

3.2.2.1. Mass rearing of spotted stem borer

Spotted stem borer larvae are reared on artificial diet in the laboratory. The

components of the diet are listed in Appendix 1 and the steps followed for preparation of diet and mass rearing are detailed here.

3.2.2.1.1. Diet preparation

1. Blend all ingredients of fraction A except sorghum leaf powder for 1minute.
2. Soak sorghum leaf powder in 2 L of warm water (70^{o}C) and blend with the ingredients of fraction A for 3 minutes.
3. Boil agar-agar in 1.6 L water and cool and to this add the blended components of fraction A.
4. Add formaldehyde (40%) and blend for 3 minutes
5. Heat the ingredients to 60^{o}C and pour 300g of this diet in different plastic jars

3.2.2.1.2. Mass rearing

1. Cool the diet to room temperature and transfer 100 sterilized black head stage egg of spotted stem borer into each of the jar and place the jar in dark for 2 days after which provide light for 12hrs daily.
2. Males emerge in the jar 25 to 26 days after inoculation and continue to emerge up to 35 days females emerge 2 to 3 days later and are larger in size than males.
3. 50 pairs of male and female moth are collected using a vacuum pump and transferred into an egg-laying cage.
4. The ovipositing cage consists of an open cylinder made up of a galvanized iron wire mesh closed at both the ends with a saucer covered with mosquito net. The iron wire mesh is wrapped with a georgette cloth with uniform holes at regular intervals, which is covered with a white butter paper at the time of moth release.
5. Female moths after mating lay eggs on the white butter paper through the holes on the georgette cloth.

6. The butter paper with egg masses attached to it are removed after 4 to 5 days of the release of moth in the cage and are incubated at 26±1^{o}C at high humidity (>80%) for 4 days for embryo to mature to black head stage.

3.2.2.2. Field infestation of spotted stem borer larvae

All the two week old seedlings were infested with the black head stage neonate larvae of spotted stem borer with the help of a bazooka applicator (Plate 1) which was developed at Centro Internacional de Mejoramiento de Maiz Y Trigo (CIMMYT), Mexico in the year 1976 (CIMMYT, 1977) for infesting maize plants with corn earworms. This instrument has been modified suitably to infest sorghum plants with spotted stem borer larvae.

For field infestation around 500 freshly hatched first instar larvae of spotted stem borer were mixed with 85 g of carrier — poppy seeds (*Papaver* sp.) and transferred into the plastic jar attached to the bazooka applicator with the help of a funnel. With the help of the bazooka applicator 14-16 first instar larvae were deposited in the whorls of each plant. To avoid larval mortality due to high temperatures, the infestation were carried out during early morning hours, and to ensure uniformity of larval distribution the bazooka applicator were gently agitated after every 10 strokes. Shoot fly infestation often interferes with the screening for resistance to spotted stem borer as they cause similar damage symptoms, hence to control the shoot fly infestation, Cypermethrin (a synthetic pyrethroid) was sprayed on the experimental plant material 1 week before artificial infestation using Electrodyne® sprayers

3.3. Phenotypic observations

Five spotted stem borer resistance component traits and eleven agronomic and

morphological traits were recorded on each entry of the RIL population in each of the two screening environments; the observations recorded are detailed here.

3.3.1. Spotted stem borer resistance component traits

3.3.1.1. Stem tunneling (cm)

This observation was recorded at maturity. The main stem of plants infested with spotted stem borer larvae were split open from the base to the apex and the tunnel length was measured in centimeters. The results are reported as tunnel length on stem per plant.

3.3.1.2. Deadheart incidence (%)

Data on number of plants with *Chilo partellus* dead hearts were recorded at 21 days after infestation for each entry. The number of deadheart is then expressed as percentage of the total number of plants for that entry.

$$\text{Deadheart incidence}(\%) = \frac{\text{Total number of plants with dead hearts in an entry}}{\text{Total number of plants in the entry}} \times 100$$

3.3.1.3. Leaf feeding damage (score)

Leaf feeding damage by *Chilo partellus* was assessed for each entry two weeks after artificial infestation. Each entry was scored on a 1-9 rating scale, based on the severity of injury on the leaves of the plant (Plate 2). The rating scale utilized for screening is detailed here

1 = No visible injury or small number of pin holes on few leaves. Leaf area damaged due to leaf feeding by stem borer is less than 150 mm^2.

2 = Small amount of shot holes on few leaves with an estimated damage of 150-300 mm^2 damage to the leaf area due to leaf feeding.

3 = Shot hole injury common on several leaves with 300-450 mm^2 of leaf area damage.

4 = Several leaves with shot holes and elongated lesions with a damage of 450-600mm^2 leaf area due to stem borer feeding.

5 = Several leaves with elongated lesions (<2.5 cm) with 600-750 mm^2 leaf area damage due to feeding by stem borer.

6 = Several leaves with long lesions (> 2.5 cm) with up to 900 mm^2 leaf area damage due to feeding by stem borer larvae.

7 = Long leisions on one- half of the leaves with leaf area damage of up to 1050 mm^2 due to stem borer feeding.

8 = Long leision on two- third of the leaves with leaf area damage of up to 1050 mm^2 due to stem borer feeding.

9 = Most of the leaves with long lesions with an leaf area damage of more than 1050 mm^2 due to feeding by stem borer larvae.

3.3.1.4. Recovery resistance (score)

Recovery resistance of an entry was assessed visually and scored on a rating scale of 1 to 9 at the time of maturity. The entries with more than 80% of damaged plants having 2 to 3 uniform tillers were scored as 1 and entries where less than 20% of plants had 2 to 3 uniform productive tiller were scored as 9.

3.3.1.5. Overall resistance (score)

Overall resistance score for each entry was assessed visually based on the overall performance of RILs to spotted stem borer damage parameters leaf feeding damage,

deadheart incidence and stem tunneling at maturity and scored on a rating scale of 1 to 9. Entries showing high level of resistance were scored as 1, while the entries with very low resistance were scored as 9.

3.3.2. Agronomic and morphological traits

3.3.2.1. Glossiness (score)

Intensity of glossiness on each entry were observed and scored on a scale of 1-5 at 14 days after emergence of seedling. This trait was observed in the morning when there was maximum reflection of light from the leaf surface. Entries with seedling showing pale green, shiny, narrow and erect leaves were scored as 1 and those with broad dull green and drooping leaves were scored as 5.

3.3.2.2. Seedling vigor (score)

Each entry was evaluated visually for seedling vigor based on the height, leaf growth and robustness of the seedlings, 12 days after germination and was scored on a rating scale of 1-5. Entries with seedlings showing maximum height, leaf expansion and robustness were scored as 1 and entry with seedlings showing poor growth, low leaf expansion and poor adaptation were scored as 5.

3.3.2.3. Seedling basal pigmentation (score)

Basal pigmentation of one-week old seedling was observed for each entry and scored on a rating scale of 1 to 3. Entries with seedlings showing no basal pigmentation were scored as 1, while the entries with pigmented base were scored as 3.

3.3.2.4. Plant color (score)

The color of the stem of the plants in each entry was observed at maturity and scored on a rating scale of 1 to 3, where entries with plants showing tan color were scored as 1 and entries with purple or dark red color were scored as 3.

3.3.2.5. Testa presence vs absence (score)

Absence or presence of a pigmented testa on the grains was visually assessed after removal of the mesocarp and scored as 1 and 2, respectively.

3.3.2.6. Mesocarp thickness (score)

The mesocarp was peeled from the grains of each entry and the thickness was assessed visually. This trait was scored on a rating scale of 1 to 3, where entries with thin mesocarp were scored as 1, while the RILs with grains having thick mesocarp were scored as 3.

3.3.2.7. Leaf angle (^{o})

The angle between the second leaf (leaf next to the flag leaf) and main stem was recorded using at time to 50% flowering on five random plants of each entry and their average was reported as the leaf angle for the entry.

3.3.2.8. Time to 50% flowering (days)

This trait was recorded as the number of days from the date of sowing to the date of anthesis in more than 50% of plants of a RIL.

3.3.2.9. Number of nodes (numbers)

Number of nodes were counted from the base of the stem to the point of initiation of panicle on five plants of each entry and were reported as the number of nodes per plant.

3.3.2.10. Plant height (cm)

Plant height was measured on five plants per entry in centimeter from ground level to the tip of the panicle of main stem and their average was reported as the height of the plant in cm.

3.3.2.11. Agronomic performance (score)

Overall agronomic performance of entries were assessed and rated on a scale of 1 to 5 at the time of agronomic maturity. Entries with plants showing poor yielding ability were scored as 1, while entries with a very high yielding capacity were rated as 5.

3.4. Molecular marker analysis

3.4.1. Laboratory procedures

3.4.1.1. DNA extraction

Around 30 selfed seeds from each of the 266 RIL and their parents were grown in pots in a green house. Bulk DNA was obtained from approximately 30 mg representative per RIL and parental line using CTAB method (Mace *et al.,* 2003) with slight modifications. DNA was further purified by RNase digestion followed by extraction with phenol:chloroform: isoamylalcohol (25:24:1) and ethanol precipitation as described by Mace *et al.* (2003). The reagents required for DNA extraction are listed in Appendix 2 and the adopted procedure for 96 well plate mini-DNA extraction are described here.

A) Preparation

1. Steel balls (4 mm in diameter and 2 numbers per extraction tube), pre-chilled at –20°C for about 30 minutes, were added to the 12×8 well strip extraction tubes with strip caps (Marsh Biomarket, USA) that were kept on ice.
2. Before starting DNA extraction, 3% CTAB buffer was preheated at 65°C in a water bath (Precision Scientific model: shaking water bath 50).
3. Leaf blades of six inches size were collected from 10 one week-old seedlings. These leaves were cut into small pieces and these pieces (approximately 30 mg) were then transferred to extraction tubes that were fitted in a box.

B) Grinding and Extraction

1. 450 µl of preheated 3% CTAB buffer was added to each extraction tube containing leaf sample.
2. Grinding was carried out using a Sigma Geno-Grinder (Spex Certiprep, USA) at 500 strokes/minute for 2 minutes.
3. Grinding was repeated until the color of the solution became pale green and leaf strip pieces were sufficiently macerated. After the first round of grinding, the boxes were checked for leakage by taking them out from the Geno-Grinder and were shaken for proper mixing of leaf tissue with buffer.
4. After grinding, the box with the tubes was fixed in a locking device and incubated at 65°C in a water bath for 10 minutes with occasional shaking.

C) Solvent Extraction

1. 450 µl of chloroform:isoamyl alcohol (24:1) mixture was added to each tube, tubes were inverted twice and the samples were centrifuged at 6200 rpm for 10 minutes (Sigma Laboratory Centrifuge 4K15C with QIAGEN rotor model NR09100:2×120 g).
2. After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube (Marsh Biomarket).

D) Initial DNA Precipitation

1. To each tube containing aqueous layer 0.7 volume (approximately 210 µl) of cold (kept at –20°C) Isopropanol was added. The solution was carefully mixed and the tubes were kept at –20°C for 10 minutes.
2. The samples were centrifuged in a centrifuge (same as earlier) at 6200 rpm for 15 minutes.
3. The supernatant was decanted under the fume hood and pellets were dried.

E) RNase A Treatment

1. In order to remove co-isolated RNA; pellets were dissolved into 200 µl of low salt $T_1E_{0.1}$ buffer and 3 µl of RNase A.
2. The solution was incubated at 37°C for 30 minutes or overnight at room temperature.

F) Solvent Extraction

1. After incubation, 200 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each tube, mixed and centrifuged (same as earlier) at 5000 rpm for 10 minutes.
2. The aqueous in each tube was transferred to a fresh tube (Marsh Biomarket) and 200 µl of chloroform:isoamyl alcohol (24:1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes (same as earlier).
3. The aqueous layer was transferred to fresh tube (Marsh Biomaket).

G) DNA Precipitation

1. 15 µl (approximately $1/10^{th}$ volume) of 3 M sodium acetate (pH 5.2) and 300 µl (2 volumes) of absolute ethanol (kept at –20°C) were added to each of the tubes and the mixtures were subsequently incubated in a freezer (–20°C) for 5 minutes.
2. Following the incubation at –20°C the tubes were centrifuged (same as earlier) at 6200 rpm for 15 minutes.

H) Ethanol Wash

1. After centrifugation, the supernatant was carefully decanted from each tube in order to ensure that the pellet remains inside the tube.
2. Subsequently, 200 µl of 70% ethanol was added to each of the tubes and this was followed by centrifugation (same as earlier) at 5000 rpm for 5 minutes.

I) Final Re-suspension

1. The supernatant was carefully decanted and pellet was allowed to air dry for one hour.
2. Dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and kept overnight at room temperature to dissolve completely.
3. The resuspended DNA samples were stored at 4°C.

3.4.1.1.1. Quantification and normalization of DNA

The quality of DNA in each sample was checked using 0.8% agarose gels stained with ethidium bromide. The reagents required for the preparation of agarose gel are listed in Appendix 3. For the quality check of the extracted DNA, each well of the agarose gel was loaded with 5 µl of sample (3 µl distilled water + 1 µl Orange dye + 1 µl DNA sample) and the gel was allowed to run at 100 V for 5 minutes. After completing the electrophoresis run, DNA banding patterns on the gel were visualized under UV light. A smear of DNA indicated poor quality whereas a clear band indicated good quality DNA. Samples of poor quality DNA were re-extracted.

The quantity of DNA in each experimental sample was assessed using a fluorescence spectrophotometer (Spectrafluor plus, Tecan, Switzerland) by staining DNA with pico green™ (1/200 dilution) (Juro, Supply Gmbh, Switzerland). Based on the relative fluorescence units (RFU) values and using a calibration graph. DNA concentration of each experimental sample was calculated (DNA concentration = -2.782763 + 0.002019 × RFU). The DNA concentration of each experimental sample was then normalized to 2.5ng/µl to produce working sample for use in PCR reactions.

3.4.1.2. Polymerase chain reaction (PCR) amplification

PCR reactions were conducted in 96 and 384-well plates in a GeneAmp PCR system

9700 Perkin Elmer (Applied Biosystem, USA) DNA thermocycler. For separation of amplicons using capillary electrophoresis M-13 tailed and direct flourophore labeled primers were used. The M-13 tailed forward primer from each primer pair was labeled with different flourophores - 6-FAM™ (Blue), Hex™ (Green) and NED™ (Yellow) (Applied Biosystems) before amplification. The reactions were performed in volumes of 5 µl using three different protocols (Table 6). A touchdown PCR program was used to amplify the DNA fragments. Reaction conditions for the PCR program were as follows:

Initial denaturation for 15 minutes at 94°C (to minimize primer dimmer formation and to activate the Taq polymerase), subsequently 10 cycles of denaturation for 10 seconds at 94°C, 35 cycles (40 cycles for m-13 labeled primers) of annealing at 61°C to 52°C for 20 seconds (the annealing temperature for each cycle being reduced by 1°C) and extension at 72°C for 30 seconds. The last PCR cycle was followed by a 20 minutes extension at 72°C to ensure amplification to equal lengths of both DNA strands (Smith *et al.,* 1995).

Table 6. PCR protocols used in DNA amplification

Protocol	Primer (2 pM)	$MgCl_2$ (10 mM)	dNTPs (2 mM)	DNA (2.5ng/µl)	*Taq* polymerase (0.5 U/µl)	Buffer (10X)	DDW (µl)
1	0.500	1.000	0.250	1.000	0.200	0.500	1.550
2	1.000	1.000	0.375	0.500	0.200	0.500	1.425
3	0.500	0.750	0.500	0.500	0.250	0.500	2.000

3.4.1.2.1. Fragment analysis

The amplified PCR products were separated by capillary electrophoresis using ABI prism of 3700 and ABI 3730 automatic DNA sequencer (Applied Biosystems Inc.). The capillary electrophoresis technique has a resolution of less than 2 bp and hence can be used to clearly distinguish polymorphisms of less than 2 bp. Moreover, as this technique is a fluorescence based detection system, it dispenses with the need for radioactive or laborious manual polyacrylamide gel screening techniques.

Prior to electrophoresis multiplexing was done *i.e.* the amplified products of primers labeled with different dyes or same flourophores-labeled primers with non-overlapping amplicons (in terms of size) were pooled. Multiplexing of numerous fragments and poolplexing of numerous samples increased the throughput of this technique. For multiplexing 1.0 µl of each of the amplified products were pooled and each of the pooled PCR product were then mixed with 0.25 µl of GeneScan 400 Rox® internal size standard (Applied Biosystems) and 7.0 µl of Hi-Di formamide (Applied Biosystems). The final volume was made upto 12 µl with sterile distilled water. This final product was then denatured for 5 minutes at 95^{o}C (Perkin Elmer 9700, Applied Biosystems) and cooled immediately on ice for ABI runs.

3.4.1.2.2. Fragment size fractionation

The denatured DNA amplicons were separated using capillary electrophoresis with the help of two automatic DNA sequencers ABI 3700 and ABI 3730. In this technique, as the DNA migrates through the detection cell, the capillaries are simultaneously illuminated from both sides of the array by an argon-ion laser. To accomplish this, a beam from a single laser source is split using a series of mirror to form a dual pathway. The fluorescent emissions are then spectrally separated by a spectrograph and focused onto a charged couple device, which are then converted to

digital information that is processed by the "collection software". The fluorescent internal size standard in each capillary eliminates variability.

In the experiment "GeneScan2 POP6 Default" run module, "G 4" filter set and "GS 400 HD" analysis module were used for all the capillary runs performed on ABI 3700 DNA sequencer. The fragments were separated in a 50cm capillary array using a POP 6 (Performance optimized polymer 6, Applied Biosystems) separation matrix. The capillary runs on ABI 3730 were performed using "Microsatellite Default" analysis method and "Genemapper-POP7" run module. The fragments were separated on a 36 cm capillary array using POP7 as a separation matrix.

3.4.2. Data Processing

For PCR products electrophoresed on ABI 3700 DNA sequencer, the Genescan® v 3.1 software (Applied Biosystems) was used to size the peak pattern in relation to the internal size standard Genescan 400 Rox®. The principle behind this is that the standards are run in the same lane or capillary as the samples that contain fragment of unknown sizes labeled with different flourophores. Genescan® analysis software automatically calculates the size of the unknown DNA fragment by generating a calibration-sizing curve based upon the migration times of the known fragments in standard. The unknown fragments are mapped on a curve and the sample data is converted from migration times to fragment size. Genotyper® v 3.7 is then used for allele calling. The peaks are then displayed with the base pairs and height in a chromatogram. The height of the chromatogram peaks obtained through capillary electrophoresis is directly propotional to the signal strength, which inturn is determined by the amount of amplified product in the sample.

For genotyping the samples electrophoresed on ABI 3730 automatic DNA sequencer GeneMapper® v.3.2 software is used. The GeneMapper® v 3.2 software

provides a series of automatic fragment sizing, allele scoring, bin-building and auto-panelizer algorithms. GeneMapper® combines the precision sizing capabilities of Genescan® software with the allele calling power of the Genotyper® software helping in accurate genotyping of the samples. Plate 3 depicts the image of PCR products after their run on a ABI sequencer.

3.4.2.1. Scoring of amplified products

The amplified PCR products of the SSR markers screened on the RILs were scored as follows.

A = Homozygote carrying allele from female parent

B = Homozygote carrying allele from male parent

H = Heterozygote carrying alleles from both parents

- = Missing data for individual at a locus

O = off type individuals carrying alleles not observed in both parents

After scoring the dataset was assembled in Microsoft excel spreadsheet and the individuals showing more than 10% off types were removed from the dataset. This final data set was then arranged on an excel sheet in a format suitable for linkage map analysis using MAPMAKER/EXP 3.0 software.

3.5. Statistical methods

3.5.1. Phenotypic analysis

3.5.1.1. Analysis of variance

Data were analysed using Residual Maximum Likelihood algorithm (ReML) procedure in GenStat 10th Ed. (GenStat, 2007) to allocate sources of variation and to estimate entry means for individual and combined environments (Patterson and Thompson, 1971; Robinson, 1987). ReML provides the Best Linear Unbiased

Predictors (BLUPs) for the performance of genotypes and estimates the components of variance by maximizing the likelihood of all contrasts with zero expectations. The predicted means for each entry was estimated with entries as fixed effect in both individual and across environment analyses; replication, error and entry × replication as random effect in individual environments and replication, error, entry × replication and entry × environment interaction as random effects in across environment analysis.

3.5.1.2. Genetic correlation

The association within spotted stem borer resistant component traits and agronomic and morphological traits, and the inter-association between these traits was worked out using both individual and combined environment dataset in the RIL population.

$$\text{Correlation coefficient } (r_{xy}) = \frac{\sigma_g XY}{\sqrt{\sigma_g^2 X} \times \sqrt{\sigma_g^2 Y}}$$

where,

r_{xy} = Genetic correlation coefficient between traits X and Y

$\sigma_g XY$ = Genetic covariance between traits X and Y

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

The significance of the of correlation coefficients was tested using 't' value at n-2 degrees of freedom.

3.5.1.3. Broad-sense heritability

Broad-sense heritability h^2 was estimated across the RIL for all the traits observed on the RIL population. In individual environment the heritability was estimated as:

Heritability (operational) $h^2 = \frac{\sigma_g^2}{\sigma_p^2}$

Heritability across the two screening environments was estimated as:

Heritability (operational) $h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_{g\times e}^2 / n_e + \sigma_e^2 / n_{e\times r}}$

where,

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

σ_e^2 = Error variance

$\sigma_{g\times e}^2$ = G × E interaction variance

n_e = Number of environments

r = Number of replications

3.5.1.4. Test of significance of means

To test whether the difference between the 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 based on the formula given by Singh and Choudhary (1999).

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{S_1^2}{n_1}} + \sqrt{\frac{S_2^2}{n_2}}}$$

where,

$$S_1^2 = \frac{\sum_{i=1}^{n} \left(x_{i1} - \overline{X_1}\right)^2}{n_1 - 1}$$

$$S_2^2 = \frac{\sum_{i=1}^{n} \left(x_{i2} - \overline{X_2}\right)^2}{n_2 - 1}$$

$\overline{X}$ = Grand mean of population / parent

$\overline{x_{ij}}$ = Individual entry mean

n = Number of entries

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

3.5.2. Genotypic analysis

3.5.2.1. Genome composition and segregation distortion

A total of 90 polymorphic SSR markers were utilized for genotyping the 266 RILs and their parents (Table 7). Few of the recombinant inbred lines (15 out of 266) exhibited presence of more than 10 % non-parental alleles; hence these lines were discarded from the dataset used for analysis. From the observed genotypic dataset marker classes at each locus were summarized for all the individuals into three different genotypic classes - A and B to mark genotypes of parent A (female parent) and parent B (male parent), and '-' to mark missing data points and heterozygotes. The segregation of each marker was tested with a chi-square goodness of fit test to the expected mendelian segregation ratio (1:1) of parental configuration.

3.5.2.2. Map construction

Linkage of all marker loci was performed with MapMaker/Exp V 3.0 (Lander *et al.,* 1987). The Haldane (1919) mapping function was used to convert the recombination frequency to genetic distances in centimorgans (cM). This analysis was carried out by evaluating the mapping populations as a RIL using two-point analysis to identify linked pairs at a LOD score of 3.0. The 'Sequence all' command was used for two-point (or pair wise) linkage analysis. While, using the "group" command all the markers were grouped in a sequence into different linkage groups, with each group

consisting of markers associated with one another with a LOD score < 3.0. The formed groups were afterwards ordered using the 'First Order' command whenever possible. Usually, the first order was aided with LOD table correlations between markers, to figure out the most-linked markers. The 'Compare' command was then used to calculate the maximum likelihood map for each specified order of markers and to report the orders stored by likelihood of their maps. The best order was indicated as having a relative *log-likelihood* of 0.0. The obtained order was further analyzed using a three-point linkage analysis using 'Ripple' command. Other markers were added using the 'Try' command and fine-tuned using again the 'Ripple' command. The 'Ripple' command was used to assign exact positions to markers. Marker loci and linkage group that were more than 50 cM apart were considered to be not significantly linked. As most of the markers used in the present study have been already mapped in previous studies and have also been used to develop an *in-silico* physical map of sorghum at ICRISAT, these markers were assigned to different linkage groups based on the known chromosomal location, and their order was verified using mapmaker.

3.5.2.3. Quantitative trait loci analysis

The genotypic dataset obtained by screening 90 SSR markers on RILs and the predicted means for each trait in individual and across environments were used to identify genomic regions associated with the traits using the composite interval mapping (CIM) approach with cofactors (Jansen and Stam, 1994). All the necessary computations for QTL mapping and estimation of their effects were performed with a software package PLABQTL ver 1.1 (Utz and Melchinger, 1996). The program performs a multiple regression on evenly distributed positions of the linkage map (Haley and Knott, 1992). Markers to serve as cofactors are determined using stepwise

regression with an F-to-enter and an F-to-delete threshold value of 3.5 (Miller, 1990). The presence of a putative QTL was determined using a critical LOD threshold as estimated by the Bonferroni χ^2 approximation (Zeng, 1994) corresponding to a genome-wise type I error of 0.25. The QTL positions were determined at local maxima of the LOD-curve plot in the region under consideration. The proportion of phenotypic variance explained by each QTL marker was estimated using the coefficient of determination (R^2), which is based on the partial correlation of a putative QTL with the trait adjusted for cofactors (Kendall and Stuart, 1961). As a RIL mapping population was used in this study, the additive model "AA" was utilized for the QTL analyses. Hence, in the final simultaneous fit both the additive and the additive × additive digenic interaction effects were estimated for the identified QTL. Two QTL were declared as congruent across traits and generations if they had the same sign and were within a 20-cM distance (Melchinger *et al.,* 1998). For across environment analyses QTL×E effects were estimated based on the fitted model to the adjusted entry means of each environment as described by Bohn *et al.* (1996) A simultaneous analysis with all the detected putative QTL was performed for each screening environment and the mean squares for QTL×E were estimated from the difference of the fits of data from individual environments and across environments. These values were then tested for significance with a sequentially rejective Bonferroni F test (SRBF).

3.5.2.4. Cross-species validation of QTL detected for stem borer resistance

Panel RFLP markers from previous maize studies were selected based on their association to regions responsible for stem borer resistance (Schon *et al.,* 1993; Bohn *et al.,* 1997, 2000; Groh *et al.,* 1998; Cardinal *et al.,* 2001; Jampatong *et al.,* 2002 and Krakowsky *et al.,* 2004). The corresponding DNA sequences for these RFLP markers

and cDNA sequences of two maize insect resistant genes *mir1* and *mir2* reported to confer resistance to lepidopteran larvae were obtained from maize genome database (http://www.maizegdb.org) and NCBI (http://www.ncbi.nih.gov), respectively. The bin map position for these sequences in maize was identified using the BLASTn search tool with the available standard servers setting on the maize genome database. These DNA sequences were then BLAST searched against the genome sequence of sorghum available at phytozome (http://www. Phytozome.net), to find the sequence similarity on each sorghum chromosome. Similarly, the physical position of all the SSR markers used in the present study was obtained and each marker was then mapped to their physical position on sorghum chromosomes for comparison with obtained maize sequences.

RESULTS

CHAPTER IV

RESULTS

The experimental study was carried out with the main objective of characterizing and understanding the genetic architecture of traits related to resistance to spotted stem borer using a recombinant inbred line (RIL) population developed by crossing sorghum genotypes ICSV 745 and PB 15520. The results obtained from genotypic and phenotypic evaluation of these traits are presented here.

4.1. Mean performance of RILs and their parents

The parental lines (ICSV 745 and PB 15520) of the recombinant inbred line (RIL) population differed phenotypically for all the traits observed across the two environments, except for the traits glossiness (2007 rainy season) and nodes per plant and overall resistance (2008 rainy season). As expected, the male parental line (PB 15520) consistently exhibited lower mean values for all of the stem borer resistance related traits across both screening environments, indicating its greater level of resistance to the spotted stem borer. The mean performance of parents and RILs for all the observed traits during the 2007 and 2008 rainy seasons are presented in Table 8 and Figure 1.

4.1.1. Spotted stem borer resistance component traits

4.1.1.1. Stem tunneling (cm)

The mean length of stem tunnel formed by the spotted stem borer larvae on female parent (ICSV 745) [30.6 cm and 98.9 cm during 2007 and 2008 rainy seasons, respectively] was significantly higher than the mean tunnel length recorded on the male parent (PB 15520) [12.5 cm and 46.9 cm during 2007 and 2008 rainy seasons, respectively]. In the recombinant inbred line (RIL) population, a mean tunnel length of 20.2 cm was observed during the 2007 rainy season, while during the 2008 rainy

season the mean tunnel length recorded was 60.2 cm.

This trait was highly influenced by the environment as revealed by significantly different mean values for the RIL genotypes across the two artificially infested screening environments. In general, the lengths of the tunnels formed by spotted stem borer larvae on the parents and the RILs were greater during 2008rainy season than observed during the 2007 rainy season.

4.1.1.2. Deadheart incidence (%)

The level of deadheart incidence due to spotted stem borer infestation on the female parent was higher (80.7% and 82.7% during 2007 and 2008 rainy seasons, respectively) than the male parent (PB 15520) (71.5% and 45.3 % during the 2007 and 2008 rainy seasons, respectively). The RIL population recorded 74.1% and 60.4% mean deadheart incidence during the 2007 and 2008 rainy seasons, respectively. The mean value of the RIL population for this trait was skewed more towards the resistant male parent during the 2007 rainy season (suggesting partial dominance of resistance), while during the 2008 rainy season it was closer to the mid-parental value (suggesting additive inheritance of resistance).

4.1.1.3. Leaf feeding damage (1-9 score)

PB 15520 exhibited less-severe symptoms of leaf feeding by spotted stem borer larvae, (mean leaf feeding damage scores of 5.6 and 4.6 during the 2007 and 2008 rainy seasons, respectively) than ICSV 745 (mean leaf feeding damage scores of 7.9 and 7.4 during the 2007 and 2008 rainy seasons, respectively). Across seasons, the RIL population tended more towards susceptibility to leaf feeding than resistance (suggesting partial dominance of susceptibility), and recorded mean leaf feeding damage scores of 6.3 and 6.0 during the 2007 and 2008 rainy seasons, respectively.

4.1.1.4. Recovery resistance (1-9 score)

Recovery resistance is a good measure of tolerance of plants to spotted stem borer infestation (Sharma *et al.,* 1997) and is visually rated based on the number of productive tillers formed even after severe infestations. In the present study, PB 15520 showed greater tolerance to spotted stem borer (recovery resistance scores of 4.3 and 3.5 during the 2007 and 2008 rainy seasons, respectively) than ICSV 745 (recovery resistance scores of 7.3 and 7.4 during the 2007 and 2008 rainy seasons, respectively). The RIL population itself recorded mean recovery resistance scores of 5.6 and 5.5 during the 2007 and 2008 rainy seasons, respectively.

4.1.1.5. Overall resistance (1-9 score)

Over all resistance score, which takes into account leaf feeding damage, deadheart incidence and number of productive tillers of the infested plant was significantly lower for PB 15520 in both the seasons (means of 5.7 and 5.4 during the 2007 and 2008 rainy seasons, respectively), indicating higher resistance to spotted stem borer infestation than ICSV 745 (means 7.7 and 6.5 during the 2007 and 2008 rainy seasons, respectively). Like deadheart incidence, the RIL population mean value for this trait tended more towards that of the resistant parent during the 2007 rainy season (mean overall resistance score of 6.3), while during the 2008 rainy season, it tended towards the mid-parental value (mean overall resistance score of 6.1).

4.1.2. Agronomic and morphological traits

4.1.2.1. Glossiness (1-5 score)

Both of the parental lines (ICSV 745 and PB 15520) were non-glossy (glossiness score > 4) and as expected phenotypic differences were not substantial among the entries of the RIL population (scores ranging between 3 and 5 with a mean value of

4.4) during the 2007 rainy season, hence observations for this trait was not recorded on the population during the 2008 rainy season trial.

4.1.2.2. Seedling vigor (1-5 score)

PB 15520 exhibited greater seedling height, leaf expansion and robustness two weeks after germination than did ICSV 745 in both the screening environments. The RIL population recorded mean values of 1.9 and 1.7 for seedling vigor score during the 2007 and 2008 rainy seasons, respectively. In general, the seedling vigor scores were marginally higher among the RIL population entries during the 2007 rainy season than during the 2008 rainy season.

4.1.2.3. Seedling basal pigmentation (1-3 score)

Seedlings of PB 15520 showed basal pigmentation, while those of ICSV 745 showed no such pigmentation. This trait was also found to segregate substantially in the RIL population, even though the mean value tended more towards the female parent (ICSV 745). The mean of RILs did not vary substantially across the two environments implicating a minimal role of environment on the expression of this trait.

4.1.2.4. Plant color (1-3 score)

ICSV 745 exhibited a tan plant color, while PB 15520 had a purple color. Similar to basal pigmentation, the recombinant inbred line population consisted of entries showing substantial variation for this trait; however, the mean value for this trait tended more towards the purple plant color of parent PB 15520. Plant color being qualitatively inherited, the RILs did not exhibit significant variation in their behavior across the two screening environments for this trait.

4.1.2.5. Testa (presence vs absence) (1-2 score)

Grains of ICSV 745 did not have a pigmented seed sub-coat, while PB 15520 had a

prominently pigmented seed sub-coat. The RIL population consisted of entries exhibiting these two variants of this trait i.e. individual entries uniform for both presence or absence of the pigmented seed sub-coat. The RIL entries were consistent for this trait across the two screening environments.

4.1.2.6. Mesocarp thickness (1-3 score)

A thick mesocarp was observed on the grains of PB 15520, while the mesocarp on the grains of ICSV 745 was very thin. The recombinant inbred lines consisted mostly of entries exhibiting either of these parental phenotypes; however, a few of the RIL entries had mesocarp of intermediate thickness. The mean of the RIL population tended towards the female parent (ICSV 745) during the 2007 rainy season; however, during the 2008 rainy season it was near the mid parental value.

4.1.2.7. Leaf angle (^{o})

PB 15520 had markedly more erect leaves (>70^{o}) compared to ICSV 745 (<51^{o}). The RIL population exhibited a mean leaf angle of 55^{o} across both the 2007 and 2008 rainy seasons suggesting that a large proportion of the RIL population entries exhibited a leaf angle similar to that of the female parent (ICSV 745). No significant effect of year was observed for the mean leaf angle values of the RIL population and its parents across the two screening environments.

4.1.2.8. Time to 50% flowering (days)

ICSV 745 flowered (69-71 days) 17-20 days earlier than PB 15520 (88-90 days) in both of the screens. The RIL population consisted of both early- and late-flowering entries with means of 75 and 77 days, during the 2007 and 2008 rainy seasons, respectively. Though the entries did not significantly differ for this trait across the two environments, the entries of RIL population and the female parent ICSV 745 flowered

earlier during the 2007 rainy season screen than during the 2008 rainy season screen, perhaps due to greater severity of stem borer damage observed on susceptible entries during the 2008 rainy season evaluation.

4.1.2.9. Nodes per plant (number)

The female parent (ICSV 745) had more nodes per plant (11 to 12) than the male parent (PB 15520) [8 to 9 nodes per plant]. The mean of the RIL population for this trait was consistent across both the seasons and ranged between 10 and 11 nodes per plant across both the 2007 and 2008 rainy seasons, respectively.

4.1.2.10. Plant height (cm)

ICSV 745 was significantly taller than PB 15520 with plant height differences averaging 64 and 86 cm across the 2007 and 2008 rainy seasons, respectively. The recombinant inbred lines recorded population means of 190 cm and 222 cm for this trait during the 2007 and 2008 rainy seasons, respectively. The mean values for plant height were higher among the RILs and parents during the 2008 rainy season compared to the 2007 rainy season, indicating a large influence of environment on this trait. The magnitude of differences across the two seasons for this trait was higher for parent ICSV 745 than for PB 15520.

4.1.2.11. Agronomic performance (1-5 score)

PB 15520 exhibited greater agronomic desirability in the infested plots compared to ICSV 745 due to its higher ability in withstanding the biotic stress. Agronomic performance did not vary substantially across the two screening environments and the RILs mean value (3.2) tended towards the mid-parental value across both the seasons.

4.2. Analysis of variance

The genotype and genotype × environment interaction variances estimated for all the traits observed on the recombinant inbred line population are presented in Table 9. In both of the screening environments as well as across these two environments, variation among the RILs was highly significant for all observed traits. Comparison of the observed parameters across the two screening environments indicated greater variation among the RILs during 2008 rainy season for most of the traits, except for leaf damage rating, seedling vigor and testa presence vs absence, for which the variances estimated were consistent across both the screening environments, and for number of nodes for which the RILs recorded significantly lower variation during the 2008 rainy season.

Environment can also often play a major role in confounding the effects and expression of the genotypes, hence it is imperative to assess the genotype × environment (G × E) interaction component of variance. The extent to which G × E affects a trait is an important determinant of the intensity of testing over years and/or locations that must be employed to satisfactorily quantify the performance of a genotype. As expected, genotype × environment were highly significant for observed traits except seedling vigor and seedling basal pigmentation, indicating the relatively environmentally-independent nature of expression of the genes governing these latter two traits. With the exception of the spotted stem borer resistance component trait "overall resistance", all the other resistance traits had G × E variance components ($\sigma^2_{g\times e}$) significantly higher than the estimated genotypic variance (σ^2_g). Among the agronomic performance and grain quality component traits, except for mesocarp thickness and plant height, all other exhibited lower $\sigma^2_{g\times e}$ than σ^2_g. In general, amongst all the traits recorded, the proportion of $\sigma^2_{g\times e}$ as compared to its respective σ^2_g in the

RIL population was highest for the trait stem tunneling.

4.3. Frequency Distribution

Variation among the RILs for various traits observed during the 2007 and 2008 rainy seasons are graphically represented by their frequency distributions in Figure 2 to 5.

4.3.1. Spotted stem borer resistance component traits

4.3.1.1. Stem tunneling (cm)

A good distribution of the RIL means was observed for stem tunneling in both the 2007 and 2008 rainy season screens. The mean and ranges for this trait varied significantly between the two screening environments indicating a high influence of environment, the peaks were observed at different regions, i.e. between 10-30 cm and 40-50 cm for the 2007 and 2008 rainy season screens, respectively.

4.3.1.2. Deadheart incidence (%)

A near-normal gaussian distribution was observed for this trait during the 2007 rainy season. Though the RIL population showed nearly continuous variation during the 2008 rainy season, a tri-modal distribution was observed indicating the presence of one or more major regions in the genome influencing this trait.

4.3.1.3. Leaf feeding damage

The distribution of leaf damage scores were nearly similar among the RILs during the 2007 and 2008 rainy season screens. A majority of the RILs showed scores between 6.0 and 7.0 in both seasons. The frequency distribution curve could be roughly classified into two major clusters, one consisting of large number of entries (90-91% of the RIL population) with higher leaf damage scores (scores between 5.0 and 8.0), while the other consisting of a few individuals entries (7-8% of the RIL population) with lower leaf damage ratings ($\leq$ 5.0 leaf damage score).

4.3.1.4. Recovery resistance

The RIL population showed a near normal distribution for this trait in the 2007 rainy season screen, while, during the 2008 rainy season the population was slightly skewed towards resistance. Large numbers of RILs exhibited recovery resistance scores between 5.0 and 6.5 across both the seasons.

4.3.1.5. Overall resistance

The RIL frequency distribution for this trait showed a wide range of variation within the mapping population. In the 2007 rainy season screen the RIL population exhibited near Gaussian distribution, while in the 2008 rainy season screen the histogram was slightly skewed towards lower scores for this trait, indicating the presence of a large number of RILs with good overall resistance.

4.3.2. Agronomic and morphological traits

4.3.2.1. Glossiness

The RIL population showed a near normal distribution for glossiness during the 2007 rainy season. However, the range of scores for glossiness among the RILs was narrow, with most of entries falling into the non-glossy phenotypic class (scores between 4.0 and 5.0), so this trait was not observed in the 2008 rainy season screen.

4.3.2.2. Seedling vigor

The distributions of the RILs for this trait were near normal and consistent in both 2007 and 2008 rainy season screens, with large numbers of RILs with means congregating in the range of 1.5 to 2.5 across the two seasons.

4.3.2.3. Basal pigmentation

A discontinuous distribution was observed for this trait among the RIL population progenies in both the 2007 and 2008 rainy season screens, with the majority of

individuals falling into two major phenotypic classes. The histograms for the 2007 rainy season and the 2008 rainy season were very similar, indicating limited influence of the environment on this trait.

4.3.2.4. Plant color

The RILs were distributed into three major classes for this trait across both the 2007 and 2008 rainy season screens. The distribution indicates the presence of a single major gene and probably a modifier for the expression of this trait.

4.3.2.5. Mesocarp thickness

The RILs were distributed into four major phenotypic classes for mesocarp thickness across both the 2007 and 2008 rainy season. The RILs were distributed in equal proportion in all the four classes during the 2007 rainy season screens; however, during the 2008 rainy season a large proportion of the RILs exhibited thick mesocarp, and the frequency of RILs with a mesocarp of intermediate thickness was relatively low. As the histogram of the RILs for this trait significantly differed in both the seasons, it may be inferred that this trait is influenced by the environment.

4.3.2.6. Testa (presence vs absence)

Similar to the trait mesocarp thickness, the distribution of RILs for this trait was not normal in either the 2007 or the 2008 rainy season screens. The histograms revealed that a large number (approximately 50%) of RILs exhibited no pigmented seed subcoat suggesting the segregation of a single major gene in the RIL population.

4.3.2.7. Leaf angle (^{o})

The RIL population showed a normal Gaussian distribution during the 2007 rainy season screen with most entries exhibiting a leaf angle ranging between 40 and 60^{o}. During 2008 rainy season the distribution of the RILs for this trait could be roughly

divided into three major clusters, with major peaks observed between 40 and 50^{o} for one cluster and 50 and 60^{o} for the second cluster (by far the largest), and a small cluster between 60 and 75^{o} for the third. The segregating pattern observed in 2008 rainy season suggests the presence of two epistatic genes controlling this trait.

4.3.2.8. Time to 50% flowering (days)

The distribution of time to 50% flowering was different in both the 2007 and 2008 rainy season screens. The population was slightly skewed towards early flowering and exhibited a slight decrease in the number of lines showing intermediate maturities during the 2007 rainy season, while during the 2008 rainy season a near normal distribution was observed for this trait with peak flowering of entries from 70-85 days. The distribution of flowering time in 2007 suggests the involvement of 3 or more epistatically interacting genes involved in late flowering.

4.3.2.9. Number of nodes

A normal distribution was observed by RIL population for this trait during the 2008 rainy season screens, but a broader peak was observed in the 2007 rainy season. Thus greater variation was observed among the RIL population progenies during the 2007 screen than during 2008 screen. Most RILs exhibited 11 to 12 nodes per plant in both the seasons.

4.3.2.10. Plant height (cm)

A wide range of variation was observed among the RILs for this trait. Clearly the distribution of RILs for this trait was different during the 2007 and 2008 rainy season screens, however in both of these screening environments the RILs could be grouped into two major phenotypic clusters. The peak of these two clusters were observed at 150-200 and 225-250 cm during the 2007 rainy season and at 150-175 and 225 and

275cm during the 2008 rainy season. The segregating pattern in 2007 suggested the presence of two to three major genes.

4.3.2.11. Agronomic performance (score)

The RIL population showed a normal Gaussian distribution for agronomic performance during both the 2007 and 2008 rainy season screens, with a majority of individual RILs having agronomic performance scores between 2.5 and 4.0 in both year's screens. The phenotypic distribution of the RILs was nearly the same in both screening environments.

4.4. Correlation

Correlation is a statistical measure used to characterize relationships between two or more variables. Correlation coefficients between all observed traits were estimated across the two screening environments. Considering the varying ranges of phenotypic values in the two screening environments, correlation co-efficients were also estimated for the observed traits in each of the individual environments. The genotypic correlation coefficients estimated using individual screening season and combined season datasets are presented in Table 10 to 13.

4.4.1. Correlations among the spotted stem borer resistance component traits

The genotypic correlations observed among the stem borer resistance component traits in individual screening seasons are listed in Table 10. Stem tunneling was found to be positively associated with leaf feeding damage (r = 0.16) and recovery resistance (r = 0.26) during the 2008 and 2007 rainy seasons, respectively. All the other five resistance component traits — deadheart incidence, leaf feeding damage, recovery resistance and overall resistance exhibited strong positive inter-associations among themselves in both 2007 and 2008 rainy seasons, with the absolute values ranging

form 0.11 to 0.47. A consistently high positive correlation across both the seasons was observed between overall resistance and recovery resistance score ($r \geq 0.44$) followed by between deadheart incidence and overall resistance ($r \geq 0.29$) and deadheart incidence and leaf feeding damage ($r \geq 0.25$).

In the joint season analysis all the associations among the resistance component traits were highly significant, except for the association between deadheart incidence and stem tunneling (Table 13). Similar to the associations observed in individual environments, the strength of the association was highest between overall resistance and recovery resistance ($r = 0.59$), followed by the association between overall resistance and deadheart incidence ($r = 0.51$), and deadheart incidence and leaf feeding damage ($r = 0.36$).

4.4.2. Correlations among the agronomic and morphological traits

The genotypic correlations observed among the agronomic and morphological traits in individual screening seasons are listed in Table 11. The trait glossiness exhibited significant and positive associations with seedling vigor ($r = 0.56$) and number of nodes ($r = 0.11$) during the 2007 rainy season. Seedling vigor showed a significant positive association with time to 50% flowering ($r = 0.13$) and number of nodes per plant ($r = 0.13$) in the 2007 rainy season; however, these associations were not found to be significant during the 2008 rainy season evaluations. Similar trend was observed for the associations between this trait and testa presence vs absence ($r = -0.12$) and mesocarp thickness ($r = -0.19$), which were negative and significant during 2008 rainy season and insignificant during 2007 rainy season.

Consistently high and significant associations were observed for the trait basal pigmentation with both plant color ($r \geq 0.38$) and plant height ($r = -0.20$) across both the seasons. Although this trait also exhibited significant association with testa

presence vs absence, the strength of this association was substantially different in the two seasons (r = 0.11 and 0.27, during 2007 and 2008, respectively). In the 2007 and 2008 rainy seasons basal pigmentation exhibited significant positive associations with agronomic performance (r = 0.13) and mesocarp thickness (r = 0.16), respectively

Positive and significant associations were also observed between the traits plant color and both presence vs absence of testa (r = 0.21 and 0.16) and agronomic performance (r = 0.17 and 0.22) during both the 2007 and 2008 rainy seasons. Plant color score also exhibited a significant negative association with plant height (r = -0.14) during the 2007 rainy season and a positive association with number of nodes (r = 0.11) during 2008 rainy season.

Presence or absence of testa, mesocarp thickness and leaf angle showed positive and significant associations ($r \geq 0.21$) during both the 2007 and 2008 rainy seasons. The consistently high values of correlation coefficients for these associations suggests a close association of the gene/s governing these traits.

Number of nodes showed a strong positive association with time to 50% flowering (r = 0.59 and 0.45) and agronomic performance (r = 0.15 and 0.12); while plant height exhibited a very strong negative association with agronomic performance score (r = -0.17 and -0.21) in both the screening environments.

All the associations observed to be significant (p = 0.05) or highly significant (p = 0.01) in either one of the screening environments were found to be highly significant across both the environments (Table 13). Among all these associations the highest correlation coefficients were observed for number of nodes and time to 50% flowering (r = 0.63), followed by testa presence vs absence with mesocarp thickness (r = 0.55), testa presence vs absence with leaf angle (r = 0.47), basal pigmentation with plant color (r = 0.44) and leaf angle with mesocarp thickness (r = 0.37).

4.4.3. Inter-correlation between resistance and agronomic/morphological traits

4.4.3.1. Individual screening season

The estimated inter-correlation coefficients between the five stem borer resistance component traits and agronomic and morphological traits observed among the RIL population progenies in individual screening environments are presented in Table 12.

4.4.3.1.1. Stem tunneling

Stem tunneling recorded a strong positive association with plant height (r = 0.24 and 0.17) and testa presence vs absence (r = 0.14 and 0.12) during both the 2007 and 2008 rainy season screens, while during the 2007 rainy season this trait showed a significant negative association with traits glossiness (r = -0.12) and basal pigmentation (r = -0.14) and a positive association with leaf angle (r = 0.14) and mesocarp thickness (r = 0.18). The highest correlation was observed between stem tunneling and plant height in both the screening seasons.

4.4.3.1.2. Deadheart incidence (%)

As expected a significant positive association was observed between deadheart incidence and time to 50% flowering (r = 0.23 and 0.15), while a consistently negative significant association was observed between this trait and nodes per plant (r = -0.12 and -0.11) and agronomic performance (r = -0.16 and -0.12) across both the screening environments. During the 2007 rainy season this trait was positively associated with seedling vigor (r = 0.18) and negatively with mesocarp thickness (r = -0.13), while during the 2008 rainy season a negative association was observed between this trait and plant color (r = -0.13) and leaf angle (r = -0.12).

4.4.3.1.3. Leaf feeding damage (score)

Leaf feeding damage score recorded significant negative associations with plant color (r = -0.17 and -0.14) during both the 2007 and 2008 rainy seasons, indicating an association of tan plant color with susceptibility. A significant negative association was also observed for this trait with other agronomic traits mesocarp thickness (r = -0.15) and leaf angle (r = -0.22) during the 2007 rainy season, while during 2008 rainy season this trait was significantly positively associated with seedling vigor (r = 0.11).

4.4.3.1.4. Recovery resistance (score)

Recovery resistance exhibited a consistently significant negative association with number of nodes per plant (r = -0.23 and -0.26) and a significant positive association with plant height (r = 0.14 and 0.15) across both of the screening seasons. All the other associations were insignificant across both seasons except those with traits mesocarp thickness (r = -0.14), leaf angle (r = -0.14) and agronomic performance (r = -0.15) during the 2008 rainy season.

4.4.3.1.5. Overall resistance (score)

Overall resistance score and number of nodes per plants were found to be negatively associated (r = -0.19 and -0.29) during both the 2007 and 2008 rainy seasons. A positive association was observed between this trait and seedling vigor (r = 0.16) during the 2007 rainy season, while during the 2008 rainy season this trait exhibited negative and significant association with mesocarp thickness (r = -0.14) and time to 50% flowering (r = -0.15). The most striking result from these associations is the consistently significant negative association across the two screening seasons of the three resistance component traits – deadheart incidence, overall resistance and recovery resistance with the single agronomic trait – number of nodes per plant.

4.4.3.2. Across-screening seasons

In the combined season analysis of the 50 correlations listed between the resistance component, agronomic and morphological traits, 25 (50%) correlations were statistically significant at minimum 5% probability level, with absolute values ranging from 0.11 to 0.36 (Table 13). Among the three major measures of spotted stem borer resistance – stem tunneling, deadheart incidence and leaf feeding damage, the association with agronomic traits was strongest between stem tunneling and plant height (r = 0.26) followed by that between deadheart incidence and agronomic performance (r = -0.22) and leaf damage rating and plant color (r = -0.20).While both overall resistance and recovery resistance exhibited strongest associations with number of nodes per plant (r = -0.36 and -0.30, respectively).

4.5. Heritability estimates

Heritability plays an important role in understanding the genetic architecture of the traits and devising various breeding strategies for exploiting or improving the traits of interest. Operational heritability was estimated for all the traits observed on the RIL population in both the screening environments (2007 and 2008 rainy seasons) and in the combined season data set of the two environments. The heritability estimates are presented in Table 14.

4.5.1. Spotted stem borer resistance component traits

4.5.1.1. Stem tunneling

The RIL population progenies recorded high heritability for this trait during 2007 (0.95) and 2008 (0.82) rainy season. However, level of heritability was low (0.17) in the across environment analysis.

4.5.1.2. Deadheart incidence

A consistently high heritability was observed among the RIL population progenies for this trait in both the environments. The estimate ranged from 0.70 to 0.75 in each of these environments. However, in the across environments analysis the level of heritability estimated was moderate (0.38).

4.5.1.3. Leaf feeding damage

Similar to deadheart incidence the RILs recorded high heritability for leaf feeding damage scores in individual environments (0.80 and 0.74, during the 2007 and 2008 rainy seasons respectively) and moderate heritability (0.47) across these environments.

4.5.1.4. Recovery resistance

During both the 2007 and 2008 rainy seasons the RIL population recorded high heritability for this trait with the estimates ranging from 0.81 to 0.91. However, the across environment analysis indicated only moderate heritability for this trait (0.40).

4.5.1.5. Overall resistance

The estimate of heritability for overall resistance was variable in the three analyses. The RILs recorded high heritability during the 2008 rainy season (0.79), while in the 2007 rainy season and the across environment analyses a moderate heritability (0.58-0.53) was observed for this trait.

4.5.2. Agronomic and morphological traits

4.5.2.1. Glossiness

The RILs recorded moderate heritability (0.54) for this trait during the 2007 rainy season. As the trait was not recorded in the 2008 rainy season, across-season heritability was not estimated for this trait.

4.5.2.2. Seedling vigor

The RILs recorded consistently high heritability both in the individual environments and in the across-environment analyses. The heritability ranged from 0.60 to 0.76.

4.5.2.3. Basal pigmentation

Consistently very high magnitude of heritability (>0.80) was recorded amongst the RIL population progenies for this qualitative trait in both the individual and the across-environment analyses, indicating low influence of environment on this trait.

4.5.2.4. Plant color

The RILs recorded high heritability for this trait in both the 2007 and 2008 rainy seasons as well as across environments. The heritability ranged from 0.83 to 0.97.

4.5.2.5. Testa (presence vs absence)

Similar to the trait seedling vigor, the RILs exhibited consistently high heritability (>0.60) for this trait in both individual and across-environment analyses. However, in individual environments the level of heritability recorded was higher than that in the across-environment analysis.

4.5.2.6. Mesocarp thickness

High heritability was recorded for this trait among the RILs in both of the screening environments (0.70 and 0.93 during the 2007 and 2008 rainy seasons, respectively); however, the across-environments analysis indicated only moderate heritability (0.46) implicating the influence of environment on expression of this trait.

4.5.2.7. Leaf angle

The RIL population recorded high heritability for leaf angle in both the 2007 (0.69) and 2008 (0.85) rainy seasons, while moderate heritability (0.59) was observed for

this trait in the across-environments analysis.

4.5.2.8. Time to 50% flowering

High estimates of heritability (>0.85) were recorded for this trait in both individual environments and also across these environments. The level of the estimates ranged from 0.87 in the across-season analyses to 0.98 in the 2008 rainy season.

4.5.6.9. Number of nodes

The heritability estimate was variable for this trait across environments and analyses. During the 2007 rainy season the estimate of heritability for this trait was high (0.92). However, during 2008 rainy season and across-environment analyses moderate heritability (0.59-0.66) were observed.

4.5.6.10. Plant height

Moderate to high heritability was recorded among the RILs for plant height in both the individual and combined environment analyses. The level of heritability estimated was variable across all the three analyses and ranged from 0.54 to 0.95.

4.5.2.11. Agronomic performance

The level of heritability among the RILs for this trait was consistently high in individual screening environments as well as across environments. During the 2007 rainy season the estimate was 0.71, while during the 2008 rainy season and in the combined analyses the estimates were 0.85 and 0.72, respectively.

4.6. Transgressive segregation

Transgressive segregation is the term used to describe the phenomenon in which some individuals in a segregating population out-perform the parents. In the current study significant transgressive segregation was observed among the RIL population for

most of the plant traits observed (Figure 6 to 9). The proportion of RILs lying outside the parental limits and the significance of t-test conducted to determine the significance of difference of RIL and the parental means are presented in Table 15.

4.6.1. Stem borer resistance component traits

Significant transgressive segregation was observed among the RIL population for all the observed resistance traits except recovery resistance. With the exception of leaf feeding damage for all the remaining three traits, segregation was towards both resistance and susceptibility. However, significantly higher proportions of segregants were observed towards resistance to spotted stem borer. Among all these traits the highest proportion of transgressive segregation was observed for the trait deadheart incidence followed by stem tunneling.

T-tests revealed significant differences between the mean value of the RIL progeny set and the mean value of its parents for all the resistance traits. However, for all these traits the RIL means tended more towards the resistant parent (PB 15520).

4.6.2. Agronomic and morphological traits

Among all the ten agronomic traits observed, significant proportions of transgressive segregation was recorded for seedling vigor, leaf angle, time to 50% flowering, number of nodes per plant and plant height. Among these five traits, transgressive segregation was significant for both the extremes only for seedling vigor. However, segregation was skewed towards the phenotype of parent PB 15520 for this trait. For the other four of the agronomic traits the RILs exhibited transgressive segregation towards the female parent phenotype (ICSV 745).

As no significant transgressive segregation was observed for the qualitative traits seedling basal pigmentation, plant color, testa presence vs absence and

mesocarp thickness, it is implicated that either the parental types exhibit the extreme forms for these traits or these traits are governed by only one or few genes, with the later explanation being likely. The lack of significant transgressive segregation for agronomic performance score suggests that the parents exhibit extreme forms as this trait is unlikely to be conditioned by a small number of genes.

Means of RILs significantly differed from the parental types for all the agronomic traits except for the traits leaf angle and number of nodes per plant, for which the difference between the RIL means and the female parental means were non-significant. The mean value for RILs tended more towards the male parent for seedling vigor, plant color, testa presence vs absence and agronomic performance, while for the rest of the traits the RIL mean value tended more towards the female parent (ICSV 745).

4.7. Molecular marker analysis

4.7.1. Segregation distortion and genome composition

A total of 90 polymorphic SSR markers were screened against the RIL population. These markers were then assessed for deviation from the expected Mendelian segregation pattern of 1:1 using a chi-square goodness of fit test. The calculated chi-square values, the probability for the deviations being due to chance for each of the marker loci and their expected map position on a sorghum genetic map are presented in Table 16.

Highly significant [$p<0.01$] deviations from the expected 1:1 Mendelian segregation ratio were observed for 25 of the 90 SSR markers (28%), while 15 of the remaining 65 SSR markers exhibited only significant deviations [$0.01 \geq p \leq 0.05$]. Among these 40 SSR markers with significantly distorted segregation pattern, an overall excess of parental alleles of ICSV 745 was observed for 26 markers (65%).

The distribution among the RIL population for homozygous ICSV 745 alleles ranged from 13.6 to 62.9%, while it ranged between 12.4 and 57.4% for PB 15520 alleles (Figure 10). The average percentage of loci homozygous for female and male parental alleles in the RIL population was similar (42.7% for ICSV 745 and for 39.8%, for PB 15520).

More than 50% of the SSR markers exhibited equal proportions of the two parental genotypes among the RIL population progenies (Figure 11). Despite the normal transmission of male and female genomes from the genome point of view, there were chromosomal segments that were significantly skewed towards either male or female parental alleles. For instance the chromosomal segments on linkage group SBI-03 [between markers *Xtxp009* and *Xtxp034* (7 markers)], on linkage group SBI-04 [between markers *Xisep0224* and *Xtxp041* (4 markers)] and on linkage group SBI-10 [between markers *Xtxp270* and *Xisp10272* (6 markers)] exhibited distortion favoring the female parental allele, while a chromosomal segment on linkage group SBI-02b [between *Xcup74* and *Xisp10228* (6 markers)] exhibited distortion favoring male parental allele.

The average proportion of parental alleles among the RIL population across the 90 SSR markers was almost equal (46.5% and 43.7% for ICSV 745 and PB 15520). The proportion of ICSV 745 genome amongst the RIL population progenies ranged from 21.7% to 78.9%, while that of PB 15520 ranged from 17.8% to 68.3%.

4.7.2. Linkage map construction

The segregation data of the 90 SSR markers for 251 recombinant inbred lines (RILs) were used for the construction of the genetic map of the (ICSV 745 × PB 15520)-based RIL population. Co-segregation analysis of the markers was performed using Mapmaker/Exp ver3.0 software. The inter-marker distances in terms of centiMorgans

(cM) were estimated using the Haldane mapping function (Haldane, 1919). The details of the linkage map constructed and the linkage distances estimated for each linkage group are presented in Table 17 and Figure 12.

The position of the SSR markers mapped across the linkage groups in the study was similar to those observed in previous studies and to the physical map developed at ICRISAT, hence the names for these linkage groups were designated as per Kim *et al.* (2005a,b) from SBI-01 to SBI-10. The constructed genetic map had 90 marker loci spanning a distance of 1289.4 cM and the number of markers assigned to each linkage group ranged from 3 to 15 with the map length of different linkage groups ranging from 32 cM to 202 cM. Though the average inter-marker distance between any pair of markers across linkage groups was 14 cM, this ranged from 8 cM on SBI-05 to 28cM on SBI-06, and there were a few gaps wider than 50 cM on SBI-01, SBI-02, SBI-04, SBI-06 and SBI-10.

4.7.2.1. Linkage group SBI-01

A total of 14 SSR markers were found to be associated across the two segments, SBI-01a and SBI-01b of this linkage group. Eight of the markers were assigned to the first segment, while six were assigned to the second segment. The average inter-marker distance across the two segments ranged from 9 cM to 11 cM. Two gaps wider than 20 cM were observed on segment SBI-01a [between marker *Xcup06* and *Xcup24* (35 cM) and between *Xcup73* and *Xtxp357* (23cM)], while one large gap was observed between the markers *Xcup44* and *Xtxp340* (23cM) on SBI-01b.

4.7.2.2. Linkage group SBI-02

The SSR markers on this linkage group were found to associate as two different segments and hence were designated as SBI-02a and SBI-02b, with 3 and 7 markers,

respectively. The total map distance covered by these markers ranged from 30 cM to 89 cM and the average inter-marker distance observed across the two segments ranged from 10 cM to 13 cM for SBI-02a and SBI-02b, respectively. One gap wider than 20cM was observed on each of the segments between markers *Xisep0747* and *Xtxp025* [24 cM on SBI-02a] and between the markers *Xtxp298* and *Xtxp56* [32cM on SBI-02b]

4.7.2.3. Linkage group SBI-03

This linkage group was the longest mapped in this study. A total of 15 markers were mapped on this linkage group [16% of the total mapped markers] covering a distance of 202 cM. An average inter-marker distance of 13 cM was recorded for this linkage group. However, three gaps wider than 25 cM was observed between the markers *Xisp10277* and *Xtxp033* [43 cM], *Xisp10231* and *Xcup38* [31 cM] and *Xcup38* and *Xisp10332* [47 cM].

4.7.2.4. Linkage group SBI-04

Six markers were found to be associated on linkage group SBI-04, spanning a total linkage distance of 147 cM. The average inter-marker distance on this linkage group was 24 cM with three large gaps (>25cM) observed between markers *Xisep0224, Xisp10230*, *Xiabtp364* and *Xtxp041*. Three markers (*Xtxp041, Xtxp327* and *Xisp10229*) were found to congregate at the distal end of this linkage group.

4.7.2.5. Linkage group SBI-05

This linkage group was the shortest among all the groups. The group comprised of only 4 SSR markers spanning a total distance of 33 cM. The four SSR markers were clustered together with an average inter-marker distance of 8 cM. The order of markers *Xtxp225* and *Xisp10257* was reversed as compared to the physical map.

4.7.2.6. Linkage group SBI-06

This group was comprised of the lowest number of SSR markers (3) covering a relatively large distance of 85 cM. Two of these markers (*Xisp10264* and *Xtxp057*) were reasonably closely associated [17.0 cM], while the third marker (*Xisep0444*) exhibited very loose association (> 65 cM) with the rest of the markers.

4.7.2.7. Linkage group SBI-07

Eleven markers constituted this linkage group (SBI-07). The total linkage distance covered by these markers was 132 cM and average inter-marker distance was 12 cM. Two large gaps (>26 cM) were observed on this linkage group between markers *Xtxp312* and *Xtxp227* (28 cM) and *Xgap342* and *Xisep0829* (29 cM).

4.7.2.8. Linkage group SBI-08

This linkage group was comprised of 8 markers spanning a distance of 94 cM. The average distance between markers was 12 cM. Two gaps wider than 20 cM were observed between *Xcup47* and *Xiabtp349* and between *Xtxp250* and *Xisp10333*.

4.7.2.9. Linkage group SBI-09

This linkage group was comprised of 8 markers covering a distance of 158 cM. The average inter-marker distance for this linkage group was 20 cM. Three large gaps (> 25cM) were observed on this linkage group, between markers *Xtxp289* and *Xisep0506* (35cM), *Xtxp010* and *Xiabtp475* (31cM) and *Xgap32* and *Xgap206* (34cM).

4.7.2.10. Linkage group SBI-10

This group was comprised of 11 SSR markers covering a distance of 172 cM. The average distance between two adjacent markers was 16 cM with large gaps on the two distal ends of this linkage group i.e. between *Xcup49* and *Xisp10359* (69 cM) and *Xtxp320.2* and *Xtxp141* (38 cM).

4.8. QTL analysis

Composite interval mapping (CIM) was performed using PLABQTL software. The additive × additive interaction genetic model was used for the QTL analysis of all the traits observed on the recombinant inbred line population. A permutation test was performed to determine the LOD threshold at 5% level for type 1 error to declare the presence of a putative QTL. Based on this a minimum LOD threshold value of 3.4 was indicated for all observed traits. However, as in this study one of the objectives was to compare the QTLs identified for the various traits with those observed in other studies, a lower LOD value of 2.5 was used.

Initially RIL mean values for screening each environment were used for the QTL analysis. Subsequently, the QTL × Environment option was used to determine, if QTL × Environment interaction was significant and if QTLs significant in the across-environment analysis were also significant in individual screening environments. The results of the QTL analysis in each environment and across environments are presented in Table18 to 21. Five-fold cross-validation runs were also performed using PLABQTL to determine the value of identified QTLs based on the frequency of the detected QTLs across the five validation runs.

Fourteen putative QTLs distributed across the 10 sorghum linkage groups were identified for the five spotted stem borer resistance component traits in the 2007 rainy season screen, while fifteen QTL were observed for these traits in the 2008 rainy season screen and in across-environment analysis. Using the 2007 phenotypic data set for eleven agronomic traits, 35 QTLs were identified during the 2007 rainy season, however 30 and 31 QTLs were identified, respectively during the 2008 rainy season and across the 2007 and 2008 rainy season environments. The position of the detected QTLs are illustrated in Figure 13 and 14.

4.8.1. Spotted stem borer resistance component traits

QTL identified for spotted stem borer resistance component traits in individual and across screening seasons are presented in Table 18 and 20.

4.8.1.1. Stem tunneling (cm)

In the 2007 rainy season trial, four QTLs were identified for stem tunneling, one each on SBI-02b, 03, 07 and 08; while during the 2008 rainy season five QTLs were identified, two each on SBI-01a and SBI-07 and one on SBI-10. These QTLs were associated with 23 to 40% of the observed phenotypic variation observed amongst the RILs for this trait in the two screening environments. Among all the detected QTLs, the QTL on SBI-08 [between markers *Xtxp292* and *Xisp10279*] (2007 rainy season) and one of the QTLs on SBI-01a [between markers *Xcup24* and *Xcup73*] (2008 rainy season) were associated with the highest proportion of the observed phenotypic variation (16%) and had large LOD scores (10.). Despite the differences in the phenotypic values and genetic variation during the two screening seasons, one of the QTLs on SBI-07 was found to have identical map position in both screening environments. The phenotypic variation explained by this QTL varied from 9% to 5% during the 2007 and 2008 rainy seasons, respectively.

In the combined season analysis, a total of five QTLs were detected explaining 36% of phenotypic variation. Four of these QTLs mapped to the same linkage groups and had similar map positions as observed for the set of QTLs detected during the 2008 rainy season. One QTL identified on SBI-04 [between markers *Xtxp327* and *Xisp10229*] was not detected in either of the two individual screening environment analyses. Except for this QTL, the QTL×E interaction was highly significant for all of the identified QTLs and this was reflected by the highly deviating QTL effects in the two screening environments.

The favorable alleles for all the QTLs identified for stem tunneling across the three analyses were inherited from the stem borer resistant male parent (PB 15520), except for the QTL on SBI-01a [between markers *Xcup01* and *Xcup06*] during the 2007 rainy season and the QTL on SBI-04 in across environment analysis, where the favorable alleles were contributed by the stem borer susceptible female parent (ICSV 745).

All the identified QTLs for this trait exhibited significant additive effects. However, the QTLs detected during the 2007 rainy season had smaller effects and were associated with less phenotypic and genotypic variation than those identified during the 2008 rainy season. No significant interaction was observed among the QTLs identified during the 2007 rainy season trial. However, a QTL on SBI-01a exhibited highly significant additive × additive interaction with both the QTLs detected on SBI-07 in the 2008 rainy season analysis and a single QTL [between markers *Xtxp227* and *Xisp10206*] on SBI-07 in the across-environment analysis. One of the QTLs identified on SBI-07 [between markers *Xtxp227* and *Xisp10206*] exhibited significant interaction with the QTL on SBI-10 in the 2008 rainy season. The two QTLs on SBI-07 also exhibited significant additive × additive interaction among themselves in both the 2008 rainy season and the across-season analysis.

The cross-validation (CV) runs for the 2007 screening environment dataset verified the presence of a putative QTL on SBI-08, as it was observed across all the five validation splits. The QTLs found on SBI-07 and 03 were observed in three and two of the five splits, respectively; while the QTL on SBI-02b was observed only in 1 of the splits. The QTL on SBI-01a observed in the 2008 and across-environment analyses, was also observed in one of the CV splits of the 2007 rainy season analysis.

One of the QTLs observed on SBI-01a [between markers *Xcup24* and *Xcup73*]

and both the QTLs on SBI-07 were observed in all the five validation splits of 2008 rainy season data set analysis; while, the QTLs on SBI-10 and SBI-01 were observed only in three and two of these five splits. The QTL on SBI-04 identified only using the full across-season dataset was also observed in one of the five CV splits of 2008 rainy season dataset.

In the across-environment analysis, a QTL on SBI-01a and another on SBI-07 [between markers *Xtxp295* and *XSbAGB*02] were observed in all the five CV splits, while the second QTL on SBI-07 was observed in four CV splits. In general, the second QTL on SBI-07 (between markers *Xtxp295-Xisep0704*) was observed in all the three analyses [2007 rainy season, 2008 rainy season and across seasons] and in majority of the cross-validation splits (3 to 5) across the three data sets.

4.8.1.2. Deadheart incidence (%)

QTLs for deadheart incidence were detected on four linkage groups [SBI-01a, 03, 07 and 08] during the 2007 rainy season accounting for 34% of the observed phenotypic variation. During the 2008 rainy season two QTLs were detected on SBI-07 and 10 accounting for 15% of the observed variation. In the combined analysis only one QTL was detected, on SBI-07, accounting for 23% of the observed phenotypic variation. The QTL on SBI-07 was common across the three datasets, explained highest phenotypic variation, and had the largest additive effects among all the identified QTLs for this trait, across the three analyses (2007 rainy season, 2008 rainy season and across-season). This QTL did not exhibit any significant interaction with the environment and hence may be considered an appropriate target for marker-assisted backcrossing.

The alleles for reduced deadheart incidence (%) were inherited from the stem borer resistant parent (PB 15520) at most of the QTLs detected in individual and

combined environment analyses, except for the QTL on SBI-01a and 08 with data from the 2007 rainy season. Significant main effects (additive) were observed among all the QTLs identified. However, significant interaction (additive × additive) interaction was observed only between the QTL detected on SBI-01a and the major QTL detected on SBI-07 during the 2007 rainy season.

Cross-validation (CV) runs for this trait strongly supported the presence of a putative QTL on SBI-07 [between markers *Xisep0829* and *Xtxp295*], as it was observed in all the five validation splits across all the three analyses. The QTL on SBI-01a [between markers *Xisp10340* and *Xiabtp450*] was observed in three of the five validation splits for the 2007 rainy and combined environment analyses, while the QTL on SBI-10 [between markers *Xisp10263* and *Xtxp320.2*] was observed in three validation splits for the 2008 rainy season data set and one of the five CV splits using the combined environment data set.

4.8.1.3. Leaf feeding damage

Two QTLs each were detected for this trait using the 2007 [on SBI-02a and 07] and 2008 rainy season [on SBI-01b and 07] data sets. These QTLs together explained a total of 11 to 12% of the observed phenotypic variation in each of the two screening seasons. The major proportion of the explained variation was provided by the QTL on SBI-07 [between markers *Xisep0829* and *Xtxp295*] during the 2007 rainy season, while both the QTLs detected during the 2008 season contributed equally to the explained variation. In the combined-season analysis, QTLs were detected on SBI-02a [between markers *Xisep0747* and *Xtxp025*] and SBI-07 [between markers *Xtxp227* and *Xisp10206* and, *Xisep0829* and *Xtxp295*], which together accounted for 17% of the observed phenotypic variation among the RIL population progeny means for this trait. No significant QTL × E interaction was observed among these detected

QTLs except for the QTL detected between markers *Xisep0829* and *Xtxp295*.

Though the QTL on SBI-07 [between markers *Xtxp227* and *Xisp10206*] accounted for high proportions of total explained phenotypic variation in all three analyses, their positions were identical only in the 2008 rainy and across-season analyses. Similarly, the genomic location of the QTLs identified on SBI-02a was similar only in the 2007 rainy and across-season analyses.

Favorable alleles (reducing leaf feeding damage) for all the QTLs identified for this trait were contributed by the stem borer resistant male parent (PB 15520). The main effects (additive) were significant for all the QTLs identified across the three analyses. However, the interaction (additive × additive) effects were significant only between the QTLs detected on SBI-02a and SBI-07 using the 2007 data set.

The five-fold CV runs weakly supported the presence of QTLs for this trait on SBI-02a [between markers *Xisep0747* and *Xtxp025*] and SBI-07 [between markers *Xtxp227* and *Xisp10206*], as these were observed in at least one of the five validation splits in individual and across-environment analyses. The QTL on SBI-02a was observed in two validation splits in the 2007 and 2008 rainy season analysis and in four of the five validation splits in across-season analysis. Similarly, the QTL on SBI-07 [between markers *Xtxp227* and *Xisp10206*], was observed in four of the five validation splits during the 2008 rainy and joint season analyses, while this QTL was also detected in one of the validation splits using the 2007 rainy season data set.

4.8.1.4. Recovery resistance

Three QTLs for recovery resistance were detected on SBI-01b, 02b and 07 using the 2007 rainy season data set. While, with the 2008 rainy season data sets two QTLs each were detected on SBI-03 and 07. The total phenotypic variation accounted for by these QTLs ranged from 28% to 38% in the 2007 and 2008 rainy seasons,

respectively. In the joint analysis across two screening seasons, three QTLs were detected. These were observed on SBI-03 (2 QTLs) and 07 (2 QTLs), and together explained 35% of the observed phenotypic variation for this trait. All the three QTLs exhibited significant QTL × E interaction except for one of the QTL on SBI-07 [between marker *Xisep0829* and *Xtxp295*].

Major proportion of the explained phenotypic variation in 2007 rainy season data set were accounted for by the QTL detected on SBI-02b [between markers *Xtxp298* and *Xtxp056.1*] and the QTL on SBI-07 [between markers *Xtxp295* and *XSbAGB02*]. The QTL on SBI-07 [between markers *XSbAGB02* and *Xcup57*] also explained the highest proportion of the phenotypic variation among all the QTLs identified using the 2008 rainy season data set and across environment data set. The genomic position of this QTL was also similar in all the three analyses. The favorable alleles for all the QTLs detected for this trait across the three analyses were contributed by the spotted stem borer resistant male parent (PB 15520). Though the additive effects of all of the detected QTL were high, no significant interaction was observed between these QTLs.

The five-fold CV test very strongly supported the presence of a putative QTL for this trait on SBI-07 (between markers *XSbAGB02* and *Xcup57*), as it was detected in all five CV data subsets of all the three analyses. The QTL on SBI-03 was also observed in all five CV splits of the 2008 rainy and across-season analysis. However, this QTL was not detected at all in the 2007 rainy season data set.

4.8.1.5. Overall resistance

For overall resistance, one putative QTL was detected on SBI-07 [between markers *Xisep0829* and *Xtxp295*] using the 2007 rainy season data set and two QTLs were identified on SBI-03 [between markers *Xisp10361* and *Xtxp034*] and 07 [between

marker *Xisep0829* and *Xtxp295*] from both the 2008 rainy and across-environment analyses. No significant QTL × E interaction was observed among the detected QTLs for this trait.

Together the putative QTLs for overall resistance explained in a simultaneous fit 15%, 11% and 21% of the observed phenotypic variation across the three analyses (2007 rainy season, 2008 rainy season and across-season), respectively. As for the recovery resistance, the highest proportion of the total phenotypic variation was accounted by the QTL on SBI-07 in the individual and across-environment analyses. The genomic location of this QTL was also similar in all three analyses.

The favorable allele for the QTL on SBI-07 was contributed by the resistant parent PB 15520. However, for the QTL detected on SBI-03 with the 2008 rainy season data set and in the across-environment analysis, the resistance allele originated from the susceptible parent (ICSV 745). Highly significant additive effects were observed for all of the identified QTLs for this trait, while no significant interaction was observed between them. Cross-validation runs performed for this trait detected the QTL on SBI-07 in all the five CV data subsets across the three analyses.

4.8.2. Agronomic and morphological traits

QTL identified for agronomic and morphological traits in individual and across screening seasons are presented in Table 19 and 21.

4.8.2.1. Glossiness

This trait was not recorded on the recombinant inbred line population during the 2008 rainy season evaluation. However, in 2007 rainy season two QTLs were observed, one each on SBI-01a [between markers *Xcup24* and *Xcup73*] and 09 [between markers *Xgap032* and *Xgap206*]. These QTL together accounted for 7% of the

observed variation in glossiness. The major proportion of the explained phenotypic variation attributable to the QTL on SBI-01a, for which the glossy alleles were contributed by the female parent (ICSV 745). For the QTL on SBI-09, the favorable glossy alleles were contributed by the male parent (PB 15520).

The QTL on SBI-01a was observed in four of the five cross-validation data sets, while that on SBI-09 was observed only in 1 of the validation splits.

4.8.2.2. Seedling vigor

Six QTLs were identified for this trait using the 2007 rainy season data set. Four of them were localized on SBI-01 (three on segment a, and one on segment b), while one each were located on SBI-03 and 09. Using 2008 rainy season data set, two QTLs each were detected on SBI-01a and 03 and a single QTL was located on SBI-04. The six QTLs identified using the 2007 rainy season data set together accounted for 27% of the observed variation in RIL means for seedling vigor score, while the five QTLs detected using 2008 rainy season data set could explain only 19% of the total phenotypic variation observed.

In the combined analysis five putative QTL were detected, three were localized on SBI-01 (two on segment a, and one on segment b), while the other two were located on SBI-03 and 04. These QTL together accounted for 19% of the phenotypic variation in the across-season RIL mean seedling vigor scores. None of the identified QTL was significantly influenced by the environment. The favorable alleles contributing to greater vigor for the QTLs detected were contributed by both the parents, with the male parent contributing favorable alleles for the QTL on SBI-01a [between markers *Xcup01* and *Xcup06*] in all the three analysis, those on SBI-01b [between markers *Xcup60* and *Xcup44*] in the 2007 rainy season and across-season analyses and that on SBI-04 [between markers *Xtxp327* and *Xisp10229*] detected in

the 2008 rainy and across-season analyses.

Two QTLs detected on SBI-01a [between marker *Xcup01*and *Xcup73*] and a single QTL identified on SBI-03 [between markers *Xisp10332* and *Xisp10361*] were common in all the three analyses. All the identified QTL exhibited highly significant additive effects. Significant additive × additive interaction effects were observed only among the QTLs identified during the 2007 rainy season. Wherein, the QTL identified on SBI-03 [between markers *Xcup38* and *Xisp10332*] exhibited significant interaction with both the QTLs detected on SBI-01a [between marker *Xcup06* and *Xcup24,* and *Xisp10340* and *Xiabtp450*]

The three genomic regions associated with SBI-01 were each observed in at least one of the five validation runs in individual season as well as across-season analyses. Similarly, one of the QTL on SBI-03 [between markers *Xisp10332* and *Xisp10361*] was detected in three validation runs using both the 2007 and 2008 rainy season data sets and in two runs in combined environment data sets.

4.8.2.3. Seedling basal pigmentation

A single major QTL accounting for nearly 40% of the observed phenotypic variation in the RIL progeny mean seedling basal pigmentation score, and relatively independent of the influence of environment, was identified on SBI-06 (between markers *Xisp10264* and *Xtxp057*), in all three analyses. The LOD score observed for this QTL was also very high (>25) in all three analyses. Two more putative QTL were identified during the 2008 rainy season screen, one each on SBI-01b [between markers *Xtxp340* and *Xtxp228*] and 04 [between markers *Xtxp327* and *Xisp10229*]. However, these additional QTLs explained only minimal proportions (<5%) of the observed phenotypic variation for this trait. The QTL identified on SBI-04 exhibited significant interaction with the major QTL on SBI-06. The alleles providing darker

basal pigmentation were contributed by the male parent (PB 15520). The QTL on SBI-06 was detected in all five runs of the cross-validation for all three data sets.

4.8.2.4. Plant color

Similar to the trait seedling basal pigmentation, one major QTL accounting for more than 38% of the phenotypic variation was detected for foliage color (tan vs non-tan) on SBI-06 (between markers *Xisp10264* and *Xtxp057*), in all three analyses. Another QTL accounting for 4% of the observed phenotypic variation in plant color detected on SBI-07 using the 2007 rainy and across-season data sets. This region may probably be harboring a modifier gene for plant color. However, no significant interaction was observed between the plant color QTLs detected on SBI-06 and SBI-07. Both these two QTLs were also relatively independent of the influence of the environment. The darker pigmentation alleles for these two QTLs were contributed by parent PB 15520.

The major QTL on SBI-06 was observed in all five validation runs of all three cross-validation analyses, while the QTL on SBI-07 was detected in two of the five runs for each of the individual environments and three of the five validation runs for the across environment analyses.

4.8.2.5. Testa (presence vs absence)

A single major QTL was observed for this trait on SBI-04 [between markers *Xtxp327* and *Xisp10229*] in all three analyses. The total phenotypic variation explained by this QTL ranged from 25% for the 2007 rainy season to more than 55% for the 2008 rainy and across-season analyses. Across all the analyses pigmented testa presence alleles at this single major QTL were contributed by the male parent (PB 15520, which itself has a pigmented testa) and no significant interaction with the environment was observed for this QTL. This QTL was observed in all five validation splits in both of

the individual environment and across-environment cross-validation runs. This QTL appears to be one of the two complementary major genes (B_1/b_1 and B_2/b_2) known to control the presence of a pigmented testa.

4.8.2.6. Mesocarp thickness

Two putative QTLs together accounting for 26% and 24% of the total phenotypic variation were detected for mesocarp thickness score using the 2007 rainy and 2008 rainy season data sets, respectively. Although for both the screening seasons the QTLs were localized on the same linkage groups (SBI-01 and 04), the map position for the QTL detected on SBI-01 was significantly different (between markers *Xcup44* and *Xtxp340* on segment b in the 2007 rainy season and between markers *Xisp10203* and *Xisp10359* on segment a in the 2008 rainy season). Major proportions of the total phenotypic variation observed among the RIL population progenies for this trait were accounted for by the QTL on SBI-04 [between markers *Xtxp327* and *Xisp10229*] in both screening seasons. This QTL accounted for 36% of the observed phenotypic variation in the combined-season analysis. The allele for thick mesocarp at this QTL was contributed by the parent PB 15520 (which itself has a thick mesocarp) and the interaction of this QTL with environment was non-significant, indicating that its phenotype was relatively easy to score. In the cross-validation test this major QTL was observed in all the five validation runs in both individual and across-environment analyses.

4.8.2.7. Leaf angle

In both individual screening environment analyses and across environment analysis only a single major QTL was detected for leaf angle and was detected on SBI-04 [between markers *Xtxp327* and *Xisp10229*]. This QTL explained 19% of the

phenotypic variation in RIL means in the 2007 rainy season data set, while for the 2008 rainy season and combined-season analyses this QTL accounted for nearly 40% of the observed phenotypic variation. Though no significant interaction with environment was observed for this trait, the main effects (additive) estimated for this QTL differed significantly in the two environments. The allele for erect leaf angle at this QTL was contributed by the male parent PB 15520.

Cross-validation tests for this trait detected this major QTL on SBI-04 in all five validation runs in the individual season and combined season analyses. A second putative QTL on SBI-07 (between markers *XSbAGB02* and *Xcup57*) was also identified in one of the five validation runs with the 2008 rainy season data set.

4.8.2.8. Time to 50% flowering

For this trait six putative QTLs were identified on SBI-01 (two on segment a and one on segment b), 04, 06 and 10 in individual screening environments and five on SBI-01 (one on segment a and one on segment b), 04, 06 and 10 in combined environment analyses. These QTL collectively explained 35 to 48% of the observed phenotypic variation across the three analyses. The QTL detected on SBI-06 [between markers *Xisep0444* and *Xisp10264*] exhibited significant interaction with the environment, while for all the other QTL, the QTL × E interaction component was non-significant. The QTL on SBI-06 likely corresponds to the Ma_1/ma_1 locus, which is a major determinant of photo period temperature response of flowering in sorghum (Quinby and Karper, 1945).

Across analyses, the highest proportion of the observed phenotypic variation was accounted for by the major QTL on SBI-06 [between markers *Xisep0444* and *Xisp10264*], followed by those identified on SBI-04 [between markers *Xisp10230* and *Xiabtp*364], SBI-10 [between markers *Xtxp270* and *Xtxp217*] and SBI-01a [between

markers *Xtxp357* and *Xisp10203*]. Though the QTL on SBI-01b [between markers *Xcup44* and *Xtxp340*] accounted for the least amount of phenotypic variation, it interacted significantly with the QTL on SBI-04 [between markers *Xisp10230* and *Xiabtp*364] in both the 2008 rainy season and combined season analyses. Though this interaction was non-significant in the 2007 rainy season, the other QTLs on SBI-01a between markers *Xcup06* and *Xcup24,* and between markers *Xtxp357* and *Xisp10203* exhibited significant interaction with the QTL on SBI-04 and 06, respectively during that season. For all the identified genomic regions associated with this trait, the alleles of male parent PB 15520 exerted a positive additive effect (late flowering), except for the QTL detected on SBI-01 (segments a and b), where the late flowering alleles were contributed by the female parent ICSV 745.

The QTL on SBI-01b, 04, 06 and 10 were detected in at least four of the five validation runs of the cross-validation test across analyses, while the two QTLs on SBI-01a were detected in three and four of the cross-validation splits in individual and across-season analyses.

4.8.2.9. Number of nodes per plant

Using the 2007 rainy season data sets, four QTLs were identified for number of nodes per plant. These were localized on SBI-04, 06, 07 and 10. The QTLs observed on SBI-06, 07, and 10 were also detected using the 2008 rainy season data set. For both seasons the detected QTLs accounted for more than 20% of the observed phenotypic variation in RIL mean node number.

In the combined-season analyses, all four QTLs identified using the 2007 rainy season were detected; however, they collectively explained a higher proportion of the phenotypic variation for this trait (31%). Similar to the trait time to 50% flowering, the QTL on SBI-06 [between markers *Xisep0444* and *Xisp10264*] exhibited

significant QTL × E interaction. This similarity is not un-expected as number of nodes on the main stem is a function of number of phyllochroms prior to panicle initiation and hence is determined by many of the same factors as flowering time, so that later flowering time is accompanied by an increased number of nodes

Alleles reducing the number of nodes for all the identified QTL were contributed by the female parent (ICSV 745). No significant interaction was observed among the detected QTLs for the 2007 analysis and across-season analysis. However, for the 2008 rainy season analysis, one of the QTL detected on SBI-10 [between markers *Xtxp270* and *Xtxp217*] exhibited significant interaction with the QTL detected on SBI-06 [between markers *Xisep0444* and *Xisp10264*] and 7 [between markers *Xtxp270* and *Xtxp217*].

The QTL identified on SBI-10 [between markers *Xtxp270* and *Xisp10263*] was detected in all the 5 validation runs for individual and across-season analyses. The QTL on SBI-06 and 07 were detected in five validation runs for the 2007 rainy season and combined season analyses and in three of the five validation runs for the 2008 rainy season.

4.8.2.10. Plant height

Individual season and combined season analyses detected three unlinked genomic regions on SBI-01a, 06 and 07 having significant effects on plant height. Collectively these three QTLs explained 33 to 42% of the total variation for plant height in the individual environment and across-environment analyses. Each of these regions accounted for 6 to 31% of the observed phenotypic variation for RIL plant height means. The highest proportion of the phenotypic variation was explained by the QTL on SBI-07 [between markers *Xtxp295* and *XSbAGB02*] in each of these three analyses. However, this QTL also exhibited significant QTL × E interaction, with substantially

larger additive effects and adjusted R^2 values in the 2008 rainy season screen than during 2007 rainy season.

The favorable alleles (for reduced plant height) for all the identified QTL were contributed by the parent PB 15520 and individually decreased plant height from 11 cm to 23 cm across analyses. Highly significant unfavorable additive × additive interaction (increasing plant height) was observed between the QTL identified on SBI-06 [between markers *Xisep0444* and *Xisp10264*] and SBI-07 [between markers *Xtxp295* and *XSbAGB02*] in the 2007 rainy season, while in the 2008 rainy season significant favorable interaction (reducing plant height) was observed between the QTL on SBI-01a [between markers *Xcup73* and *Xtxp357*] and 06 [between markers *Xisep0444* and *Xisp10264*].

The QTLs detected on SBI-06 and 07 were detected in all five runs of cross validation, while the QTL on SBI-01a was detected in four validation runs for the 2008 rainy season analyses.

4.8.2.11. Agronomic performance

Seven QTLs were detected for this trait using the 2007 rainy season data set. Two QTLs each were located on SBI-01a, 2 (segment b) and 4, while a single QTL was detected for this trait on SBI-07. These QTLs together accounted for 32% of the phenotypic variation observed among the RILs for this trait in 2007. In the 2008 rainy season only 5 QTLs were detected together explaining nearly 40% of the variation. These were localized on SBI-01a, 04, 07 (two QTLs) and 10.

In the across-environment analyses seven QTL were detected on SBI-01a [two QTLs], 02b [2 QTLs], 04 and 07 [two QTLs] accounting for nearly 43% of the observed phenotypic variation. QTL × E interaction were significant for all the identified QTLs except for the ones on SBI-04, and 07.

A comparison of the genomic locations of the QTLs identified from the three analyses revealed identical positions for three QTL one each on SBI-01a [between markers *Xcup01* and *Xcup24*], 04 [between markers *Xtxp327* and *Xisp10229*] and 07 [between markers *Xisep0829* and *Xtxp295*]. The highest proportion of the total phenotypic variation was accounted for by the QTLs on SBI-07 [between markers *Xisep0829* and *Xtxp295*] followed by the QTLs on SBI-01a [between markers *Xcup01* and *Xcup24*], and 04 [between markers *Xtxp327* and *Xisp10229*] across analysis

Favorable alleles (increasing agronomic performance score) for all the identified QTL were contributed by the parent PB 15520 except for the two QTL, identified on SBI-02b during the 2007 rainy season and across environment analysis and a single QTL detected on SBI-10 during the 2008 rainy season. Significant additive × additive interaction was observed between the QTLs on SBI-01a [between markers *Xcup01* and *Xcup06*] and the QTL on SBI-04 [between markers *Xtxp327* and *Xisp10229*] during the 2007 rainy season. Cross-validation tests detected the QTL on SBI-07 [between markers *Xisep0829* and *Xtxp295*] in all five validation runs across all the three analyses, while the other major QTL on SBI-04 was observed in five validation runs for the 2008 rainy and across-season analyses and two of five validation runs for the 2007 rainy season analysis.

DISCUSSION

CHAPTER V

DISCUSSION

Sorghum ranks among the world's most important cereal crops and is well adapted to growth under semi-arid conditions, but its yield is constrained by many factors including insect pests. Spotted stem borer is one of the major insect pests of sorghum and can cause considerable losses in yield and quality of grain and stover. The most economical and environmentally acceptable means of reducing losses to sorghum insects is the use of genetically resistant cultivars. However, resistance breeding programmes aiming to incorporate spotted stem borer resistance to agronomically desirable cultivars have met with limited success to date. Lack of comprehensive genetic characterization of stem borer resistance due to the large environment effects and genotype by environment (G×E) interactions resulting from insect pressure variability and variability in environmental conditions that are critical for insect infestation is one of the major reasons for the slow progress in stem borer resistance breeding. Many early reports have suggested that the inheritance of resistance is controlled by several genes (Rana and Murty, 1971; Kulkarni and Murty, 1981; Pathak and Olela, 1983; Pathak, 1983, 1990; Rana *et al.,* 1984). However, it is difficult to characterize these genes through conventional genetic analysis, because the individual effects of each of these genes on phenotype are relatively small. Even in cases where a few genes may exert strong effects, genetic analysis for determination of the number of genes involved and their chromosomal locations, has been difficult because of the lack of discrete phenotypic segregation among the progeny from crosses of resistant and susceptible parental stocks.

The recent use of molecular markers in quantitative genetic studies has greatly facilitated the study of complex quantitative traits and made it possible to dissect polygenes for such traits into individual Mendelian factors (Paterson *et al.,*

1991). Using molecular-linkage genetic maps and quantitative trait locus (QTL) mapping technology, it is now possible to estimate the number of loci controlling genetic variation of a trait in a segregating population and to determine the map positions of these loci in the genome. The identification of QTLs underlying traits of spotted stem borer resistance, agronomic and morphological traits in sorghum could provide an impetus to spotted stem borer resistance breeding programmes. Thus, the present study was primarily aimed to map and characterize QTLs associated with spotted stem borer resistance and other agronomic and morphological traits using a well-characterized mapping population of random inbred lines (RILs) derived from a cross of susceptible and resistant parental lines. The results of phenotypic and genotypic analysis of spotted stem borer resistance traits and other agronomic and morphological traits are discussed here.

5.1. Phenotypic analysis

Reliable phenotypic characterization of the quantitative traits is a pre-requisite to the application of molecular genetic knowledge for broadening our understanding of their genetic control. In the present study, a recombinant inbred line (RIL) population was evaluated at ICRISAT-Patancheru under conditions of artificial stem borer infestation during the 2007 and 2008 rainy seasons, with a major objective of identification of genomic regions associated with spotted stem borer resistance. Phenotypic observations were recorded on the RILs for five spotted stem borer resistance component traits — leaf feeding injury, deadheart incidence, stem tunneling, recovery resistance and overall resistance. Among these traits, leaf feeding injury, deadheart incidence and stem tunneling have been reported as the most important measures of resistance to spotted stem borer, while “recovery resistance” (a good measure of the number of productive tillers formed by the plants consequent to deadheart formation)

is suggested as an important parameter in screening for spotted stem borer tolerance. The other resistance measures, overall resistance score, provides an indication of the overall response of a RIL to infestation by spotted stem borer, which takes into account both resistance (leaf feeding damage, deadheart incidence and stem tunneling) and tolerance (number of productive tillers and the overall plant stand) parameters. Apart from spotted borer resistance component traits, five agronomic and six morphological traits were also observed on the RIL population, for which the parental lines showed sufficient phenotypic differences. The data collected for observed traits during 2007 and 2008 rainy seasons were subjected to statistical analysis. The results obtained are discussed here, under the following topics: Mean performance, Genetic variance, heritability and G×E interaction, Frequency distributions and transgressive segregation, and character association.

5.1.1. Mean performance

5.1.1.1 Spotted stem borer resistance component traits

An insight into the mean performance of the parents of the RILs indicated substantial differences between them for all observed stem borer resistance parameters (leaf damage rating, deadheart incidence, stem tunneling, recovery resistance score and overall resistance score). Although neither of the parental lines was free from spotted stem borer damage, the observed low mean values for all of the resistance component traits for the male parent and high mean values recorded for the female parent are indicative of the greater resistance of the male parent (PB 15520) and extreme susceptibility of the female parent (ICSV 745) to this pest. The contrasting features of the parents for spotted stem borer resistance component traits satisfy the requirement of sufficient phenotypic polymorphism between the parental lines for an efficient segregation analysis. This was accompanied by the necessary marker-genotype

polymorphism required for linkage map construction (Young, 2001). Among the RILs a wide range of expression for resistance to spotted stem borer damage was observed ranging from very high resistance (low to damage levels) to very low (severe damage levels). The high degree of variation observed can be attributed to the frequent breakage and reshuffling of linked genetic loci controlling these traits through several meiotic events during the development of the RIL mapping population.

While high levels of variation were observed between the RILs for all the five resistance component traits, it is notable that the observed ranges among the RILs for most of these parameters, particularly stem tunneling and deadheart incidence, greatly differed across the two screening seasons. A comparison of the two environments indicated that in general, the level of "stem tunneling" recorded on RILs was substantially greater during 2008 than during the 2007 rainy season, while lower deadheart incidence, greater recovery and better overall resistance was observed among the infested plants in the 2008 rainy season. One of the reasons for this substantial difference in the phenotypic expression for the observed traits could be the influence of environment. Of all the environmental factors, availability of moisture is one of the most important limiting factor influencing survival and growth of both spotted stem borer larvae and sorghum plants (Berger, 1992; Kumar, 1997; Sharma *et al.,* 2005). A long dry spell after infestation can increase mortality among early instar larvae or can induce long diapause (Berger, 1992; Kfir, 1997; Taley and Thakrey, 1980) reducing the incidence of damage and increasing apparent resistance of the plants. In the present study, the two screening environments (2007 and 2008 rainy seasons) did not vary significantly for most of the important weather parameters except for the amount of rainfall received (Table 22). The relatively dry weather of 2007, particularly during the month of July (soon after the artificial infestation of the

sorghum seedlings with neonate spotted stem borer larvae), due to very scanty rainfall might have caused greater mortality among the neonate larvae, hence reducing the larval density, incidence of tunneling and tunnel length. Further, the higher precipitation during the 2008 rainy season, especially during the initial stages of crop growth in July and August (after artificial infestation) could have increased plant growth rate resulting in pushing of the growing tip upwards relatively faster preventing the larvae from causing deadheart formation, but providing conditions favorable for larvae to survive until the stem tunneling phase of the infestation. In addition, the higher moisture levels could have induced rapid initiation and development of auxiliary tillers and better uptake of nutrients, hastening recovery of stem borer damaged plants and thereby influencing tolerance expression. The influence of environment on these traits can further be confirmed by estimating the G×E variance.

The mean performance of the RILs compared to that of their parents indicates the nature of genetic effects (additive, dominance or epistatic effects) for a trait. A value near to the mid-parental value suggests absence of dominance effects (Mather and Jinks, 1971), while a significant difference between the population mean and mid-parental value indicates predominance of dominance or epistatic gene effects (Jinks and Pooni, 1981). In the present study, the mean performance of RILs across environments for most of the resistance component traits except stem tunneling remained near the mid-parental value, ruling out dominance for these traits (Table 15). Predominance of additive gene effects for foliar damage, deadheart incidence and both additive and dominance effect for overall resistance score and recovery resistance score have been reported by Sharma *et al.,* (2007). Pathak (1990) also emphasized the role of both additive and dominance gene effects for the expression of

resistance to spotted stem borer, and reported that leaf feeding damage is controlled primarily by additive effects, while both additive and dominance gene effects contribute to the inheritance of deadheart incidence.

For stem tunneling the mean performance of RILs was slightly skewed towards the resistant parent suggesting either partial dominance of resistance or the role of epistatic interaction. Hagi (1984) reported predominance of non-additive gene action for stem tunneling, while additive gene action for stem tunneling has also been reported by Pathak and Olela (1983) and Nour and Ali (1998).

5.1.1.2 Agronomic and morphological traits

Substantial difference for mean performance was observed among the parental lines for all the observed agronomic and morphological traits except glossiness (both the parental lines were non-glossy). The mean performances of RILs for most of the agronomic and morphological traits observed were skewed towards one of the parental mean phenotypes, except in case of testa score (presence or absence) and agronomic performance score across screening seasons (Table 15). The mean performance of the RIL population for seedling vigor score neared that of the male parent suggesting partial dominance or epistasis for this trait. A similar trend was also observed for plant color score in agreement with the known dominance of red color to tan color. Mean performance of the RILs for basal pigmentation score was skewed towards that of the female parent (non-pigmented) indicating the role of epistasis or dominance for this morphological marker trait. Previous studies have reported that two genes each, exhibiting significant epistatic effects control plant color and basal pigmentation in sorghum (Woodworth, 1936; Ayyangar and Reddy, 1942; Laubscher, 1945; Stephens, 1947; Doggett, 1988). Skewness of the RIL population towards the female parent phenotype was also observed for the trait mesocarp thickness,

suggesting at least partial dominance of thin mesocarp over thick mesocarp. This result corroborates earlier findings, which suggested thin mesocarp can be governed by a single gene "*Z*" exhibiting dominance over thick mesocarp (Rooney *et al.,* 1980; Doggett, 1988). The mean of the RIL population for leaf angle was skewed towards that of the low value female parent and their difference was non-significant. Apart from dominance this non-significant difference between the female parent and the RIL population mean could be due to association of flat leaf angle with a fitness trait. Erect leaf angle is largely controlled by a single recessive gene "*lg*" (Ayyangar *et al.,* 1935) and previous study has reported a close association of genes governing leaf angle and lethality (yellow seedling mutant) in sorghum (Karper and Conner, 1931; Doggett, 1988). This association might have resulted in the increased frequency of plants with flat leaf angle during the development of the RIL population.

The mean performance of the RIL population was skewed towards the female parent for time to 50% flowering and number of nodes. The relatively low mean performance of RILs for these traits might be due to earlier initiation of flowering in response to the biotic stress (spotted stem borer). Previous studies in sorghum have also reported a high influence of environment on six major maturity loci influencing both floral initiation and number of nodes in sorghum (Quinby and Karper, 1945; Quinby, 1966; Quinby, 1973; Childs *et al.,* 1997; Rooney and Ayden; 1999). The mean performance of RILs for plant height, though significantly different from the parental mean, was slightly skewed towards the female parent suggesting partial dominance of tallness over dwarfness and/or epistatic interaction between loci governing plant height in this population. Similar results for plant height have also been obtained in sorghum (Quinby and Karper, 1954).

A perusal of the mean performances of RILs in terms of agronomic and morphological traits indicated that differences among the RILs were not substantial across environment for most of these traits. However, the most unstable trait was plant height. In general, the RILs exhibited greater plant height during the 2008 rainy season as compared to 2007; as described in the previous section, this difference in the plant phenology can be explained based in part on the higher availability of moisture due to higher precipitation during July and August of the 2008 rainy season than during these months of the 2007 rainy season, which might have induced faster growth and development of plants.

5.1.2 Genetic variance, heritability and G×E interaction

The reliability of a QTL mapping largely depends upon the variation and heritability of individual traits (Kearsey and Farquhar, 1998). Phenotypic variations observed between individuals are a result of both genetic and environment differences (Walsh, 2001). Expression of a quantitative trait is largely determined by the interaction of the genotype with the environment, also known as (G×E). G×E interactions may be defined as the varying performance of individual genotypes across environments. While a portion of the varying performance observed might be a result of data noise (*i.e.,* experimental error), some may actually represent the involvement of different loci with slightly different expression for the trait in response to different environmental condition. These variations are quantified using variance; a high genetic variance (relative to the phenotypic variance) indicates that the variation observed in a population is mainly due to the sum of genotypic values and their interactions at all loci associated with the trait. In a selfed population such as RILs, where the frequency of heterozygotes is minimal, the genetic variance is a fair indicator of the importance of additive effects and additive × additive interactions.

Heritability is another useful quantitative parameter that provides a measure of the relative importance of the genetic component in determining the expression of characters (Allard, 1960). A high heritability indicates a high genetic basis for variation while, a low heritability implies that the observed variation lacks genetic basis and is largely environmental (Walsh, 2001). Hence, estimation of the genetic variance and heritability for a quantitative trait allows one to evaluate the degree to which genetics influences expression of the trait.

In general, quantitative variances caused by genetic factors can be concealed by G×E interaction. Hence, G×E often results in reduction of the association between the phenotypic and genotypic values and leads to variable levels of significance of QTL effects across environments (Hayes *et al.,* 1993). Thus, a prior knowledge of G×E is essential for determining appropriate strategies for improving these traits.

5.1.2.1 Spotted stem borer resistance component traits

Individual environment analysis of RILs documented highly significant genetic variance for all of the observed spotted stem borer resistance component traits. In both the screening environments broad-sense heritability estimates for all the observed resistance component traits were also high, indicating a genetic basis for the observed variation and a good control of within-trial environment variation and experimental error.

Significant genetic variances observed for all the stem borer resistance component traits in the across-environment analysis indicate that the differences observed among the RILs should persist across environments. However, the high and significant G×E variance and low to moderate heritability for these resistance component traits indicate that a major portion of the variation observed among the RILs for these traits was due to interaction of genes with the screening environments.

Among all the resistance component traits observed, the level of G×E variance was highest compared to genetic variance, and heritability was lowest for stem tunneling and deadheart incidence, suggesting a major role of environment and genotype × environment in the expression of these traits. The poor correspondence of the individual RIL means across environments for these traits further corroborates these results. Studies dealing with G×E interaction across crops have suggested that high and significant G×E effects are usually due to inconsistent genotypic responses to photoperiod, temperature, soil moisture, soil type, or fertility level from location to location and year to year (Liang *et al.,* 1966; Paterson *et al.,* 2002). In the present study the difference in the amount and distribution of rainfall received across the two environments might have been the most probable reason for the variation in the expression of resistance in the genotypes as the trials were sown on the same date, the same field and under optimal soil fertility conditions for the two years evaluations.

5.1.2.2 Agronomic and morphological traits

Highly significant genetic variation and high heritability estimates were observed among the RILs for most of the observed agronomic and morphological traits in the individual environment and across-environment analyses, indicating a substantially genetic basis for the observed variation among the RILs and low within trial environmental variation and error.

In the across-environment analysis, G×E variance ($\sigma^2_{g\times e}$) values were non-significant for seedling vigor score and seedling basal pigmentation score, while for the plant color score the magnitude of this parameter was substantially lower than the genetic variance, suggesting a very limited influence of environment on these traits. Limited environmental influence coupled with high heritability estimates for these traits indicate the possibility of identifying major genetic loci controlling these traits

through molecular marker analysis. Previous studies in sorghum have identified several major genetic loci associated with these traits (Woodworth, 1936; Doggett, 1988; Sajjanar, 2002; Deshpande, 2005). Among all the observed agronomic and morphological traits, only for plant height G×E variance recorded was substantially higher than the observed genetic variance, so across-environment heritability for this trait was moderate. High G×E variance coupled with moderate and inconsistent levels of plant height across the two screening environments indicates a substantial influence of environment on this trait.

5.1.2. Frequency distributions and transgressive segregation

RILs are inbred lines derived from a cross between two diverse parents. From the frequency distribution on such a population, the number of genes and their interaction controlling a trait can be inferred. The number of major phenotypic classes is directly related to the number of genes determining the trait. Rao *et al.* (2007) opined that if genes controlling a trait are additive and explain equal variance, then there must be N+1 number of phenotypic classes for N number of genes. In addition, a perfect normal frequency curve indicates predominance of additivity, while a skewed distribution and presence of large numbers of transgressive segregants implicates epistatic interaction (Pooni and Jinks, 1982; de Vicente and Tanksley, 1993; Rieseberg *et al.,* 1999; Dickinson *et al.,* 2003). Presence of a large proportion of transgressive segregation for a trait also indicates that both favorable and unfavorable alleles for a trait are dispersed in the parents.

5.1.2.1 Spotted stem borer resistance component traits

Assesement of the frequency distribution of the five major stem borer resistance component traits among RILs in individual environment and across-environment data

sets indicated a continuous distribution for these traits implicating their polygenic inheritance. These results are in conformity with several previous studies (Rana and Murty, 1971; Kulkarni and Murty, 1981; Pathak and Olela, 1983; Pathak, 1985; Agrawal *et al.,* 1990; Prem Kishore, 2001; Sharma *et al.,* 2007). The distributions were skewed for most of the spotted stem borer resistance component traits, except recovery resistance, indicating a significant role of epistatic effects among the loci controlling these traits. Transgressive segregation observed for all of these traits further confirms the likely role of epistasis in the genetic control of these traits. Predominance of epistatic effects for spotted stem borer resistance in sorghum, particularly under artificially infested conditions has been reported in previous studies (Hagi, 1984; Pathak, 1990).

The distribution of RILs for stem tunneling clustered into five classes — three segregative and two transgressive segregant groups. These five classes for tunneling could be explained by the presence of at least four major genetic loci, contributing to the expression of this trait. The presence of transgressive segregation indicates the presence of both favorable and unfavorable alleles dispersed in the parents.

For deadheart incidence and leaf damage rating, two broad phenotypic classes were observed across environments, assuming that the alleles interact in an additive manner, at least one major genetic locus is expected to be controlling each of these traits. However, the unequal proportion of the distribution among the phenotypic classes, particularly for leaf feeding damage, indicates the presence of one more parallel locus contributing to reduction in leaf feeding damage. A high frequency of transgressive segregation was observed for both these traits. This result indicates an association of favorable and unfavorable alleles in repulsion phase in the parents and of the distribution of RIL means more towards the values of the resistant parent might

be due to favorable additive × additive interactions. Additive × additive interaction effects for these traits were also reported in several previous studies (Rana and Murty, 1971; Hagi, 1984; Nour and Ali, 1998; Sharma *et al.*, 2007).

A near normal distribution with very low levels of transgressive segregation was observed among the RIL population for recovery resistance. Thus recovery resistance is expected to be governed by several genes with alleles linked in a coupling phase in the parents, and the expression of this trait is expected to be purely governed by additivity. Additive gene action for recovery resistance has also been reported by Sharma *et al.* (2007). The RILs for overall resistance exhibited significant transgressive segregation towards both the parental extremes. The histogram for this trait could be divided into three broad phenotypic classes implicating the presence of at least two major genetic loci controlling the trait. The presence of transgressive segregation towards both extremes suggests a role of complementary gene action.

5.1.2.2 Agronomic and morphological traits

A discontinuous distribution was observed among the RIL for the four simply inherited morphological marker traits — plant color, basal pigmentation, testa (presence vs absence) and mesocarp thickness indicating that these traits are indeed governed by only few major genes. Absence of transgressive segregants for these traits further indicates that the alleles for these traits are fixed in the parental lines in coupling phase with no scope of recombinations through meiotic events. Among the other traits, RILs exhibited a normal distribution for seedling vigor with large transgressive segregation towards both the parental types. Thus seedling vigor is expected to be governed by several genes with complementary interactions. The presence of at least two major phenotypic classes for both basal pigmentation and plant color is an indicative of single gene inheritance, but their unequal proportion

could be explained by the presence of at least one more gene contributing to the inheritance of seedling base color and plant foliage color. Previous studies in sorghum have suggested a two factor control for both seedling basal pigmentation (Karper and Conner, 1931; Woodworth, 1936; Ayyangar and Reddy, 1942) and plant color (Stephens, 1947; Doggett, 1988). For testa (presence vs absence) and mesocarp thickness, the distribution of RILs could be broadly grouped into three major phenotypic classes, implicating at least two major genes associated with these traits. The presence of testa has been reported to be controlled by a pair of complementary genes B_1 and B_2 (Doggett, 1988), while mesocarp thickness has been suggested to be largely controlled by a single gene (*Z*) (Rooney *et al.,* 1980). A near normal distribution with a large frequency of transgressive segregation towards the ICSV 745 parental mean was observed for the trait leaf angle. Even though the distribution among the RILs for this trait seems to be continuous, the recombinant inbred lines in the present study can be categorized into two major phenotypic groups [flat ($<55^o$) and erect leaf habit ($>55^o$)], suggesting the presence of at least one major genetic locus associated with this trait. A single major gene associated with leaf angle has also been reported in previous studies in sorghum (Ayyangar *et al.,* 1935; Hart *et al.,* 2001). Frequency distribution for time to 50% flowering indicated a high frequency of transgressive segregation towards early maturity, indicating the role of epistasis in inheritance of this trait. The distribution for this trait could be divided into at least five phenotypic groups suggesting the presence of at least four major genetic loci for this trait. Previous reports in sorghum have identified and classified six maturity maturity loci each with several alleles interacting with one another (Quinby and Karper, 1945; Quinby, 1966; Quinby, 1973; Childs *et al.,* 1997; Rooney and Ayden, 1999). For number of nodes, the RILs exhibited a normal distribution with a large proportion of

segregation towards the higher trait value parental mean, again implicating the role of epistasis in governing this trait. The distribution curve could be divided into two segregative and one transgressive phenotypic class, suggesting this trait to be associated with at least two major genetic loci. The distribution for plant height indicated a large proportion of transgressive segregation towards the higher trait value parent (ICSV 745), indicatting a major role of epistasis for this trait. The phenotypic distribution could be divided into three broad segregative and one small transgressive group, indicating the presence of at least three major genetic loci associated with variation in this trait. Previous studies in sorghum have identified four major genes with additive effects controlling this trait (Quinby and Karper, 1954). The distribution among the RILs for the agronomic performance was near normal, with an insignificant proportion transgressive segregation. The histogram for this trait could be broadly grouped into four major phenotypic classes indicating the presence of at least three major genetic loci determining the much of variation observed among the RILs for this trait.

5.1.3. Character association

Correlation is the measure of the tendency of traits to be inherited together. If correlation between two traits is significant, selection for one would lead to simultaneous selection of the associated traits. Falconer (1989) suggested that the association between selected traits and traits targeted for improvement determines the efficiency of indirect selection. Significant associations are a result of linkage, pleiotropy or cause and effect relationships between traits. In pleiotropy, the same gene determines the expression of several characters and the association is unbreakable. However associations arising from linkage are transient and can be manipulated, provided that sufficiently large segregating populations are used

As resistance to spotted stem borer is the end point measurement of several component traits acting independently and in concert, knowledge of the association between resistance component traits would facilitate better understanding of the genetic architecture of resistance. In the present study correlations were estimated between all the observed traits in the individual environment and across-environment analyses. High correlation between these traits is expected to be accompanied by identification of co-mapped QTLs. Significant associations observed among traits in both the individual environment screens and across-environment analysis in the present study are discussed here.

5.1.3.1. Spotted stem borer resistance component traits

Among all the resistance component traits, significant positive association was observed among deadheart incidence, leaf feeding damage score, recovery resistance score and overall resistance score, while association of these traits with stem tunneling were in general non-significant and inconsistent. These associations suggest that the inheritance for stem tunneling is significantly different from that of the other resistance component traits. Sharma *et al.* (2007) reported significant positive association between leaf feeding damage and deadheart incidence, while non-significant associations were recorded between primary (leaf feeding damage and deadheart incidence) and secondary damage parameters (stem tunneling) (Singh *et al.,* 1983; Rana *et al.,* 1985).

The results of the present and various previous studies suggest that the inheritance for stem tunneling is independent of other sorghum stem borer resistance component traits. Among all the associations recorded between resistance component trait, the degree of correlation between overall resistance score and recovery

resistance score was substantially high across environments suggesting a common genetic basis governing both these traits.

5.1.3.2. Agronomic and morphological traits

Consistently significant positive association was observed among scores of morphological traits seedling basal pigmentation and plant foliage color and testa (presence vs absence) suggesting pleiotropy or a close linkage between genetic loci governing these traits. Previous studies in sorghum have also reported a close association of genes governing seedling basal pigmentation, plant color and presence of testa (Stephens and Quinby, 1939; Casady, 1975). Significant positive associations were also observed between seedling basal pigmentation and plant height and between plant color and agronomic performance. These associations might be the manifestation of stem borer infestation rather than due to common genetic loci (*i.e.* in a stem borer-infested field, plants exhibiting red color and colored basal pigmentation performed better than plants with tan plant color and non-pigmented seedlings due to the significant association of these traits with resistance parameters, probably as a result of genetic linkage, although pleiotropic effects might also be involved).

A significant association was also observed among mesocarp thickness, testa (presence vs absence) and leaf angle. Earlier studies have also reported the association of the genes governing testa and mesocarp (Rooney *et al.,* 1980; Doggett, 1988). A highly significant and positive association was observed between time to 50% flowering and number of nodes. The strength of this association indicates a high probability of same genetic loci governing both the traits. This result is in conformity with the earlier observation made by Doggett (1988), who suggested that the genes controlling duration of growth would determine the number of leaves (and therefore the number of nodes). Agronomic performance recorded a significant positive

association with number of nodes, while a negative association was observed between this trait and plant height, indicating close associations of loci governing these traits.

5.1.3.3. Spotted stem borer resistance component vs agronomic/morphological traits

Consistently high and significant positive associations were observed between stem tunneling and plant height, and between stem tunneling and testa (presence vs absence). In various previous studies, both in sorghum as well as in maize, it has been observed that both number of tunnels and tunnel length formed by stem borers are greater in taller plants (Singh and Rana, 1984; Khurana and Verma, 1985; Prem Kishore, 1991a; Cardinal *et al.,* 2001; Krakowsky *et al.,* 2004).

Deadheart incidence was found to be positively associated with time to 50% flowering and negatively with agronomic performance (score). This association is largely expected because deadheart formation on the main shoot would induce formation of new tillers on which initiation of flowering would start comparatively later. Similarly large incidence of deadhearts would drastically affect the yielding capacity of an individual line. Several studies in sorghum have indicated deadheart incidence to be the most important component trait associated with the poor agronomic performance of a stem borer-damaged sorghum genotype (Singh *et al.,* 1968; Taneja and Leuschner, 1985; Sharma *et al.,* 2007).

A significant negative association was observed between leaf feeding damage and plant color implicating that tan colored plants were more susceptible than plants with red foliage color. This association might be the result of pleiotropy. Doggett (1988) observed that plant color (black purple, red purple and brown) in sorghum is mainly controlled by two genes, which express particularly on infestation by insect pests or diseases, confirming the observation made in the present study. Hence, it may

be inferred that in resistant plants genes responsible for production of antibiotic factors are expressed upon infestation and these factors in turn result in the characteristic color of the plant foliage.

Overall resistance and recovery resistance scores were found to be negatively associated with numbers of nodes. Sharma *et al.* (2007) also observed similar association between these traits. The trait recovery resistance was found to be positively associated with plant height. This observation is in contrast to the results obtained by Sharma *et al.* (2007), wherein a significant negative association was recorded between plant height and recovery resistance.

5.2. Molecular marker / Genotypic analysis

Determination of the number and distribution of genetic loci controlling a polygenic trait is facilitated when knowledge on genome organization of a species is available. Genetic linkage maps provide the basis for understanding of genome organization of an organism and help in locating genetic loci associated with various traits. An efficient molecular marker system and a large segregating population are perquisites for the construction of a reliable linkage map.

In the present study, a subset of 90 polymorphic SSR markers well distributed across the sorghum linkage map were used to genotype a recombinant inbred line population developed from a pair of genetically diverse inbreds for construction of a linkage map and subsequent QTL analysis. The results obtained through molecular marker analysis are discussed here.

5.2.1. Mapping population and genome composition

In the present study a recombinant inbred line population was utilized for map construction and QTL analyses. RILs developed from an F_2 using single-seed descent

are expected to allow a better resolution of linked QTLs than the F_2 population and its derived F_3 progenies because of additional recombination during development of lines (Burr *et al.,* 1988; Cowen, 1988; Lander and Botstein, 1989; Knapp and Bridges, 1990). Furthermore, RILs should allow an increased power of QTL detection, because of complete homozygosity at QTLs and marker loci (Moreno-Gonzalez, 1993). However, in the present study the average RIL was heterozygous in at least 8% of the marker loci and homozygous for female and male parent alleles at 43 and 40% of the marker loci. The high residual heterozygosity observed could be due to substantial inter-crossing among the segregating lines during post-rainy generation advance. Higher than expected heterozygosity among RILs, has also been reported in previous studies in sorghum (Xu *et al.,* 2000; Sajjanar, 2002; Deshpande, 2005). Of the 266 RILs utilized in the present study, fifteen were excluded from the analyses because of detection of non-parental alleles at more than 10% of the marker loci. Several reasons can be attributed to the presence of non-parental alleles including contamination of RILs during generation advance, mutation, or use of parental lines still segregating at some of the marker loci. Even though there are certain advantages in using an RIL population, there are some disadvantages also, such as additional time needed to develop these sets of near-homozygous lines and inability to measure dominance effects of QTLs. However, previous studies in sorghum have suggested that spotted stem borer resistance is predominantly controlled by additivity (Pathak and Olela, 1983; Hagi, 1984; Nour and Ali, 1998; Sharma *et al.,* 2007); hence the inability to detect dominance effects must not be a major concern in the present study.

5.2.2. Segregation distortion

The average percentage of loci homozygous for the male and the female parent alleles were nearly equal indicating the transmission of parental alleles in the expected 1:1

ratio. Despite the normal transmission of both the male and female genomes from a genome-wide point of view, there were markers that exhibited substantial segregation distortion. Skewed distribution, were observed on the RIL population for 44% of the SSR markers screened. Large number of markers exhibiting segregation distortion has been observed in several previous studies in sorghum (Pereira *et al.,* 1994; Sajjanar, 2002; Deshpande, 2005). Several reasons may explain the segregation distortion observed. The type and size of the mapping population used, is one of the reasons suggested for distortion observed among the markers. Theoretically, all mapping population can show distorted segregation. However, Liang *et al.* (2006) suggested that recombinant inbred lines usually show extreme segregation distortion, because during the development of the RIL population, many recessive lethal genes become homozygous, are expressed and individuals expressing these fail to contribute seeds to the subsequent generation, thereby resulting in a skewed distribution in the population. In addition, a low to moderate sized mapping population is more likely to show greater distortion than a large size of population. However, the present mapping population size of 266 RILs seems sufficiently large to minimize the effects of drifts and hence could not be attributed as the major reason for the observed segregation distortion.

A variety of physiological and genetic factors could also cause segregation distortion (Grant, 1975). Residual heterozygosity in parental lines (Cloutier *et al.,* 1997) or mechanisms of preferential segregation such as linkage to a heterochromatic region, or genes responsible for pollen tube competition (Liedl and Anderson, 1993), pollen lethals, preferential fertilization and selective elimination of zygotes (Kreike and Stiekema, 1997) have been reported for the observed distortion of various markers.

In the present study, the segregation distortion did not affect the overall parental allele frequencies, which were nearly equal in the population. While distortion was observed towards both the parental types, it is notable that clusters of markers observed on linkage groups SBI-03, SBI-04 and SBI-10 were all skewed towards the alleles of female parent (ICSV 745). There are numerous reasons for the distorted segregation ratios repeatedly observed in several sorghum mapping experiments (Chittenden *et al.,* 1994; Xu *et al.,* 1994; Dufour *et al.,* 1997; Tao *et al.,* 1998a). Tao *et al.* (1998a) suggested that the most likely reason for this skewness might be the presence of genes or loci near the markers that influences gametophytic or zygotic competitiveness and /or viability (segregation distortion loci).

Markers that show segregation distortion are often excluded from use in the linkage map development, however, this treatment usually reduces the coverage of the genome by the linkage map and there is a high probability that loci governing quantitative traits or qualitative traits on this region can be missed (Wang *et al.,* 2005). For a correctly inferred map (map distance and marker order) influence of QTL analysis could be negligible; however, if the recombination fraction or the order of the marker loci are inferred incorrectly, the basic assumptions of QTL analysis do not hold and the results may be seriously imprecise (Sandbrink *et al.,* 1995). The detection of the QTLs through composite interval mapping would not be affected by segregation distortion of marker loci because the final output is the result of a prior run of step-wise regression (He *et al.,* 2005).

5.2.3. Linkage map

The genetic linkage map of sorghum generated in the present study spans 1289 cM and consists of 90 SSR marker loci. The total length of the linkage map is comparable to the distance observed in many previous linkage maps of sorghum (Pereira and Lee,

1995; Bhattramakki *et al.,* 2000; Subudhi and Nguyen, 2000; Haussmann *et al.,* 2002, 2004). As the linkage groups SBI-01a and 01b, and 02a and 02b represent chromosomes 1 and 2 of the physical map, respectively, it can be said that the present linkage map is a good representation of the whole nuclear genome of sorghum with all ten expected linkage groups. A wide genome coverage of sorghum was achieved mainly because of the prior knowledge of the position of all SSR markers used, based on the consensus map developed for sorghum at ICRISAT (Hash, 2008 pers. comm.), and the selection of polymorphic SSR markers evenly distributed in the centromeric regions along with distally-located markers across linkage groups for map construction.

The length of the mapped linkage groups varies from 33 cM for SBI-05 to 202 cM for SBI-03. Linkage groups SBI-04, SBI-06 and SBI-07, with total genomic lengths of about 147, 85 and 132 cM, respectively, were comparable to the map lengths for these chromosomes reported by Bhattramakki *et al.* (2000). The two linkage groups that were shorter than expected were SBI-05 and SBI-06. Similar results were also obtained by Wu and Huang (2007) in sorghum, wherein among all the linkage groups constructed SBI-05 and SBI-06 were shortest, with only 7 and 3 SSR markers contributing to them. The unexpectedly short length for these two linkage groups is probably due to the lack of detectable marker polymorphism between the parents used in the present study and not an indication of low recombination or short physical lengths on these linkage groups. As the number of markers on a linkage group is the direct indication of the variation observed in the population and the distance coverage is in part a reflection of the genetic variation observed among the linkage groups, it may be inferred from the present study that for SBI-05 and SBI-06 the parents exhibit relatively limited dissimilarity.

Gaps wider than 20 cM have been observed on various linkage groups in the present study. The most probable reason could be attributed to the lack of polymorphic markers in these regions. However, the gaps on the distal ends of linkage groups, particularly on SBI-07, 08, 09 and 10 suggest that the recombination is highly localized in these regions and infrequent in centromeric segments.

In general, the marker order observed in the present study was similar to the order observed for these markers in the consensus map, however, there were few rearrangements on SBI-05 (between markers *Xtxp225* and *Xisp10257*), on SBI-07 (between markers *Xtxp312* and *Xisp10233*) and on SBI-10 (between markers *Xisp10321* and *Xisp10359*). Flips or rearrangements are a common feature between closely spaced markers when comparing linkage maps derived using small to moderate sized mapping populations and have been reported in previous studies in sorghum (Feltus *et al.,* 2006; Wu and Huang, 2007). These rearrangements or inversions can be attributed largely to differences in the frequencies of relatively rare recombinations in the populations being compared.

5.2.4. QTL mapping

A total of 45-49 QTLs have been detected for spotted stem borer resistance component and agronomic and morphological traits across three analyses, individually accounting for 1.5 to 55% of the observed phenotypic variation. The detection of large numbers of QTLs, even those explaining very low phenotypic variation is indicative of the effectiveness of RILs and CIM for QTL analysis. Increased power of detection of QTLs using RILs has been reported in previous studies in maize (Burr *et al.,* 1988; Austin and Lee, 1996). In addition, Jansen (1993) opined that the use of CIM reduces the error variance, hence would increase the power of detecting the QTL as compared to other methods Composite interval

mapping is perhaps the best tool available in QTL characterization because it uses multiple regression and background markers to avoid detection of any ghost QTLs. Efficiency of CIM for QTL detection has been reported in several previous studies in sorghum (Rami *et al.,*1998; Kebede *et al.,* 2001; Nagaraj *et al.,* 2005).

5.2.4.1. QTL mapping for spotted stem borer resistance component traits

Resistance to spotted stem borer is polygenic. Because of its complex nature involving interaction of many genes, conventional breeding appears to have had little effect in improving the level of spotted stem borer resistance in elite cultivars. In addition, breeding strategies are impeded by the extremely time- and labor-consuming procedures required for effective field screening of materials. The identification of markers associated with stem borer resistance component trait QTLs, would assist breeders to construct beneficial allelic combinations and accelerate breeding programmes for development of agronomically elite resistant cultivars. Further, the analysis of these traits in two environments would help in identifying stable QTLs. The most practical application of this analysis would be to form an ensemble of different putative QTLs favoring resistance to further assess by attempting marker-assisted selection.

5.2.4.1.1. Stem tunneling

Stem tunneling was found to have a very complex inheritance. Four QTLs were identified for this using the 2007 rainy season phenotyping data set, while five QTLs were detected in the 2008 and across-environment analyses (Figure 15 and 17). These QTLs together explained 23% to 40% of the observed phenotypic variation and were associated with reduction in the tunnel length. Conventional genetic analysis, has previously suggested this trait to be controlled by both additive and epistatic gene

interaction effects (Hagi, 1984; Nour and Ali, 1998). Significant additive and additive × additive interaction effects observed among most of the detected QTLs, particularly for the 2008 rainy season analysis, corroborates the predominance of additivity for this trait.

Most of the QTLs detected for this trait exhibited significant interaction with screening environment. This presumably indicates that the environment makes an appreciable difference in the expression of this trait. Different sets of QTLs identified across the 2007 and 2008 rainy seasons further confirm the major role of environment on the expression of genes controlling this trait. Discrepancies in stem tunneling QTLs detected for the same population evaluated in different environments have been reported previously in maize (Cardinal *et al.,* 2001; Krakowsky *et al.,* 2002). In general, the QTLs from the 2007 rainy season data set exhibited smaller additive effects and explained less of the observed variation than did those detected with the during 2008 rainy season data set. Disparity in the QTLs detected and their effects across environments are thought to be due to the differences observed in an important climatic factor (rainfall) between the 2007 and 2008 rainy seasons. The relatively dry period of during July and August of the 2007 rainy season might have resulted in increased larval mortality, hence reducing the larval density on the plants. The relatively low larval density in turn would have caused lower expression and variability for stem tunneling among the RILs during the 2007 rainy season. Similar observations have been made in maize for stem tunneling by European corn borers (Jampatong *et al.,* 2002; Krakowsky *et al.,* 2004).

Consistent identification of QTLs across different environments provides confirmation on location of alleles that are less influenced by the environment and could greatly increase the efficiency of selection of a relatively low heritability trait.

Melchinger *et al.* (1998) suggested that QTLs across environment can be declared as common or congruent, if they were present within a 20 cM interval. In the present study, only one QTL on SBI-07 (between markers *Xtxp295* and *XSbAGB02*) was found to be common across the two screening seasons, and this QTL accounted for 5 to 9% of observed phenotypic variation and additive effects from -4.0 to -5.0 cm, across analyses. The substantially low number of common QTL identified and the lower phenotypic variation explained, further confirms the high influence of environment on this trait. Similar results have been obtained in previous studies in maize, wherein very few common QTLs were observed for stem tunneling by European corn borers across several environments (Krakowsky *et al.,* 2004). Though the stem tunneling QTL on SBI-07 also exhibited significant interaction with the environment, the interaction was of the non-crossover type, *i.e.* the difference between environments was observed only in the strength of the effects but not in the direction of these effects. Such interaction does not have any serious implications in plant breeding (Yadav *et al.,* 2003). Hence, this QTL on SBI-07 can be considered stable and could warrant further evaluation and fine-mapping of this region, facilitating its subsequent use in marker-based spotted stem borer resistance sorghum breeding programmes.

5.2.4.1.2. Deadheart incidence

Four QTLs were detected for deadheart incidence using the 2007 rainy season data set, one each on SBI-01a, 03, 07 and 08. For the QTLs on SBI-03 (between markers *Xtxp228* and *Xisp10331*) and 07 (between markers *Xisep0829* and *Xtxp295*), the male parental alleles were associated with a reduction in the level of deadheart incidence, while for the QTLs on SBI-01a and 08, they were associated with increase in deadheart incidence (Figure 15). Using the 2008 rainy season data set, only two QTLs

were identified one each on SBI-07 and 10, for which the male parental alleles were associated with decrease in deadheart incidence, while in the across-environment analysis only one QTL was identified on SBI-07 (Figure 17). The position of this QTL (between markers *Xisep0829* and *Xtxp295*) was similar across all three analyses. This QTL was also the most stable QTL, as it did not exhibit any significant QTL × E interaction effect, and was consistently shown to contribute a very high portion of the observed phenotypic variation (10 - 28%) across all three analyses. Incidentally, the position of this QTL was observed to be similar to that of the stable QTL identified for stem tunneling. The significant additive effects observed among the detected QTL indicates a major role of additive gene action in controlling this trait. Previous studies in sorghum have also suggested additive gene action for deadheart incidence (Nour and Ali, 1998; Sharma *et al.,* 2007).

5.2.4.1.3. Leaf feeding damage

As observed from the frequency distribution, two QTLs, were detected for this trait in both the screening environments. Both QTLs had favorable alleles from the male parent reducing the leaf feeding damage rating (*i.e*. increasing the resistance). Khairallah *et al.* (1998) opined that in tropical maize, resistance to south western corn borer (SWCB) foliar damage might be the result of expression of a large number of genes with small genetic effects acting additively, rather than due to a few major genes. In the present study, the QTLs identified for leaf damage rating explained only a minimal proportion of the observed phenotypic variation (<10%) and individually exhibited small but significant additive effects. This result also indicates the major role of additive gene action for leaf feeding damage by spotted stem borer. Previous studies in sorghum have also revealed a major role of additive genetic effects for control of spotted stem borer foliar damage (Rana and Murty, 1971; Hagi, 1984; Nour

and Ali, 1998; Sharma *et al.,* 2007). Most of the QTLs identified for leaf feeding damage by southern corn borer and southwestern corn borer larvae (Böhn *et al.,* 1997; Khairallah *et al.,* 1998) in maize have also exhibited significant additive gene effects.

In the present study, consistent QTLs were not detected for this trait across the three analyses. The inconsistencies might be a result of the differences in amount of rainfall received during the 2007 and 2008 rainy seasons, which might have altered the expression of resistance mechanisms of the plants and affected the growth and survival of spotted stem borer larvae. Among the QTLs identified in across environment analysis, the position of QTL detected on SBI-02 (segment a) (between markers *Xisep0747* and *Xtxp025*) and 7 (between markers *Xisep0829* and *Xtxp295*) was similar to the QTL observed only in 2007 rainy season, while the other QTL on SBI-07 (between markers *Xtxp227* and *Xisp10206*) mapped to the same position as one of the QTLs identified in the 2008 rainy season. Even though these QTLs failed to map congruently in both the screening environments, there is a clear indication of their presence in both of these environments, at least at a lower threshold (Figure 15 and 17). Despite the use of a large mapping population size, the lower LOD scores for these QTLs and the low portion of observed phenotypic variation explained by the identified QTLs from the across-environment analysis can be explained by the fact that different environmental factors during the 2007 and 2008 rainy seasons might not have been strong enough to permit these QTLs to be expressed at their maximum level. The identification of only few QTLs with minor effects for leaf feeding further suggests the likely presence of several additional undetected QTLs in genomic regions that have been sparsely covered by SSR markers with current available marker data set. The QTLs identified on SBI-02a (between markers *Xisep0747* and *Xtxp025*) and 7 (between markers *Xtxp227* and *Xisp10206*) in the across environment

analysis were stable as they did not exhibit any significant interaction with the environment and hence may be promising candidates for MAS (marker-assisted selection) for spotted stem borer leaf feeding resistance.

5.2.4.1.4. Recovery resistance

Three QTLs were observed for this trait using the 2007 rainy season phenotyping data set, while four QTLs were detected during 2008 rainy season and across-environment data sets (Figure 16 and 18). For all the QTLs detected the alleles derived from the male parent were associated with an increase in the level of recovery resistance expression (decreasing the level of recovery resistance score). The identified QTLs exhibited significant additive effects indicating the role of additive gene action for this trait. Sharma *et al.* (2007) also suggested the predominance of additive gene action in controlling this trait. In the present study, the QTL on SBI-07 (between markers X*SbAGB*02 and *Xcup57*) was consistently observed across all three analyses and it also explained a major proportion of the total observed phenotypic variation (12-20%) for this trait across three analyses. This QTL exhibited a significant interaction with the environment, however, the interaction was a non-crossover type, and hence this QTL may be considered putative QTL for MAS. As recovery resistance is an indirect measure of the number of productive tillers formed following infestation, recovery resistance must be associated with genomic regions in sorghum that controls the development of productive tillers. The position of the major QTL identified in the present study on SBI-07 for recovery resistance also corroborates with the position of QTLs detected for productive tillers in previous studies in sorghum by Paterson *et al.* (1995) [LG J] and Hart *et al.* (2001) [LG E].

5.2.4.1.5. Overall resistance

A single QTL was detected for overall resistance on SBI-07 using the 2007 rainy season data set, while in the 2008 and across-environment analyses, two QTLs were detected on SBI-03 and 07 (Figure 16 and 18). The allele from PB 15520 contributed to an increase in overall resistance (decrease of overall resistance score) for the QTL identified on SBI-07, while for the QTL on SBI-03 the allele from this parent contributed to reduction of overall resistance (increase of overall resistance score). The map position of the QTL on SBI-07 (between markers *Xisep0829* and *Xtxp295*) was common across all three analyses and this QTL explained the highest portion of observed phenotypic variation across analyses (8 to 17%). This QTL too did not exhibit significant interaction with the two screening environments indicative of its stable expression across environments. As overall resistance measures both resistance and tolerance to spotted stem borer, this QTL on SBI-07 was found to co-localize with the regions associated with the stable QTLs identified for recovery resistance, leaf feeding and deadheart incidence. This clearly makes this region an excellent candidate for future exploratory marker-assisted selection and fine-mapping studies.

5.2.4.1.6. QTL clusters

Identification of multiple stable QTLs for spotted stem borer resistance component traits on SBI-07 particularly on the distal end of the chromosome indicates this region of the chromosome to be a gene-rich region contributing to resistance (Figure 15 to 18). This chromosome has also been identified to contain QTLs providing resistance to midge (Tao *et al.,* 2003), grain mold (Klein *et al.,* 2001a) and shootfly (Deshpande, 2005) in sorghum. Hence MAS (marker-aided selection) directed at this region could greatly help in improving not only spotted stem borer resistance but also in developing a complex linkage block of combining this with resistance to other major

sorghum insect pests. However, further genotyping at a larger number of locations across this region, and phenotyping at least a selected subset of the RIL population in additional screening environments would be desirable to confirm the stability of the identified QTL before using the markers linked to this region for applied introgression breeding via MAS.

5.2.4.2. QTL mapping for agronomic and morphological traits

Genetically controlled variation for several morphological and physiological traits influences yield and quality of sorghum. Classical genetic studies have contributed information regarding the manner of genetic control of some of these characters. The recent use of molecular markers in sorghum has also made it possible to map many of the genetic loci that control variation in these traits. However, many of the genes with major effects for these traits are unmapped as yet. In this study an effort has been made to characterize various agronomic and morphological traits for which the RILs exhibited substantial variation, using molecular markers.

5.2.4.2.1. Glossiness

QTL analysis using the 2007 rainy season data set revealed the presence of only two QTLs on SBI-01a (between markers *Xcup24* and *Xcup73*) and 09 (between markers *Xgap032* and *Xgap206*), each accounting for minor portions of phenotypic variation (<10%) for this trait (Figure 19). However, previous studies in sorghum have reported the presence of a major QTL for this trait on SBI-05 *i.e.* LG J (Sajjanar, 2002; Deshpande, 2005). The identification of only minor QTLs is in agreement with the narrow range of variation observed among the RILs for this trait. Presence of both positive and negative additive effects for the identified QTLs indicates the dispersed nature of alleles enhancing glossiness in both the parents of the mapping population.

5.2.4.2.2. Seedling vigor

The frequency distribution for seedling vigor suggested polygenic inheritance for this trait. The identification of several QTLs with minor effects explaining a total phenotypic variation of <15% across seasons further confirms the quantitative nature of inheritance of this trait (Figure 19 and 23). The presence of both negative and positive effects indicates that favorable alleles for this trait are dispersed in the two parental lines. The QTLs were identified on SBI-01 (a and b), 03, 04 and 09 in the present study across three analyses. The QTL identified on SBI-01 (segment a) (between markers *Xcup01-Xcup24*) and the QTL detected on SBI-03 (between markers *Xcup38* and *Xisp10332*) were common across all three analyses and did not exhibit significant interaction with the environment. The QTL on SBI-01 (segment b) (between markers *Xcup60-Xcup44*) was identified only from the 2007 and across-season analyses; however, there is an indication of its presence in the 2008 rainy season analysis also, but at a lower LOD score. Sajjanar (2002) also reported minor QTLs for this trait on chromosomes 1 (LG A) and 3 (LG C).

5.2.4.2.3. Basal pigmentation

Previous genetic studies in sorghum have indicated this trait to be controlled by two genes with complementary gene action (Woodworth, 1936; Ayyangar and Reddy, 1942). One of the major genetic loci for coleoptile color (Rs_2/rs_2) has been mapped to chromosome 10 (LG H) (Boivin *et al.,* 1999); while a second major gene for seedling color (*SDCR* / *sdcr*) has been mapped on SBI-04 (LG J) (Tao *et al.,* 2000) and a third gene (Rs_1/rs_1) has been mapped to SBI-06 (LG I) (Subudhi and Nguyen, 2000). In the present study, only a single major stable QTL was detected for this trait on SBI-06 (between markers *Xisp10264* and *Xtxp057*) across all the three analyses, explaining nearly 40% of the observed phenotypic variation (Figure 19 and 23). The identified

major QTL, which appears to correspond to (Rs_1/rs_1) mapped very near to the major QTL for plant color in the present study (previously identified as *P/p* by Tao *et al.,* 1998), suggesting a common genomic region responsible for the control of both these traits.

5.2.4.2.4. Plant Color

Discrete phenotypic classes observed for this trait indicated that only few major genes control this trait. In the present study, two QTLs one each on SBI-06 (between markers *Xisp10264* and *Xtxp057*) and 07 (between markers *Xisep0829* and *Xtxp295*) were detected from the 2007 rainy season and across-season analyses (Figure 20 and 23). In the 2008 rainy season analysis, only a single QTL on SBI-06 was detected; however, there is a clear indication of the presence of the QTL on SBI-07 at a lower LOD in 2008 rainy season analysis also. The position of the QTL identified on SBI-06 was common across all three analyses and this QTL exhibited the highest phenotypic variation (38 to 42%), the QTL on SBI-07 contributed to only a small phenotypic variation. The positive additive effects for the identified QTL indicated that the alleles from male parent (PB 15520) were responsible for increasing the plant color score. These identified QTL most probably corresponds to the major gene *P/p* identified for this trait on SBI-06 (LG I) (Klein *et al.,* 2000) (LG B) (Boivin *et al.,* 1999), and the intensifier gene *I/i* that has been reported on SBI-07 (LG J) (Tao *et al.,* 2000).

5.2.4.2.5. Testa (presence vs absence)

Presence of a pigmented testa causes high tannin levels in sorghum seeds (Menkir *et al.,* 1996). The presence of high levels of tannin in the caryopsis is an undesirable trait for food and feed processing, and therefore important consideration has to be given to

permitting presence of a pigmented testa in any sorghum breeding programme. The presence of a pigmented testa has been reported to be governed by a pair of complementary genes (B_1/b_1 and B_2/b_2) (Doggett, 1988). In the present study, a single major genetic locus contributing to more than 25% of the phenotypic variation has been observed on SBI-04 (between markers *Xtxp327* and *Xisp10229*) across all three analyses (Figure 20 and 24). This locus also did not exhibit significant interaction with the environment indicating it to be stable across environments. In a previous study, the major gene B_2/b_2 for the presence of testa has been located on SBI-02 (LG F) of sorghum (Dufour *et al.,* 1997; Rami *et al.,* 1998). The lack of correspondence of locus for this trait as compared to other studies might be due to differences in the genetic backgrounds utilized to map this trait or due to lack of coverage of the genomic regions by the markers. The later is unlikely since the region containing the B_2/b_2 locus is represented in SBI-02b However, as the identified QTL on SBI-04 explained a large proportion of the phenotypic variation, it may be considered as another major loci contributing to the presence of testa.

5.2.4.2.6. Mesocarp thickness

Two QTLs have been detected for mesocarp thickness score using the data sets from 2007 (on SBI-01b and 4), and 2008 rainy and across season (on SBI-01a and 04) (Figure 20). The QTL on SBI-04 (between markers *Xtxp327* and *Xisp10229*) explained the highest portion of observed phenotypic variation (>20%) and exhibited a similar map position across all three analyses, indicating this region to be ideal candidate for identifying a major gene influencing mesocarp thickness. The positive effect observed for this score indicates that the alleles from the male parent are associated with a thicker mesocarp. Previous studies have reported a single major recessive gene governing mesocarp thickness (Z/z) (Rooney *et al.,* 1980; Doggett,

1988) and this genetic locus has been mapped on SBI-02 of sorghum (Boivin *et al.,* 1999; Tao *et al.,* 1998). In the present study the QTL identified for this trait on SBI-04 shared a common position with the QTL identified for testa presence vs absence, indicating a close association between these two traits and in agreement with previous findings of a strong association between mesocarp thickness and presence vs absence of pigmented testa.

5.2.4.2.7. Leaf angle

A single major QTL accounting for nearly 20% of the observed phenotypic variation for RIL mean was observed on SBI-04 (between markers *Xtxp327* and *Xisp10229*) across all three analyses (Figure 21 and 24). This QTL was highly significant and did not exhibit any interaction with the environment; the positive significant additive effect of this QTL indicates that the alleles from male parent PB15520 contributed to a more erect the leaf angle. Previous genetic study in sorghum by Ayyangar *et al.* (1935) revealed that the erect leaf habit of the sorghum plant is due to absence of ligules and auricles at the base of the leaves and is mainly governed by a single recessive gene "*lgs*". QTL mapping results of Hart *et al.* (2001) indicated the presence of a major stable QTL for this trait on SBI-07 and a second major gene (*lg*) has been mapped to SBI-03 (Zwick *et al.,* 1998). In the present study, even though a QTL had not been identified in either of these position, there is a clear indication of the presence of the QTL on SBI-07 across-environments at a lower LOD value.

5.2.4.2.8. Time to 50% flowering

Days to 50% flowering is considered to be an important trait for planning a breeding programme. Early flowering genotypes are often preferred when a cultivar is to be sown late or early in a particular growing season. A series of six major "maturity"

genes have been recognized for flowering time in sorghum - Ma_1 to Ma_6 (Quinby and Karper, 1945; Quinby, 1966; Quinby, 1973; Childs *et al.,* 1997; Rooney and Ayden, 1999). Dominant alleles at the first four genes are reported to cause long days to inhibit flowering, but allow early flowering in short days. While mutation at Ma_1 causes the greatest reduction in sensitivity to long days, mutations at other loci have modest effects. The genes Ma_5 and Ma_6 represent a special case because when both of them are present in dominant form, they inhibit floral initiation regardless of day length. In the present study, six QTLs have been identified to contribute to time to 50% flowering across both the screening environments, collectively explaining around 34 to 48% of the total phenotypic variation (Figure 21). These QTLs mapped to common genomic regions across environments and only the QTL on SBI-06 (between markers *Xisep0444* and *Xisp10264*), exhibited significant interaction with the two screening environments. Significant additive × additive interaction was observed between the QTLs on SBI-01b (between markers *Xcup44* and *Xtxp340*), and on SBI-04 (between markers *Xisp10230* and *Xiabtp364*), for both the 2008 and across-season analyses. The results of the present study agree with the prior reports for the location of most of the QTLs for maturity. The major QTL on SBI-06 (between markers *Xisep0444* and *Xisp10264*), SBI-01(b) (between markers *Xcup44* and *Xtxp340*) and SBI-10 (between markers *Xtxp270* and *Xtxp217*) are likely to include maturity loci Ma_1, Ma_3 and Ma_4 respectively. The QTL on SBI-04 might correspond to Ma_2 loci, while the QTL on SBI-01a might be the maturity loci Ma_6 as Ma_5 has recently been mapped to SBI-02 (Mace and Jordan, 2010, pers. comm.)

5.2.4.2.9. Number of nodes

Genomic regions associated with maturity must also be controlling the number of nodes (Doggett, 1988). In the present study, four QTLs were detected for this trait in

the 2007 and across-season analyses (Figure 25). In the 2008 analysis, only three QTLs were detected; however there was an indication of the presence of the QTL on SBI-04 also at a low LOD value (Figure 21). The position of the QTLs on SBI-04 (between markers *Xisep0224* and *Xisp10230*), 06 (between markers *Xisep0444* and *Xisp10264*) and 10 (between markers *Xtxp217* and *Xisp10263*) were similar to those obtained for maturity QTLs in the present study, and hence likely correspond to Ma_2, Ma_1 and Ma_4 respectively. As observed among the maturity QTLs, the QTL on SBI-06 for this trait exhibited a significant interaction with the environment. An additional QTL was also detected for this trait on SBI-07 that has not been detected as a maturity QTL in the present study.

5.2.4.2.10. Plant height

Lodging resistance is one of the major objectives of any breeding programme. Plant height and culm stiffness are reported to be the two most important traits determining lodging resistance in cereal plants (Keller *et al.,* 1999). In sorghum, plant height is reported to be governed largely by four independent genes (Quinby and Karper, 1954) Dw_1, Dw_2, Dw_3 and Dw_4. Dw_3 has been reported to have a pleiotropic effect on the number of kernels per panicle and kernel weight as well as on tiller number and panicle size (Casady, 1965). Dw_2 has been reported to have a pleiotropic effect on panicle length, main head yield, seed weight and leaf area (Graham and Lessman, 1966), while no pleiotropic effect has been observed for the other two dwarfing loci. Periera and Lee (1995) associated the locus identified on linkage group A (SBI-07) with Dw_3. As Dw_2 is reported to be linked to Ma_1 maturity gene the location of this gene has been suggested to be on LG I (SBI-06) (Rami *et al.,* 1998; Chittenden *et al., 1994;* Paterson *et al.,* 1995). Previous studies have also identified QTLs for plant height on other genomic regions such as on SBI-04, SBI-09 and SBI-10 (Pereira and

Lee, 1995; Lin *et al.,* 1995; Rami *et al.,* 1998; Klein *et al.,* 2001a; Hart *et al.,* 2001; Feltus *et al.,* 2006; Ritter *et al.,* 2008).

In the present study, three major putative QTLs have been identified on SBI-01a, SBI-06 and SBI-07 across the three analyses accounting for 33 to 42% of the total observed phenotypic variation (Figure 22 and 25). The results of the present study agree with prior reports for the locations of these QTLs for plant height. The genetic loci identified on SBI-06 (between markers *Xisep0444* and *Xisp10264*) and 07 (between markers *Xtxp295* and *XSbAGB02*) are likely to correspond to the dw_2 and dw_3 dwarfing gene, due to their proximity to a major maturity locus (Ma_1) and recovery resistance (productive tiller number) loci, respectively, on the same chromosomes. The alleles reducing plant height for all the QTL identified were contributed by the male parent (PB 15520). The QTL on SBI-07 was found to be significantly influenced by the environment, while the other QTLs were relatively free from environment interaction.

5.2.4.2.11. Agronomic performance

Agronomic performance of a genotype is an indication of its overall desirability for crop production. It is an indirect measure of general adaptability and yield capacity of a genotype. In the present study, several QTLs were detected for this trait across all three analyses suggesting the relatively stable performance of genes affecting this trait in the screening environments used in this study. Seven QTLs (on SBI-01a, 02b, 04 and 07) were detected for this trait using the 2007 rainy season and across-environment data sets, while with the 2008 rainy season data set five QTLs were detected (on SBI-01a, 04, 07 and 10) (Figure 22 and 26). Three of these identified QTLs on SBI-01a (between markers *Xcup01* and *Xcup73*), 04 (between markers *Xtxp327* and *Xisp10229*) and 07 (between markers *Xisep0829* and *Xtxp295*) accounted

for the highest portions of phenotypic variation and exhibited similar map positions across the three analyses. However, these QTLs were also substantially influenced by the environment. Some of the identified QTLs, particularly those identified on SBI-01 and 07, correspond to the QTLs for grain yield detected in previous studies in sorghum. Tuinstra *et al.* (1998) detected QTLs for yield on SBI-01 (LG F), 03 (LG G), and 10 (LG E), while Rami *et al.* (1998) reported a major QTL for yield on SBI-07 (LG A), and Ritter *et al.* (2008) reported a major QTL on SBI-02. Present results and those of other researchers indicate that the QTLs for overall agronomic performance are widespread across the sorghum genome. However, the QTLs on SBI-01 and 07 play an important role in the expression of this trait.

5.2.4.3. Co-mapped QTLs

Significant correlation among traits indicates common genomic regions influencing the expression of the trait. Pleiotropism and linkage are the two major components responsible for observed correlations. In crop plants, genetic loci once thought to have a single specific effect are now known to influence several other traits. To the extent that these loci also harbor naturally occurring variants with quantitative effects, one might expect effects on several phenotypes. However, it is not possible to distinguish between pleiotropy and linkage as a cause of correlated effect on two traits, until one has mapped QTN (Quantitative Trait Nucleotides) governing these traits.

Identification of genetic loci co-mapped for different traits will have a great implication on marker-assisted breeding approaches. QTLs co-mapped for different traits across all three analyses are discussed here. Among the resistance component traits, co-mapped QTLs were identified for leaf feeding, overall resistance and deadheart incidence (between markers *Xisep0829* and *Xtxp295*) on SBI-07. All these QTL exhibited a negative additive effect indicating complementary relationships

between these pairs of traits. Few of the agronomic and morphological trait QTLs were also found to co-map with spotted stem borer resistance component traits. A stem tunneling QTL was found to co-map with a QTL for seedling vigor, and agronomic performance (between markers *Xcup24* and *Xcup73*) on SBI-01a, with a QTL for testa, mesocarp thickness, leaf angle and agronomic performance (between markers *Xtxp327* and *Xisp10229*) on SBI-04, a QTL for plant height on SBI-07 (between markers *Xtxp295* and *XSbAGB02*) and a QTL for time to 50% flowering on SBI-10 (between markers *Xtxp270* and *Xtxp217*). A cluster of QTL for other resistance component traits — deadheart incidence, leaf feeding damage, overall resistance and recovery resistance were found to co-map with the QTL for agronomic performance and plant color on SBI-07 (between markers *Xisep*0829 and *Xtxp295*) .

Among the rest of the agronomic and morphological traits co-mapped QTLs were identified for agronomic performance and plant height on SBI-01 (between markers *Xcup06* and *Xcup24*); time to 50% flowering and number of nodes on SBI-06 (between markers *Xisep0444* and *Xisp10264*); and basal pigmentation and plant color on SBI-06 (between markers *Xisp10264* and *Xtxp057*). Based on the direction of the additive effects of the co-mapped QTLs, the most undesirable association was observed on SBI-10 (between markers *Xtxp270* and *Xtxp217*), wherein a QTL reducing tunnel length was found to be closely associated with a QTL increasing the time to 50% flowering. Such undesirable associations between stem tunneling and anthesis also have been reported in maize (Khairallah *et al.,* 1998; Bohn *et al.,* 2000; Krakowsky *et al.,* 2002). If this association is caused by pleiotropy, rather than linkage, it will be impossible in conventional breeding to combine the desired alleles for both traits into a single genotype. Similar unfavorable associations were also detected on SBI-04 (between markers *Xtxp327* and *Xisp10229*), wherein a QTL

region responsible for better agronomic performance and erect leaf habit was found to be associated with increased tunnel length and reduced grain quality (presence of testa and thick mesocarp).

Many of the genomic regions appear to affect multiple traits. Further research needs to be done to determine whether there is a single gene with pleiotropic effects underlying such common loci or there is a cluster of tightly linked genes that may be recombined to obtain desirable segregants if a sufficiently large F_2 population is used. As expected the co-mapped QTLs for the traits in the present study were supported by significant correlations among the co-mapping traits.

5.2.4.4. Selection of superior RILs based on phenotypic and genotypic data

Across-season means of the RIL population progenies for spotted stem borer resistance component traits revealed that certain lines are superior phenotypically and may be good candidates for utilization in stem borer resistance breeding strategies. However, selection of lines based primarily on phenotypic value might not be accurate; hence selection of individual lines based on the combined phenotypic and genotypic data set could greatly increase the probability of their successful utility in a breeding programme.

In the present study, 12% of recombinant inbred lines exhibiting desirable phenotype based on phenotypic value for each of the spotted stem borer resistance component traits were determined. These lines were then scored for presence of putative QTL with favorable alleles based on their genotypic values for the flanking markers. Loci with homozygous favorable alleles and unfavorable alleles for the markers flanking a putative QTL were scored as 3 and 1, respectively, while heterozygous loci were scored as 2. Each line selected based on the phenotypic value was then ranked based on its total genotypic scores (Table 23). Most of the identified

QTLs with favorable alleles for stem tunneling were present in RILs 135, 108, 24, 93, 212 and 47. However, there was no correspondence among all the selected RILs for stem tunneling and other resistance component traits except leaf feeding damage, providing further supporting evidence for the independent genetic control of these traits. Several of the RILs selected for low stem tunneling also exhibited lower leaf damage by spotted stem borer larvae. Among these, RIL108 was found to be superior for both stem tunneling and leaf feeding resistance. Several lines were detected to be superior for both deadheart incidence and leaf feeding damage score; however, significant phenotypic correlation and identification of co-mapped QTL for these traits suggests that this may be due to common genetic control of deadheart incidence and leaf damage. Among the identified entries RILs 239, 35, 19 and 69 had most of the favorable alleles for QTL for both of these traits. Similarly, RILs 253, 250, 196 and 185 performed best for both overall resistance score and recovery resistance score. Based on the combined phenotypic and genotypic data sets, none of the RILs were found to be superior for all of the resistance component traits. However, RIL 188 had accumulated favorable alleles for most of the QTLs identified for resistance component traits except stem tunneling. These lines may be utilized as donor parents in marker-assisted breeding programmes for development of resistant varieties or hybrids or could be utilized as components of a base population for various population improvement strategies such as marker-assisted recurrent selection schemes.

5.2.5. Cross-species validation of identified spotted stem borer resistance QTLs

Comparative mapping based on regions of substantial colinearity of regions provides a powerful tool for cross-species validation and confirmation of conservation of gene order and function (Ahn and Tanksley, 1993). The first comparative genetic

experiments in plants were performed on members of Solanaceae family (Bonierbale *et al.,* 1988; Tanksley *et al.,* 1988, 1992) and several conserved genomic regions have been identified in these species. A remarkable degree of genome conservation has also been established in comparative genetic mapping experiments in the Poaceae family (Ahn and Tanksley, 1993; Kurata *et al.,* 1994; Moore *et al.,* 1995; Devos and Gale, 1997; Gale and Devos, 1998), although genome sizes vary as much as 40-fold between some species, and despite the fact that they diverged as long as 60 million years ago. The conserved molecular and genetic composition suggests that the identified common genomic regions between species could code for similar functions. Hence, macrosynteny must be viewed in context of microsyntenic relationship between species. Paterson (1995) suggested that correspondence of QTL across genera that have been reproductively isolated for millions of years has many implications. Firstly, QTL analysis in one genus may predict results in other genera. Such predictive values would make QTL mapping results more broadly applicable, enabling research on facile systems to be extrapolated into more difficult ones (Paterson, 1995). Further, correspondence of QTLs in different species would appear to indicate that only a small number of genes play a major role in controlling important traits and such regions are conserved across species. Among the grasses, the maize genome has been found to exhibit substantial homology with sorghum and rice genomes (Gale and Devos, 1998; Paterson, 1995). In spite of the enormous differences in genome size, comparative genome mapping using DNA markers between sorghum and maize have indicated remarkable conservation (Hulbert *et al.,* 1990; Whitkus *et al.,* 1992; Berhan *et al.,* 1993; Pereira *et al.,* 1994; Pereira and Lee, 1995) and presence of orthologous genes. The first comparative study of local gene content and order in the grasses revealed that several genes are conserved in order and

orientation in *sh2/a1*-homologues of the maize, rice and sorghum genomes (Chen *et al.,* 1997; 1998). Similarly orthologous regions for alcohol dehydrogenase (*adh1*) have been identified in sorghum, maize and rice (Tikhanov *et al.,* 1999; Tarchini *et al.,* 2000). Early comparative studies involved mapping of RFLP markers on sorghum and maize mapping population to identify common genomic regions. However, with the availability of the sorghum genome draft sequence (Paterson *et al.,* 2009), it is now relatively easier to identify orthologous genomic regions of sorghum *in-silico* using the bioinformatics search tool BLAST (Basic Local Alignment Search Tool). In the present study an attempt was made to validate the QTLs for sorghum spotted stem borer by comparing the genomic regions associated with spotted stem borer resistance in sorghum to regions identified to confer resistance to various stem borer species in maize and hence deduce orthologous regions for resistance between maize and sorghum. The comparative genomic regions of maize and sorghum associated with the stem borer resistance component traits are presented in Table 24 and Figure 27.

A large number of QTLs identified for stem borer resistance are concentrated on maize chromosome 1. This chromosome exhibits co-linearity with SBI-01 and SBI-07. The RFLP markers *umc*58 and *umc*33 (bin position 1.06 and 1.07) associated with QTLs for leaf feeding by sugarcane borer (SCB) and south western corn borer (SWCB), and tunneling by European corn borer (ECB) respectively (Schon *et al.,* 1993; Bohn *et al.,* 1997, 2000), were found to exhibit close similarity to a region on SBI-07 of sorghum (between 56 Mbp-63 Mbp), identified to harbor a large number of stable QTLs contributing to spotted stem borer resistance (leaf feeding damage, deadheart incidence and stem tunneling). As this genomic region in sorghum has also been identified to harbor putative QTL for several other insect pests, it could be

considered ideal for identifying conserved candidate genes conferring resistance to insect pest across grass species.

Four of the RFLP markers (between maize bin position 5.04 and 5.06) associated with resistance to leaf feeding by ECB, SCB and SWCB and stem tunneling by ECB (Bohn *et al.,* 1997; Groh *et al.,* 1998; Jampatong *et al.,* 2002) concentrated to a small region on sorghum SBI-04 (between markers *Xtxp327* and *Xisp10229*) spanning the region from 60.0 to 63.0 Mbp on SBI-04 aligned sorghum genome sequence. As this region was also found be associated with a QTL for stem tunneling across environments, this genomic region can also be considered as containing potentially orthologous stem borer resistance genes. The correspondence of an apparently large sequence of maize (bin 5.05 to 5.06) to a relatively smaller genomic region in sorghum is probably a result of ancestral allotetraploidization of the maize genome (Gaut and Doebley, 1997) and differences in the expansion and dispersion of repetitive DNA between the two species (White and Doebley, 1998). Chen *et al.* (1997), while comparing *sh2*/*a1* homologous region in maize, rice and sorghum found that while in maize the region between *sh2* and *a1* were 140 Kb apart, the difference was only 19 Kb in sorghum, indicating that while gene content and order are frequently conserved between species, genome size (and thus intergenic distance) is subject to massive variation. Two more genomic regions with QTLs detected for stem tunneling on SBI-02 (between markers *Xtxp207* and *Xtxp296*) and for leaf feeding damage (between markers *Xisep0747* and *Xtxp025*) were found to correspond to genomic regions on chromosome 2 (between bins 2.06 to 2.08 and 2.10) of maize associated with ECB stem tunneling (Groh *et al.,* 1998; Krakowsky *et al.,* 2004) and ECB leaf feeding (Jampatong *et al.,* 2002), respectively. These regions probably may also contain orthologous genes for stem borer resistance across species

The correspondence of the identified genomic regions conferring resistance to stem borer in both maize and sorghum suggests strongly that these genomic regions may indeed contain genes conferring resistance to stem borers that might have been conserved and fixed across the many years of evolutionary history and divergence of sorghum from maize. Several more genomic regions showing synteny between maize and sorghum have been identified in this study; however, these regions were reported to contribute to different functions across species. For instance, RFLP marker *bnl*5.62 (bin position 1.01) associated with a ECB stem tunneling resistance QTL, and *umc*161 and *bnl*6.32 (bin positions 1.11 and 1.12, respectively) associated with leaf feeding resistance QTLs for SCB and SWCB on maize chromosome 1 exhibited close synteny to regions on sorghum SBI-01 that were associated with stem tunneling and leaf feeding during the 2008 rainy season by spotted stem borers, respectively. Such concordance of map positions for different damage parameters across different stem borer insect species has been reported in previous studies in maize (Cardinal *et al.,* 2001; Krakowsky *et al.,* 2001, 2004; Bohn *et al.,* 1997). These results suggest that resistance to leaf feeding and stem tunneling by spotted stem borers in sorghum and other stem borers in maize are conferred in part by common genomic regions.

In the present study *mir*1 and *mir*2 (maize insect resistant) gene cDNA clones were also used for similarity searches of the aligned sorghum genome sequence. These genes are responsible for the production of defensive cystein protease upon infestation of maize by lepidoteran insects (Lopez *et al.,* 2007). In the present study, the genomic sequence for these genes exhibited similarity with regions of sorghum SBI-10. However, this region were not found to be associated with any of resistance parameter in the field screens conducted in the present study, implying a possible different mechanism of antibiosis in sorghum as compared to maize or a failure of

identification of a QTL for leaf feeding on this chromosome due to poor genome coverage in this population or lack of polymorphism for homologues of these genes between the parents of the phenotyped sorghum RIL population.

Based on this comparison a few more genomic regions not sufficiently covered in the present sorghum genetic map have been identified, that may harbor additional QTLs for stem borer resistance, such as those on SBI-01 (between *Xiabtp450* and *Xtxp229*), the distal end of SBI-04 (65-70 Mbp below *Xisp10229*) and on SBI-09 (between markers *Xgap032*, *Xiabtp475* and *Xtxp010*). Hence prioriy must be given to filling these gaps and extending the linkage map by using appropriate SSR and DArT ™ markers for these regions.

5.2.6. Breeding strategies

5.2.6.1. Conventional breeding approach

The estimates of across-season heritability for spotted stem borer resistance component traits were not as high as one would prefer; however, they were higher than one would expect for highly complex traits such as yield in sorghum. The low to moderate heritability estimates for spotted stem borer resistance traits coupled with the high influence of environment as evidenced from their high G×E variances renders individual plant selection for improving spotted stem borer resistance ineffective or environment specific.

Starks and Doggett (1970) suggested population improvement as the most efficient method for improving spotted stem borer resistance in sorghum. Previous studies have indicated the predominance of additive gene action for governing this trait; in the present study also most of the QTLs detected for these traits exhibited significant additive main effects and additive × additive interaction effects. Hence, an efficient breeding programme for improving spotted stem borer resistance would be

the one that would allow accumulation of desirable genes such as mass selection or recurrent selection. Mass selection has not been found to be very effective for improvement of spotted stem borer resistance in sorghum, while substantial success has been reported through recurrent selection (Agrawal *et al.,* 1990). Conventional recurrent selection requires more time for improvement, if the selection is based on primary resistance component traits such as leaf feeding and deadheart incidence, the nature of borer reaction can be determined prior to pollination hence only one or two seasons would be required for completing each cycle of recurrent selection. However, one more additional season would be required if the selection is based on stem tunneling, recovery resistance score or overall resistance score, as these traits can be measured only after flowering has been completed; or if the reconstituted population is random mated a second season prior to another round of selection. In addition, a simultaneous positive correlated response of selection might not be observed for various other agronomic traits that are governed by polygenes such as yield, through recurrent selection. Further, as early generation selection for spotted stem borer resistance in the field is difficult and unpredictable, and genotype screening requires replications and is resource intensive; marker-assisted selection is likely to be the best available method to facilitate the transfer of the genes for spotted stem borer resistance into well-adapted genotypes. Similarly, among the agronomic and morphological traits observed, many exhibited polygenic inheritance suggesting that marker-assisted breeding may be the most reliable approach for improving these traits. Seedling basal pigmentation, plant color, testa pigmentation (presence vs absence), mesocarp thickness and leaf angle were found to be controlled by single major genes, and hence single plant selection based on phenotype could be effective for these traits. However, phenotypic selection for traits such as leaf angle would be

difficult due to the recessive nature of desirable alleles and the cumbersome screening process required for assessing mesocarp thickness and testa pigmentation implicating the use of marker-based selection as the most appropriate method for rapid advancement of generations (especially when trying to introgress favorable recessive alleles into an adapted recurrent parent background.

5.2.6.2 Prospects of marker-assisted selection

Molecular marker-assisted selection (MAS) is an approach that has been developed to avoid the problems connected with conventional plant breeding, changing the criterion of selection from phenotype towards selection of specific favorable alleles at genes controlling phenotype with direct or indirect selection (Francia *et al.,* 2005). Marker-assisted selection can accelerate breeding by reducing the time to develop new cultivars (Tanksley and Hewitt, 1988; Paterson *et al.,* 1991). Identification of putative QTLs for traits is the first step towards conventional use of MAS for improvement of traits. However, there are certain considerations that need to be taken when deciding which QTLs should be emphasized in a MAS strategy. First of all, it is necessary to decide which region(s) has enough evidence for the presence of a major QTL. This can be achieved by setting appropriate thresholds (LOD more than 2.5) for the identification of putative QTLs. Secondly, only those QTLs consistently expressed across appropriate testing environments can be recommended for use in MAS. Finally, the selected QTL for MAS should have high levels of contribution to the phenotypic variance. In this study, QTLs identified on SBI-07 for spotted stem borer resistance component traits fulfilled these criteria. All these putative QTLs had high values of LOD scores and were detected in both the screening environments. In addition, this region was also found to contribute to stem borer resistance in maize, confirming the association of this region with resistance. While these QTLs will

contribute to only a small improvement in resistance to spotted stem borer, integration of MAS with a transgenic approach *i.e.* pyramiding of these QTL with toxin protein genes from *Bt* (*Bacillus thuriengensis*) would increase the probability of success in development of spotted stem borer resistant sorghum varieties or hybrids.

Besides MAS for resistance, selection can also be done for agronomic and morphological traits such as grain and leaf morphology, plant color, height, flowering time etc. In the present study, the QTLs on SBI-06 for plant color and basal pigmentation; on SBI-04 for mesocarp thickness, testa (presence vs absence), leaf angle; on SBI-01, 06 and 10 for number of nodes and time to 50% flowering; and on SBI-01, 06 and 07 for plant height exhibited stability and contributed substantially to observed phenotypic variation across environments, suggesting them to be promising candidates for MAS. Molecular markers closely linked to genes of agronomic importance can be useful tools for indirect selection in sorghum resistant breeding programmes. In the present study association of QTL for plant height and agronomic performance with QTLs for resistance component traits suggests that MAS for these simply inherited agronomic traits might permit indirect selection for spotted stem borer resistance component traits.

Marker-assisted selection (MAS) is time-efficient, non-destructive and depending on linkage relationships, characterized by low selection error rates. MAS should be applied on a case by case basis Even though MAS has several advantages it is still a cost-intensive process, hence for it to be successful, robustness of the targeted QTL is essential. Thus, saturation of the map particularly near the genomic regions identified for the targeted spotted stem borer resistance component, agronomic or morphological traits is required to minimize the likelihood of false positive QTLs and increase the probability of success of MAS. Marker saturation of these regions will

also facilitate selection of recombinants with minimal negative linkage drag due to linkage of the target genomic region with unfavorable alleles from their common donor parent. Further investigation for spotted stem borer resistance will be required to establish the importance of the identified genomic regions in other backgrounds and under natural infestation. Also, several more seasons of field evaluations would be required to establish the effectiveness of the screening system in evaluating the stability of QTLs across environments. In addition, validation of markers associated with the resistance component traits and agronomic and/or morphological traits in an independent population or by backcrossing the putative QTLs into a range of cultivars would provide much stronger support for marker-QTL linkage across different genetic backgrounds.

Although QTL analysis has been of tremendous use in the identification of genomic regions pertaining to the resistance, it fails to identify the complete sets of genes of a biochemical pathway leading to the expression of the trait. Our results indicate the existence of genes or gene clusters with major effects on spotted stem borer resistance particularly on SBI-07, and several single major QTLs for agronomic and morphological component traits on SBI-06 and SBI-04. Hence marker saturation of these regions would be particularly useful for detection of candidate genes that might be responsible for variation in these traits.

SUMMARY

CHAPTER VI

SUMMARY

The present investigation was carried out with the primary objective of characterizing and understanding the genetic architecture of spotted stem borer resistance in sorghum through QTL analyses. In addition, this opportunity was also taken to locate chromosomal regions responsible for the variation observed among the RILs for several other agronomic and morphological traits. The experimental material for this study constituted of 266 $F_{9:10}$ RILs produced from a cross between ICSV 745 and PB 15520. The female parent was susceptible while the male parent was resistant to spotted stem borer. The 266 RILs along with their parents and resistant (IS 2205) and susceptible (ICSV 1) control entries were evaluated under artificial infestation during the 2007 and 2008 rainy seasons. The experiment was laid out in 28×10 alpha-lattice designs and the phenotypic observations were recorded in the population on five resistance component traits and eleven agronomic and morphological traits for which the parental lines exhibited substantial differences. The best linear unbiased predictors (BLUPs) for each RIL were obtained by subjecting the phenotypic data set to ReML (residual maximum likelihood algorithm) analysis. These recombinant inbred lines were also genotyped with 90 polymorphic SSR markers and a linkage map was constructed using MAPMAKER/EXP v 3.0 software. The phenotypic and genotypic data sets were then utilized for identification of QTLs for the observed traits following a composite interval mapping approach using PLABQTL software. The salient findings of the study are briefly summarized here:

- The parental lines of the RIL population differed substantially for spotted stem borer resistance component traits and most of the agronomic and morphological traits. A wide variation was also observed among the recombinant inbred lines for

all of the observed traits across both screening environments. The mean performance of RILs for most of spotted stem borer resistance component traits approached the mid-parental value indicating a minimal role of dominance or genetic interactions for any of these traits except for stem tunneling. Among the observed agronomic and morphological traits except for testa pigmentation (presence vs absence) and agronomic performance score, the mean performance were skewed towards one or other of the two parents indicating a role of genetic interaction in their expression.

- A high G×E interaction variance was observed for all of the spotted stem borer resistance component traits and most of the observed agronomic and morphological traits, except plant color score and seedling basal pigmentation score. Across environment heritability for the resistant component traits were low to moderate, while for most of the observed agronomic/morphological traits it was high, suggesting that environment played greater role in expression of spotted stem borer resistance components than for the other observed agronomic and morphological traits, presumably because the G×E interaction component of resistance to spotted stem borer involves not only the interaction of plant with environment but also interaction of environment with the insect pest population that infested the experiment each season.
- A near normal distribution of RILs for most of the spotted stem borer resistance and agronomic traits indicates polygenic inheritance for these traits. In addition, the observed histograms and large proportion of transgressive segregation for these traits indicates major roles of epistasis and strongly suggests the dispersed nature of favorable and unfavorable alleles for these traits among the two parents, respectively.

- Correlation analysis indicated significant and positive associations among all the resistance component traits, except stem tunneling, suggesting independent genetic control for this trait. Significant association was also observed between various pairs of agronomic/morphological traits and resistance component traits such as plant height with stem tunneling, deadheart incidence with time to 50% flowering, plant color score with leaf feeding damage and overall resistance score, and recovery resistance score with plant height and number of nodes.
- The 266 RILs were genotyped using 90 polymorphic SSR markers and the basic linkage map constructed for this population spanned 1289.4 cM representing all 10 sorghum chromosomes. The linkage group SBI-03 contained the highest number of SSR markers (15) followed by SBI-01 (14 markers, across segments a and b), SBI-07 (11 markers) and SBI-10 (11 markers). Average inter-marker distance across the ten linkage groups was 14 cM, which is ideal for an efficient QTL mapping study if marker distribution is uniform across the linkage map. However, a few wide gaps (>20 cM) remained despite the attempts to fill them, particularly on SBI-04, 06 and 10.
- Different subsets of QTLs were detected for the spotted stem borer stem tunneling resistance component across the two environments due to the substantial influence of environment on expression of this trait. Only the QTL on SBI-07 (between markers *Xtxp295* and *Xisep0704*) was observed on similar positions in the individual and across-environment analyses. Incidentally, this QTL also accounted for the largest portion of observed phenotypic variation; and even though it exhibited significant QTL×E, this QTL could be considered to be stable for stem tunneling because of non-crossover type of interaction and hence provide an excellent initial target for marker-assisted selection.

- For all the other resistance component traits, several QTLs were detected accounting from 1.6% to 27% of the observed phenotypic variation. Surprisingly, for most of these resistance component traits, stable QTLs were also detected on the distal end of SBI-07 (region between markers *Xisep0829* and *Xisep0704*), near to the identified stable QTL for stem tunneling. In addition, these QTLs also explained largest proportion of the observed phenotypic variation for the respective traits across the putative QTLs identified.
- Among the observed morphological traits, single major QTLs explaining more than 20% of the observed phenotypic variation across environments were detected for seedling basal pigmentation score (SBI-06), plant color (SBI-06), testa pigmentation (presence vs absence) score (SBI-04), mesocarp thickness score (SBI-04), and leaf angle (SBI-04) suggesting the presence of candidate genes for these traits on SBI-04 and 06. The positions of the major QTLs for testa pigmentation, mesocarp thickness and leaf angle have not been previously reported This study replicated several previous findings of six putative QTLs for time to 50% flowering and three for plant height. In addition, several QTLs each with minor effects for seedling vigor and agronomic performance were also identified confirming the polygenic nature of inheritance of these traits.
- Based on the phenotypic observations and QTLs detected for the resistance component traits, the RILs 135, 108, 24, 93, 212 and 47 were found to harbor favorable alleles for most of the detected QTLs for spotted stem borer resistance, while RILs 239, 35, 19 and 69 had favorable alleles for most of the QTLs detected for resistance to leaf feeding damage and deadheart incidence. Similarly, RILs 253, 250, 196 and 185 had favorable alleles for most of the detected QTLs for recovery resistance score and overall resistance score. These RILs would hence be

best available candidates for use as donor parents for marker-assisted breeding.

- *In-silico* comparison of genomic regions associated with spotted stem borer resistance and regions identified in maize with stem borer resistance indicated that the genomic regions on the distal ends of SBI-07 (between markers *XSbAGB02* and *Xisep0829*) and SBI-04 (between markers *Xtxp327* and *Xisp10229*) and on SBI-02 (between markers *Xisep0747* and *Xtxp025*) are orthologous to maize genomic regions containing stem borer resistance QTLs. Based on this comparison, a few more genomic regions that are not adequately covered in the present sorghum RIL population have been identified that may harbor a few more putative QTLs for spotted stem borer resistance, including those on SBI-01 (between markers *Xiabtp450* and *Xtxp229*), the distal end of SBI-04 (65-70 Mbp below *Xisp10229*) and on SBI-09 between (between markers *Xgap032*, *Xiabtp475* and *Xtxp010*). Hence future work must be prioritized on filling these gaps and extending the linkage map by using appropriate markers derived from the EST sequences present in these genomic regions.

The results of this QTL study are a first step towards the design of marker-assisted selection programmes for spotted stem borer resistance improvement in sorghum. Several QTLs for spotted stem borer resistance component traits have been identified under conditions of artificial infestation in the present study. Clusters of stable QTLs identified for most of the spotted stem borer resistance component traits particularly, on SBI-07 and their homology with maize genomic regions conferring resistance to stem borers, are particularly interesting and hence may be useful as initial targets for marker-assisted varietal improvement and positional cloning of the cloned genes. However, since these QTLs were identified under artificial infestation the usefulness of these QTLs should further be evaluated under natural infestation conditions. In

addition to stem borer resistance, putative QTLs for various agronomic and morphological traits were also detected in the present study; these identified QTLs could also play a major role in marker-assisted selection programmes for sorghum improvement.

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APPENDICES

Appendix 1. Artificial diet for rearing spotted stem borer larvae

S.No	Ingredient	Quantity
FRACTION A		
1	Water	2000.0 ml
2	Chick pea flour	438.4 g
3	Brewers yeast	32.0 g
4	Sorbic acid	4.0 g
5	Vitamin E	4.6 g
6	Methyl parhydroxy benzoate	6.4 g
7	Ascorbic acid	10.4 g
8	Sorghum leaf powder	160.0 g
Fraction B		
1	Agar-agar	40.8 g
2	Water	1600.0 ml
3	Formaldehyde (40%)	3.2 g

Appendix 2. Reagents required for DNA extraction.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	3% CTAB (Cetyl Trimethyl Ammonium Bromide) buffer	10 mM Tris 1.210 g 1.4 M NaCl 8.180 g 20 mM EDTA 0.745 g 3% CTAB 3.000 g Distilled water 100.000 ml Adjust to pH 8.0 using HCl. Add 0.17 ml mercaptoethanol only at the time of keeping the buffer in boiling water
2	Chloroform:Isoamyl Alcohol (24:1)	Chloroform 96 ml Isoamyl alcohol (IAA) 4 ml Store in dark at room temperature.
3	Isopropanol	Keep isopropanol at –20°C. Use only ice cold isopropanol.
4	RNase A (10 mg/ml)	Dissolve 100 mg of pancreatic RNase A in 100 ml of 10 mM Tris (pH 7.5) and 15 mM NaCl. Heat in boiling water bath for 15 minutes and allow to cool slowly to room temperature. Dispense into aliquots and store at –20°C. Working stocks may be stored at 4°C.
5	Phenol:Chloroform:Isoamyl Alcohol (25:24:1)	Phenol (equilibrated) 50.0 ml Chloroform:IAA (24:1) 50.0 ml Store at 4°C.
6	Sodium Acetate (3 M, pH 5.2)	Dissolve 40.824 g of sodium acetate in 60 ml distilled water and adjust to pH 5.2 using glacial acetic acid. Make the volume up to 100 ml with distilled water and autoclave.
7	Absolute Ethanol	Store at –20°C
8	70% Ethanol	Absolute ethanol 70 ml Distilled water 30 ml
9	$T_1E_{0.1}$ Buffer	10 mM Tris 121.0000 g 1mM EDTA 0.0372 g Distilled water 100.0000 ml
10	$T_{10}E_1$ Buffer	0.5 M Tris 6.050 g 0.5 M EDTA 9.306 g 2 M NaCl 11.688 g Distilled water 100.000 ml

Appendix 3. Reagents required for DNA quantification.

S.No.	Chemicals / Reagents	Chemical composition / Remark
1	0.8% agarose	0.8 g agarose was dissolved in 100 ml of 1X TBE buffer.
2	1X TBE buffer	For 10X TBE buffer – dissolve 109 g of Tris and 55 g of boric acid one by one in 800 ml distilled water and add 40 ml of 0.5 M EDTA (pH 8.0). Bring volume to 1 litre with distilled water. Sterilize by autoclaving and store at 4°C.
3	Ethidium bromide (10mg/ml)	Dissolve 100 mg of ethidium bromide in 10 ml distilled water. Wrap the tube in aluminium foil and store at 4°C.
4	Orange loading dye	0.5 M EDTA (pH-8.0) 10 ml 5 M NaCl 1 ml Glycerol 50 ml Distilled water 39 ml Add orange dye powder till the colour becomes sufficiently dark.

Table 1. Sources of resistance to spotted stem borer (*Chilo partellus*)

Germplasm lines / cultivars	References
IS Nos. 704, 1019, 1150, 1242 and 2194	Pant *et al.* (1961)
Jonna AKP-1	Kalode and Pant (1966)
IS Nos. 1054, 1060 (Sundhia), 1150, 2194 and T / 263	Kalode and Pant (1967)
Line 477	Kundu and Jotwani (1977)
E 302 (BP 53 × Kafir B) and E 303 (BP 53 × IS 3954)	Jotwani *et al.* (1974)
IS 5629	Jotwani *et al.* (1978a)
E 302, E 303, P 37, NCL 3 and P 151	Jotwani *et al.* (1978b)
DU 19, DU 98, DU 245, DU 291, U 218, U 373, U 358 and U 376	Jotwani *et al.* (1979)
D 168, D 172, D 259, D 358, D 367 and D 369	Singh *et al.* (1980)
E 302, D 303, D 114 and IS 4308	Lal and Sukhani (1982)
E 501, E 502, E 503, E 504, E 601, E 602, E 603 and E 604	Jotwani (1982)
IS Nos. 2122, 2146, 2195, 2205, 4337, 4660, 18551	Seshu Reddy (1983)
Serena	Pathak and Olela (1983)
E 302 and E 303	Dalvi *et al.* (1983)
E 302, E 303, IS Nos. 1044, 1151, 2162, 4660, 17739, 18328, 18349, 18479, 18849	Dabrowski and Kidiavai (1983)
E 303, E 501, E 502, E 601 and E 641	Mote and Bapat (1983)
CSV 8R, SPV 35, SPV 103, SPV 140 and SPV 192	Singh *et al.* (1983)
SPV 31, SPV 101, SPV 110, SPV 232, SPV 257, SPV 209, SPV 291, SPV 301 and SPV 311, E 302, E 701, CSV 3, CSV 6, CSV 8R and Aispuri	Singh and Rana (1984)
CSV 5 × CSV6, CSV 5 × CSV4 and CSV 5 × IS 4664	Prem Kishore *et al.* (1984)
E 302, SPV135, IS 2312, IS 4664, CSV 8 R and SPV 104	Rana *et al.* (1984)
E 304	Kundu (1985)
LC 119/83-3	Alghali (1985)
IS Nos. 10364, 10370, 1044, 3962, 4213, 12497, 18479, 18326, 4405, 18676, 5613, 18517, 18323, 4881, 1151, 1847, L l, L 2 and S 178	Seshu Reddy (1985)
IS 2146 × Serena	Pathak (1985)

Contd.,

Table 1 (Contd.,). Sources of resistance to spotted stem borer (*Chilo partellus*)

Germplasm lines / cultivars	References
IS Nos. 1044, 1082, 1119, 2122, 2123, 2146, 2168, 2169, 2291, 2309, 2312, 2375, 2376, 4273, 4546, 4637, 4576, 4757, 4776, 4881, 4981, 5075, 5253, 5429, 5469, 5470, 5480, 5538, 5566, 5571, 5585, 5604, 5619, 5622, 7223, 8811, 9608, 10711, 12308, 13100, 13674, 17742, 17745, 17747, 17750, 17948, 17966, 18333, 18366, 18551, 18573, 18577, 18578, 18579, 18580, 18548, 18585, 18662, 18667, 20643, 21969, 22039, 22091, 22145, 22507,23411, 23962 and 24027	Taneja and Leuschner (1985)
IS 1044, IS 1151, IS 2205, IS 12308	Saxena (1986)
Serena, LC 119/80-2 and P 101	Alghali (1987)
P 311	Prem Kishore (1987a)
P 217, P 297, P 500, P 84, P 296, P 467, P 471 and P 495	Prem Kishore (1987b)
IS Nos. 2205, 1833, 18551, 13100	Woodhead and Taneja (1987)
IS 5469 and IS 2205	Singh and Verma (1988a)
IS 2123 and IS 5469	Singh and Verma (1988b)
IS 178, Improved Ramkel, S 136, PC 6, SPV 238	Dhaliwal *et al.* (1988)
IS 2146	Alghali and Saxena (1988)
ICSV 700, 701, 825, 826, 827, 828 and 829; IS Nos. 1082, 2312, 3962, 5604, 5622 and13681	Agrawal and Taneja (1989)
IS Nos. 3962, 18584, 2235, 1054, 5469, 5619, 18577, P 37 and SPV 102	Patel and Sukhani (1989)
IS Nos. 1044, 2122, 2123, 2263, 2291, 2309, 2312, 4756, 4776, 5469, 5480, 5538, 5566, 5571, 5585, 10711, 12308, 13100, 13674, 18551, 18577, 18579, 18662, SB 8536	Agrawal *et al.* (1990)
P 921, P 930, P 933, P 934, P 936	Prem Kishore (1991)
IS Nos. 18584, 18577, 2205	Patel *et al.* (1995)
MASV 33/93, IS 2205, PB 15438, PB 15221-1.2-2, PB 15826, PB 15881-3, ICSV 700	Singh and Shankar (2000)
SPV 1155, SPV 1359, SPV 1466, SPH 1162, SPH 1078, SPH 1079, SPH 1165, P-84, P-217, P-291, P-296, P-467, P-471, P 500 and P 297	Prem Kishore (2001)
SPV 462, SPV 1489, SPV 1526, SPV 1563, SPV 1566, SPV 1567, SPV 1576, CSV 13, CSV 15, CSH 17, SPH 1148, SPH 1280	Prem Kishore *et al.* (2002)
DS 1, DS 2, DS 3, DS 4, DS 5 and DS 6	Prem Kishore (2005)
SP 55301 (A&B), ICSV 700, IS 18551, SFCR 151	Sharma *et al.* (2006)
SSV 7073, SSV-53, SSV 6928, HES-4 and IS 2312	Marulasiddesha *et al.* (2007)

Table 2. Details of linkage maps constructed in sorghum

Sl No	Reference	Year	Mapping population	Parental lines	Molecular markers	Linkage group	Map length (cM)	Purpose / Remark
1	Hulbert *et al.*	1990	55 F_2 plants	Shanqui Red and M 91051	37 RFLP maize probes	8	283 R	omparative mapping
2	Binelli *et al.*	1992	149 F_2 plants	S 18729 and IS 24756	1 RFLP maize probes	5	440 U	omparative mapping
3	Whitkus *et al.*	1992	81 F_2 plants	IS 2482 and IS 18809	85 RFLP maize probes and 7 isozymes	13	949 H	omparative mapping
4	Berhan *et al.*	1993	55 F_2 plants	Shanqui Red and M 91051	96 RFLP maize probes	15	709 R	omparative mapping
5	Chittenden *et al.*	1994	56 F_2 plants	BTx623 and *S. propinquum*	256 RFLP sorghum probes, 20 maize, rice and oat probes	10	1445 U	volutionary study
6	Pereira *et al.*	1994	78 F_2 plants	CK 60 and PI 229828	191 RFLP maize probes and 10 sorghum probes	10	1530 U	omparative mapping
7	Xu *et al.*	1994	50 F_2 plants	BTx623 and IS 3620C	179 RFLP sorghum probes, 11 maize probes	14	1789 K	TL analysis
8	Ragab *et al.*	1994	93 $F_{2:3}$ families	BSC 35 and BTx631	38 RFLP sorghum probes and 33 maize probes	15	633 H	omparative mapping
9	Lin *et al.*	1995	370 F_2 plants	BTx623 and *S. propinquum*	8 RFLP sorghum probes, 124 aize, rice and oat probes	11	935 K	evelopment of high ensity map
10	Pereira and Lee	1995	152 F_2 plants	CK 60 and PI 229828	6 RFLP sorghum probes, 34 maize genomic probes and 66 maize cDNA probes	10	1299 H	QTL analysis
11	Tuinstra *et al.*	1996	98 RIL ($F_{5:7-8}$)	Tx7078 and B35	150 RAPD, 20 RFLP maize/ sorghum probes	17	1580 R	QTL analysis
12	Dufour *et al.*	1997	98 RIL (F_5)	IS 2807 and IS 379	126 RFLP maize probes, 19 sugarcane probes, 4 cloned genes and 2 morphological markers	13	977 H	Comparative mapping

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Table 2 (Contd.,). Details of linkage maps constructed in sorghum

Sl No	Reference	Year	Mapping population	Parental lines	Molecular markers	Linkage group	Map length (cM)	Purpose / Remark
12	Dufour *et al.*	1997	91 RIL (F_5)	IS 2807 and IS 249	115 RFLP maize probes and 8 RFLP sugarcane probes	12	878 H	QTL analysis
			Composite map from the two populations		164 RFLP maize probes, 19 RFLP sugarcane probes, 3 cloned genes and 2 morphological markers	13	1095 H	
13	Taramio *et al.*	1997	68 F_2 plants	CK 60 and PI 229828	191 RFLP maize probes, 10 sorghum probes and 7 SSR markers	10	1575 U	Utility of SSR for mapping in sorghum
14	Ming *et al.*	1998	56 F_2 plants	BTx623 and *S.propinquum*	328 RFLP probes from sorghum and other cereals	10	1750 K	Comparative mapping
15	Tao *et al.*	1998a	120 RIL (F_5)	QL 39 and QL 41	68 RFLP sorghum probes, 87 probes of maize, sugarcane, rice, oat and barley, 8 SSR and 3 morphological markers	21	1400 U	Comparative and QTL mapping
16	Boivin *et al.*	1999	110 RIL (F_5)	IS 2807 and IS 379	128 RFLP of sorghum, rice, oat or barley, 176 AFLP, 151 loci of RFLP, cloned genes and morphological markers	11	1899 H	Comparative mapping
		1999	Composite map from 110 RIL (F_5) and 91 RIL (F_5)	IS 2807 and IS 379; and IS 2807 and IS 249	343 RFLP probes and morphological markers	11	1352 H	
17	Peng *et al.*	1999	137 RIL (F_{6-8})	BTx623 and IS 3620C	323 RFLP from sorghum, maize, rice, oat and barley	10	1347 K	Comparative mapping

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Table 2 (Contd.,). Details of linkage maps constructed in sorghum

Sl No	Reference	Year	Mapping population	Parental lines	Molecular markers	Linkage group	Map length (cM)	Purpose / Remark
18	Bowers *et al.*	2000	65 F_2 plants	BTx623 and *S. propinquum*	2399 loci using 1925 RFLP probes	10	1200 U	Development of high density sorghum map
19	Kong *et al.*	2000	137 RIL (F_{6-8})	BTx623 and IS 3620C	33 SSR, 111 RFLP probes from sorghum, maize, rice, oat or barley	10	1287 K	Development of high density sorghum map
20	Tao *et al.*	2000	152 RIL (F_5)	QL 39 and QL 41	267 RFLP, 17 SSR, SSRs and morphological markers	14	1871 U	QTL analysis
21	Xu *et al.*	2000	98 RIL (F_7)	B35 and Tx7000	162 RFLP sorghum and maize probe, cloned genes and sequenced DNA probes	10	837 H	QTL analysis
22	Bhattramakki *et al.*	2000	137 RIL ($_{F6-8}$)	BTx623 and IS 3620C	116 SSR, 354 RFLP and SSR markers	10	1406 K	Development of high density sorghum map
23	Subudhi and Nyugen	2000	98 RIL (F_7)	B 35 x Tx7000	214 RFLP, SSR and RAPD	10	1200 H	Comparative mapping
24	Klien *et al.*	2001a	125 RIL (F_5)	RTx430 and Sureno	44 SSR, 85 AFLP and 1 morphological marker	10	970 K	QTL analysis
25	Hausmann *et al.*	2002	225 RIL (F_{3-5})	IS 9830 and E 36-1	125 AFLP, 45 SSR, 14 RFLP and 3 RAPD	10	1265 H	Comparative mapping and QTL analysis
			226 RIL (F_{3-5})	N13 and E 36-1	158 AFLP, 54 SSR and 16 RFLP	12	1410 H	
			Composite map of the two populations		339 AFLP, SSR, RFLP and RAPD	11	1424 H	
26	Menz *et al.*	2002	137 RIL (F_{6-8})	BTx623 x IS 3620C	336 RFLP, 136 SSR and 2454 AFLP	10	1713 K	High density map

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Table 2 (Contd.,). Details of linkage maps constructed in sorghum

Sl No	Reference	Year	Mapping population	Parental lines	Molecular markers	Linkage group	Map length (cM)	Purpose / Remark
27	Agrama *et al.*	2002	93 RIL (F_{5-6})	GBIK and Redlan	38 SSR and 75 RAPD	12	1530 K	QTL analysis
28	Bowers *et al.*	2003	65 F_2 plants	BTx623 and *S. propinquum*	2512 loci using 2050 RFLP probes	10	1059.2 K	High density map
29	Tao *et al.*	2003	120 RIL (F_5)	ICSV 745 x 90562	235 RFLP and 8 SSR	12	1472 U	QTL analysis
30	Haussmann *et al.*	2004	226 RIL ($F_{3:5}$)	IS 9830 and E 36-1	137 AFLP, SSR and RFLP	11	1498 H	QTL analysis
			226 RIL ($F_{3:5}$)	N 13 and E 36-1	157 AFLP, SSR and RFLP	11	1599 H	
31	Nagaraj *et al.*	2005	88 RIL (F_6)	96-4121 and Cargill 607E	60 SSR	13	603.5 K	QTL analysis
32	Feltus *et al.*	2006	Bridge map	BTx623 x IS 3620C and BTx623 and *S. propinquum*	106 SSR and RFLP	10	1367.4 K	Comparative and QTL mapping
33	Wu and Huang	2007	277 F_2	Westland A line and PI 550610	118 SSR	16	997.5 K	Comparative mapping
34	Mace *et al.*	2008	92 RIL (F_5)	R 931945-2-2-2 and IS 8525	358 DArT, 229 AFLPs, 55 SSRs and 2 morphological markers	10	1431.6 K	Comparative and QTL mapping
35	Ritter *et al.*	2008	184 RIL (F_6)	R9188 and R 9403463-2-1	228 AFLP and SSR	16	2012.9 U	QTL analysis
36	Mace *et al.*	2009	Consensus map	BTx623 × IS 3620C R 890562 × ICSV 745 R 931945-2-2-2 × IS 8525 B 923296 × SC 170-6-8 BTx642 × QL 12 SAR 10 × SSM 249	1190 DArT, 839 SSRs and RFLPs	10	1355.4 K	Comparative and QTL mapping

Map function K: Kosambi; H: Haldane; R : Recombination frequency; U: Not specified

Table 3. QTLs associated with various traits in sorghum

Component traits	References
Agronomic traits	
Plant height	Ritter *et al.* (2008); Feltus *et al.* (2006); Hart *et al.* (2001); Klein *et al.* (2001a); Kapran and Axtell (2000); Rami *et al.* (1998); Lin *et al.* (1995) and Periera and Lee (1995),Srinivas *et al.* (2009)
Maturity	Ritter *et al.* (2008); Feltus *et al.* (2006); Hart *et al.* (2001); Chantereau *et al.* (2001); Kapran and Axtell (2000); Trouche *et al.* (1998); Childs *et al.* (1997) and Lin *et al.* (1995)
Leaf architecture	Feltus *et al.* (2006) and Hart *et al.* (2001)
Panicle architecture	Brown *et al.* (2006); Feltus *et al.* (2006); Hart *et al.* (2001) and Rami *et al.* (1998)
Yield	Ritter *et al.* (2008); Feltus *et al.* (2006); Kapran and Axtell (2000); Tuinstra *et al.* (1997a) and Rami *et al.* (1998)
Grain quality	Feltus *et al.* (2006); Klein *et al.* (2001a) and Rami *et al.* (1998)
Sugar content	Ritter *et al.* (2008) and Yun-long *et al.* (2006)
Tillering	Paterson *et al.* (1995) and Hart *et al.* (2001)
Pre-harvest sprouting resistance	Lijavetzky *et al.* (2000)
Fertility restoration	Wen *et al.* (2002) and Klein *et al.* (2001b)
Abiotic Stress tolerance	
Drought tolerance	Feltus *et al.* (2006); Sanchez *et al.* (2002); Kebede *et al.* (2001); Subudhi *et al.* (2000), Tao *et al.* (2000); Xu *et al.* (2000); Ejeta *et al.* (2000); Crasta *et al.* (1999) and Tuinstra *et al.* (1996,1997a)
Biotic stress resistance	
Parasitic weed	
Striga	Haussmann *et al.* (2004) and Ejeta *et al.* (2000)
Disease resistance	
Anthracnose	Singh *et al.* (2006); Mehta (2002); Klein *et al.* (2001a) and Boora *et al.* (1998);
Grain mold	Klein *et al.* (2001a)
Head smut	Oh *et al.* (1994)
Leaf blight resistance	Boora *et al.* (1999)
Bacterial leaf stripe	Klein *et al.* (2001a)
Downy mildew	Oh *et al.* (1996)
Rust	Tao *et al.* (1998b)
Stalk rot	Reddy *et al.* (2008)
Zonate leaf spot	Klein *et al.* (2001a), Mohan *et al.* (2009)
Insect pest resistance	
Green bug	Wu and Huang (2008); Nagaraj *et al.* (2005); Agrama *et al.* (2002) and Katsar *et al.* (2002)
Midge	Tao *et al.* (2003)
Shoot fly	Sajjanar (2002) and Deshpande (2005),Satish *et al.* (2009)

Table 4. QTLs identified conferring resistance to stem borers in maize

Sl No	Pest	Reference	Year	Mapping population	Parental lines	Trait/s	QTLs identified	Linkage group	Phenotypic variation explained (%)
1	ECB	Schőn *et al.*	1993	300 F_3	B73 x B52	Stalk tunneling	7	1,2,3,7,10	3.4 - 15.7
2	ECB	Lee and Veldbloom	1993	150 $F_{2:3}$	Mo17 x H99	Leaf blade feeding	2	1,4	16.0 - 17.0
3	ECB	Beavis *et al.*	1994	112 F_2	B73 x Mo17	Leaf and stalk damage	3	7, 8, 9	8.0 - 20.0
4	SCB	Bohn *et al.*	1996	171 F_2	CML131 x CML67	Leaf feeding	10	1,2,5,7,8,9,10	7.2 - 15.4
5	SWCB, SCB	Bohn *et al.*	1997	171 F_2	CML131 x CML67	Leaf feeding by SWCB	6	1,5,7,9	1.6 - 14.9
						Leaf feeding by SCB	10	1,2,3,5,7,8,9	3.8 - 30.8
6	SWCB	Khairallah *et al.*	1998	472 F_2	Ki3 x CML139	Leaf feeding	7	3,5,6,8,9	11.3 - 29.9
7	SCB, SWCB	Groh *et al.*	1998	215 $F_{2:3}$	CML131 x CML67	Leaf feeding by SWCB	9	1,5,7,8,9	3.2 - 14.0
						Leaf feeding by SCB	8	1,5,7,8,9	2.3 - 25.8
	SWCB			475 $F_{2:3}$	Ki3 x CML139	Leaf feeding by SWCB	5	1,6,8,9	2.6 - 14.6
8	ECB	Bohn *et al.*	2000	226 $F_{2:3}$	Do6 x D408	Stalk damage rating	5	1,5,6,8	3.5 - 8.1
						Stem tunneling	6	1,3,5,9,10	5.5 - 13.5
9	ECB	Cardinal *et al.*	2001	200 $F_{6:8}$	B73 x B52	Stem tunneling	9	2,3,5,7,8,9	4.4 - 13.8
10	ECB	Jampotong *et al.*	2002	244 $F_{2:3}$	B73 Ht x Mo47	Leaf feeding	9	1,2,4,5,6,8	4.4 - 25.0
						Stem tunneling	7	2,5,6,8,9	4.1 - 15.1
11	SWCB	Willcox *et al.*	2002	277 BC_1F_2	CML67 x CML204	Leaf feeding	3	7,9,10	4.9 - 17.3
12	ECB	Krakowsky *et al.*	2002	147 F_3	B73 x De811	Stem tunneling	7	1,3,4,5,8	5.4 - 24.7
13	ECB	Krakowsky *et al.*	2004	183 $F_{6:8}$	B73 x De811	Stem tunneling	16	1,3,5,6,7,9,10	4.7 - 21.3
14	ECB	Krakowsky *et al.*	2005	183 $F_{6:8}$	B73 x De811	Stalk sheath component	44	1,2,3,4,5,6,7,9,10	5.0 - 31.0
15	ECB	Krakowsky *et al.*	2006	183 $F_{6:8}$	B73 x De811	Leaf sheath component	42	1,2,3,4,5,6,7,8,9,10	4.0 - 29.0
16	ECB	Krakowsky *et al.*	2007	183 $F_{6:8}$	B73 x De811	Cell wall component	20	2,5,6,7,9,10	-

ECB: European corn borer, SCB: Sugarcane borer, SWCB: South western corn borer,

Table 5. Details of the parental lines used in the development of RILs

Parents	Pedigree	Description
ICSV 745	(PM 11344 x A 6250)-4-1-1-1	Highly resistant to midge, susceptible to spotted stem borer, released in Karnataka as DSV 3, Dual purpose variety, has tan plant color, and medium thick and non-juicy stem. Leaves are broad, thick, and drooping with white midrib. Boot leaf is short and erect. It is a medium-duration cultivar (60-85 days to 50% flowering).Its panicle is compact at the base and semi-loose at the apex. Glumes are short, straw-colored, and cover about one third of the grain. Seed is lustrous, asymmetrical, without subcoat, with a thin pericarp and a beak.
PB 15520	(PM 14386-1-6 × IS2205)-14-2-2-2-1	Resistant to spotted stem borer, has red plant color, and thick and non-juicy stem. Leaves are broad, thick, and erect with white midrib. Boot leaf is short and erect. It is a late maturing line (> 90 days to 50% flowering) with shorter plant height. Its panicle is compact and the seed has a subcoat and a thick mesocarp

Table 7. Sequence information of selected polymorphic SSR markers

Markers	Forward primer sequence	Reverse primer sequence	Allele size (bp)		Reference
			ICSV 745	PB 15520	
Xcup01	CATGGGCGGGTTGAAGAC	TGCAGGAAGGGAGGATGTAG	197	223	Schloss *et al.* (2002)
Xcup06	GGCAGTAGCAGGCGTTTAAG	AACTGAATCAGGTCATGGGC	202	212	Schloss *et al.* (2002)
Xcup14	TACATCACAGCAGGGACAGG	CTGGAAAGCCGAGCAGTATG	206 (235)	208	Schloss *et al.* (2002)
Xcup24	AAACTGGATGCCACACCAAG	AGCTATACCAACACGGGCAG	173	169	Schloss *et al.* (2002)
Xcup38	CTCTCACGGAAAGGAAGCAC	TACCGAAGCGGAAGCTACTC	155	148	Schloss *et al.* (2002)
Xcup44	CATGCATGCGTGTACCTGAG	TAGCTGTGTCCGTCGATGTC	217	219	Schloss *et al.* (2002)
Xcup47	TGAGCAATGAACTTAGGGGG	CTACCCTTTGATGGCAGTACC	241(248)	236	Schloss *et al.* (2002)
Xcup49	TCCACCTCCATCATCTTTCC	CTCCACCACCTCCATGACTC	151	146	Schloss *et al.* (2002)
Xcup50	TGATTGATTGAGGCAGGCAC	TTCCGGTCTCTGTCCATTTC	*315*	*310*	Schloss *et al.* (2002)
Xcup57	CTGCAGAGAGCTAATTGTGC	TCTTGGAAGAGACGGACCTG	174	171	Schloss *et al.* (2002)
Xcup60	GTATGCATGGATGCCTGATG	GCGAGGGTATGTAGCTCGAC	163	150	Schloss *et al.* (2002)
Xcup61	TTAGCATGTCCACCACAACC	AAAGCAACTCGTCTGATCCC	196	202	Schloss *et al.* (2002)
Xcup73	GGTTCTGTCGTCATCACCAG	ATCTTTAGCCGCCACATGAC	213 (180)	205	Schloss *et al.* (2002)
Xcup74	GTCGCCATTGTGATGAAGAG	CAGTAGTCCAGCAAAACGGC	235	229	Schloss *et al.* (2002)
Xgap015	GCTGCTAAGCCGTGCTGA	TTATTTGGGTGAAGTAGAGGTGAACA	125	129	Brown *et al.* (1996)
Xgap032	CTCGGCGGTTAGCACAGTCAC	GCCCATAGACAGACAGGAAAGCC	210	194	Brown *et al.* (1996)
Xgap206	ATTCATCATCCTCATCCTCGTAGAA	AAAAACCAACCCGACCCACTC	162	146	Brown *et al.* (1996)
Xgap342	TGCTTGTGAGAGTGCCTCCCT	GTGAACCTGCTGCTTTAGTCGATG	278	288	Brown *et al.* (1996)
Xiabtp349	ATAGTTGGCGTGCTCCTGAC	GCCATCATCCATCCATATCC	321	304	Folkerstema (Unpublished)
Xiabtp364	GCACTAATGCCATGCAAATG	CAGCAGGCAGAGTGTAGGTG	145	140	Folkerstema (Unpublished)

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Table 7 (Contd.,). Sequence information of selected polymorphic SSR markers

Markers	Forward primer sequence	Reverse primer sequence	Allele size (bp)		Reference
			ICSV 745	PB 15520	
Xiabtp450	GCAAATCCTCAAACGAGAGC	ATATCATGCGTGCCAGACAA	243	255	Folkerstema (Unpublished)
Xiabtp475	TTCGTTTGCTCGATCAGTTG	CCAAGCGCAAGGGTACATTG	292	278	Folkerstema (Unpublished)
Xisep0131	CACGACGTTGTAAAACGACTCAGTCTTGACACAAGCAAGC	CGCTTCTTCCTGAGCTTGAG	232	248	Ramu *et al.* (2009)
Xisep0224	CACGACGTTGTAAAACGACACTGGGGTTCCTTTTCCTGT	TCCCTGATTTCCCCTCTTTT	229	221	Ramu *et al.* (2009)
Xisep0444	CACGACGTTGTAAAACGACATGATCCGTCGGAGTTAGCA	GGATGCAGGACAGCATCTCT	228	222	Ramu (Unpublished)
Xisep0506	CACGACGTTGTAAAACGACCGTGCAAGTTTGGAATTTGTC	CGGGCAGGTATAAGGTGTTG	232	228	Ramu *et al.* (2009)
Xisep0704	CACGACGTTGTAAAACGACCAAGTCCGTCGTGCTAGAGG	CCCTTTAATTAGCCCCAAACA	223	219	Ramu *et al.* (2009)
Xisep0747	CACGACGTTGTAAAACGACAGGCAGCCTGCTTATCACAA	ACAAGCTCAGGTGGGTGGT	222	208	Ramu *et al.* (2009)
Xisep0829	CACGACGTTGTAAAACGACCGCTGCCAAAATCTAAGCTC	CACGGTGGTCACATCAGAAG	197	207	Ramu *et al.* (2009)
Xisp10203	CGAACCCGTATATGTGGA	TTGTTGCTTGTGGTTCCT	209	211	Folkerstema (Unpublished)
Xisp10206	GTGTGCTTTGCTTCGTTT	GTTGAACCCGATTGCTG	175 (180)	179	Folkerstema (Unpublished)
Xisp10207	CCTTGTTGGGATCCTTCT	ATATCGGTCCATTGCCTT	310	262	Folkerstema (Unpublished)
Xisp10228	TTGGAAACCAGTCAGAGC	ACACTGCAACTGCAACAA	210	194	Folkerstema (Unpublished)
Xisp10229	CTGTTAGGTTTGCTGCGT	CCACCATCTCAATTGCTC	275 (300)	271	Folkerstema (Unpublished)
Xisp10230	TTGGTTGAAGTCGCTGTT	CGGCCTTCACAACATAAG	217	207	Folkerstema (Unpublished)
Xisp10231	CGCAGCGTATGTGTATCA	AGCGTTTCTCTCCTCCAT	263 (210)	267	Folkerstema (Unpublished)
Xisp10233	TGCTTCACGATAAAAGCC	TCTGGGTTTGCATAGCAT	152	160	Folkerstema (Unpublished)
Xisp10254	ACCCAAACACAGAGCAAA	AGAGCTGCTGAGAGANGG	211	201	Folkerstema (Unpublished)
Xisp10257	TGGGAATTACATCANGCA	TTTGAACTGGCATGACCT	244 (245)	289	Folkerstema (Unpublished)
Xisp10259	CAAGGTTTCACTTTATTTTACCA	TGGAATGCAACATAGCAA	208	206	Folkerstema (Unpublished)

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Table 7 (Contd.,). Sequence information of selected polymorphic SSR markers

Markers	Forward primer sequence	Reverse primer sequence	Allele size (bp)		Reference
			ICSV 745	PB 15520	
Xisp10263	TATCTTCTCCGCCCTTTC	TAAGNGCCAAGGGAATG	344	325	Folkerstema (Unpublished)
Xisp10264	TCATTCACTCTCTTTCCCC	AGAATCTGCCATGAACGA	207 (190)	153	Folkerstema (Unpublished)
Xisp10272	GGTCGTCCAGNCATATCA	ACGACAAGGTTGTGTGCT	284	284+290	Folkerstema (Unpublished)
Xisp10277	GATGCTCTCCACAACAGG	ACGGCAGACACTTTTTCA	230	228	Folkerstema (Unpublished)
Xisp10279	CCGGGAACGACATTATTT	AGCATGCATGACGAATTT	135	150	Folkerstema (Unpublished)
Xisp10307	TTTGTGCTTTGGGTGTTT	GGTGCACCATCTTCTCCT	349	342	Folkerstema (Unpublished)
Xisp10321	GGACTCCTCGACTTCGTT	CTGCAGACACCGGTAAGT	205	228	Folkerstema (Unpublished)
Xisp10331	TTAGAGCGCATCAGGAAG	GCACAAAGGAGGACACAA	279	286	Folkerstema (Unpublished)
Xisp10332	GCTTCTGCACGACAAATC	TGCGAGGAACCTGTGTAT	181	183	Folkerstema (Unpublished)
Xisp10333	TCTCCACCGATTCGAGTA	CCTTCCTACAGCAAAGCA	190	204	Folkerstema (Unpublished)
Xisp10340	ACCTCCCCCTCCTAACTC	GCACTAGCGGTAACATGG	203	196	Folkerstema (Unpublished)
Xisp10350	TTGTTCTTTCTCGGTTGC	TGCTTCAGGAAGTGGAAG	216	212	Folkerstema (Unpublished)
Xisp10358	ACCCAAATCTGCAAATCA	GAGTCGGCAGGCTGG	104	116	Folkerstema (Unpublished)
Xisp10359	CACCTGTCTCCCCACATA	TCTTTCACCACAAATGCC	194	184	Folkerstema (Unpublished)
Xisp10361	GTCGATTCCTTCCCTGTT	ACTCCAATAGTGGTGCGA	209	197	Folkerstema (Unpublished)
Xtxp009	AATAGCACCGCCGCGCG	CATTGTGGAGTCCCTGATAC	158	116	Kong *et al.* (2000)
Xtxp010	ATACTATCAAGAGGGGAGC	AGTACTAGCCACACGTCAC	146	141	Kong *et al.* (2000)
Xtxp018	ACTGTCTAGAACAAGCTGCG	TTGCTCTAGCTAGGCATTTC	226 (230)	232	Kong *et al.* (2000)
Xtxp025	CCATTGAGCTTCTGCTATCTC	CATTTGTCACCACTAGAACCC	150 (160)	114	Kong *et al.* (2000)
Xtxp033	GAGCTACACAGGGTTCAAC	CCTAGCTATTCCTTGGTTG	233 (215)	227	Kong *et al.* (2000)

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Table 7 (Contd.,). Sequence information of selected polymorphic SSR markers

Markers	Forward primer sequence	Reverse primer sequence	Allele size (bp)		Reference
			ICSV 745	PB 15520	
Xtxp034	TGGTTCGTATCCTTCTCTACAG	CATATACCTCCTCGTCGCTC	362	360	Kong *et al.* (2000)
Xtxp041	TCTGGCCATGACTTCTCAC	AAATGGCGTAGACTCCCTTG	278	272	Kong *et al.* (2000)
Xtxp056	TGTCTTCGTAGTTGCGTGTTG	CCGAAGGAGTGCTTTGGAC	330	326	Bhattramakki *et al.* (2000)
Xtxp057	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	249	247	Bhattramakki *et al.* (2000)
Xtxp141	TGTATGGCCTAGCTTATCT	CAACAAGCCAACCTAAA	184(190)	172	Bhattramakki *et al.* (2000)
Xtxp207	ACACATCTACTACCCTCTCACCCT	TGATAGACTTGTGAGCAGCTCC	166	154	Bhattramakki *et al.* (2000)
Xtxp210	CGCTTTTCTGAAAATATTAAGGAC	GATGAGCGATGGAGGAGAG	185	205	Bhattramakki *et al.* (2000)
Xtxp217	GGCCTCGACTACGGAGTT	TCGGCATATTGATTTGGTTT	176	178	Bhattramakki *et al.* (2000)
Xtxp225	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	165	169	Bhattramakki *et al.* (2000)
Xtxp227	TGAAAGTTTTGGCATTGA	TGTAGGATAGCCCAGGTT	176	179	Bhattramakki *et al.* (2000)
Xtxp228	ACAGGTTGGCGATGTTTCTCT	TTCTTTTTCGAATTCATTCCTTTT	245 (250)	235	Bhattramakki *et al.* (2000)
Xtxp229	TGCCCAAGAGGATAAAAGGT	AGCGACGGCACATCAAT	175	173	Bhattramakki *et al.* (2000)
Xtxp248	GGGTGTCCAATGTTGTCTGC	GGCCGTTACTGTCCCTTACTCA	236	243	Bhattramakki *et al.* (2000)
Xtxp250	GCACATCCTCTAAAACTACTTAGT	GAACAGGACGATGTGATAGAT	278	273	Bhattramakki *et al.* (2000)
Xtxp262	TGCCTGCCCGACCTG	TTGCTGTCTCCGCTTTCC	170	166	Bhattramakki *et al.* (2000)
Xtxp270	AGCAAGAAGAAGGCAAGAAGAAGG	GCGAAATTATTTTGAAATGGAGTTGA	266	307	Bhattramakki *et al.* (2000)
Xtxp289	AAGTGGGGTGAAGAGATA	CTGCCTTTCCGACTC	270	294	Bhattramakki *et al.* (2000)
Xtxp292	CATTTGCGAAGTTACAACATTGCT	CATTCCTGACTGCCCTCTCC	361	356	Bhattramakki *et al.* (2000)
Xtxp295	AAATCATGCATCCATGTTCGTCTTC	CTCCCGCTACAAGAGTACATTCATAGCTTA	178 (190)	180	Bhattramakki *et al.* (2000)
Xtxp296	CAGAAATAACATATAATGATGGGGTGAA	ATGCTGTTATGATTTAGAGCCTGTAGAGTT	166	168	Bhattramakki *et al.* (2000)

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Table 7 (Contd.,). Sequence information of selected polymorphic SSR markers

Markers	Forward primer sequence	Reverse primer sequence	Allele size (bp)		Reference
			ICSV 745	PB 15520	
Xtxp298	GCATGTGTCAGATGATCTGGTGA	GCTGTTAGCTTCTTCTAATCGTCGGT	182	190	Bhattramakki *et al.* (2000)
Xtxp304	ACATAAAAGCCCCTCTTC	CTTTCACACCCTTTATTCA	216 (210)	300	Bhattramakki *et al.* (2000)
Xtxp312	CAGGAAAATACGATCCGTGCCAAGT	GTGAACTATTCGGAAGAAGTTTGGAGGAAA	154	138	Bhattramakki *et al.* (2000)
Xtxp316	CCAGCTTCACTTACGAGGAGATG	ATGCCCGTTTTCTAATTCTTCTACT	419 (350)	340	Bhattramakki *et al.* (2000)
Xtxp320.1	TAAACTAGACCATATACTGCCATGATAA	GTGCAAATAAGGGCTAGAGTGTT	273 (285)	261	Bhattramakki *et al.* (2000)
Xtxp327	ACCACTGCTCACGCTCAC	GCGGTGTACAGCTTCGTC	179	173	Bhattramakki *et al.* (2000)
Xtxp331	AACGGTTATTAGAGAGGGAGA	AGTATAATAACATTTTGACACCCA	264	237	Bhattramakki *et al.* (2000)
Xtxp340	AGAACTGTGCATGTATTCGTCA	AGAAACTCCAATTATCATCCATCA	218	215	Bhattramakki *et al.* (2000)
Xtxp357	CGCAGAAATACGATTG	GCTATCTGGAGTAACTGTGT	275	248	Bhattramakki *et al.* (2000)
SbAGB02	CTCTGATATGTCGTTGTGCT	ATAGAGAGGATAGCTTATAGCTCA	97	95	Taramino *et al.* (1997)

Table 8. Mean performance of RILs, parents and checks for spotted stem borer resistance component, agronomic and morphological traits

S.No.	Traits	Year	Mean ± SE					Range
			ICSV 745	RILs	PB 15520	ICSV1	IS2205	RILs
1	Stem tunneling (cm)	2007	30.56 ± 1.25	20.21 ± 0.77	12.47 ± 1.42	21.83 ± 3.45	7.11 ± 0.64	3.22 - 64.00
		2008	98.91 ± 5.86	60.24 ± 1.53	46.95 ± 3.54	97.12 ± 9.81	39.83 ± 3.94	12.81 - 148.79
2	Deadheart incidence (%)	2007	80.70 ± 8.56	74.05 ± 0.72	71.53 ± 0.89	63.04 ± 3.53	44.24 ± 6.71	43.98 - 98.34
		2008	82.71 ± 3.25	60.44 ± 0.93	45.33 ± 0.99	83.30 ± 1.62	19.36 ± 1.86	28.77 - 99.98
3	Leaf feeding damage (score)	2007	7.95 ± 0.63	6.29 ± 0.06	5.63 ± 0.55	7.83 ± 0.21	4.42 ± 0.26	3.66 - 7.94
		2008	7.42 ± 0.15	6.00 ± 0.06	4.62 ± 0.33	7.58 ± 0.15	4.58 ± 0.19	3.19 - 8.32
4	Recovery resistance (score)	2007	7.46 ± 0.22	5.58 ± 0.06	4.30 ± 0.19	5.42 ± 0.15	4.00 ± 0.17	3.05 - 8.62
		2008	7.36 ± 0.16	5.55 ± 0.07	3.55 ± 0.12	7.58 ± 0.15	3.42 ± 0.15	2.92 - 8.04
5	Overall resistance (score)	2007	7.69 ± 0.13	6.30 ± 0.04	5.67 ± 0.18	6.25 ± 0.18	4.50 ± 0.19	3.93 - 8.05
		2008	6.46 ± 0.32	6.14 ± 0.04	5.45 ± 0.23	7.92 ± 0.15	4.83 ± 0.21	4.01 - 8.65
6	Glossiness (score)	2007	4.37 ± 0.23	4.37 ± 0.03	4.61 ± 0.05	3.67 ± 0.19	2.08 ± 0.08	2.90 - 5.29
7	Seedling vigor (score)	2007	2.44 ± 0.11	1.93 ± 0.03	1.90 ± 0.11	1.58 ± 0.15	1.33 ± 0.14	0.97 - 3.03
		2008	2.22 ± 0.22	1.75 ± 0.03	1.31 ± 0.19	2.00 ± 0.17	1.50 ± 0.15	0.96 - 2.99
8	Basal pigmentation (score)	2007	1.03 ± 0.04	1.76 ± 0.04	2.82 ± 0.13	2.00 ± 0.17	2.75 ± 0.13	0.89 - 3.06
		2008	1.11 ± 0.11	1.72 ± 0.05	2.79 ± 0.11	1.58 ± 0.23	2.17 ± 0.17	0.96 - 3.04

Contd.,

Table 8 (Contd.,). Mean performance of RILs, parents and checks for spotted stem borer resistance component, agronomic and morphological traits

S.No.	Traits	Year	Mean ± SE					Range
			ICSV 745	RILs	PB 15520	ICSV1	IS2205	RILs
9	Plant color (score)	2007	1.00 ± 0.01	2.20 ± 0.05	3.00 ± 0.00	1.00 ± 0.00	3.00 ± 0.00	0.97 - 3.03
		2008	0.99 ± 0.02	2.27 ± 0.06	2.98 ± 0.01	1.00 ± 0.00	2.83 ± 0.14	0.96 - 3.67
10	Testa presence vs absence (score)	2007	1.00 ± 0.01	1.57 ± 0.03	2.00 ± 0.00	1.11 ± 0.11	1.57 ± 0.20	0.94 - 2.07
		2008	1.00 ± 0.00	1.47 ± 0.03	2.00 ± 0.00	1.00 ± 0.00	2.00 ± 0.00	1.00 - 2.00
11	Mesocarp thickness (score)	2007	1.56 ± 0.22	1.94 ± 0.04	3.00 ± 0.01	2.00 ± 0.00	2.42 ± 0.15	0.97 - 3.04
		2008	1.34 ± 0.19	2.11 ± 0.05	3.00 ± 0.01	2.00 ± 0.00	3.00 ± 0.00	0.99 - 3.02
12	Leaf angle (^{o})	2007	50.96 ± 3.91	54.98 ± 0.47	73.08 ± 1.74	54.17 ± 1.02	51.81 ± 0.75	34.35 - 78.69
		2008	49.32 ± 2.50	55.51 ± 0.61	70.37 ± 0.20	59.79 ± 1.31	52.74 ± 1.98	28.97 - 81.97
13	Time to 50% flowering (days)	2007	69.55 ± 0.13	75.09 ± 0.44	90.87 ± 0.94	67.83 ± 0.30	72.83 ± 0.58	60.40 - 94.61
		2008	71.44 ± 0.30	76.58 ± 0.39	88.86 ± 0.81	56.75 ± 0.52	66.33 ± 1.30	55.98 - 93.41
14	Number of nodes (nos)	2007	12.19 ± 1.00	10.55 ± 0.10	7.99 ± 0.35	8.49 ± 0.19	11.00 ± 0.30	5.48 - 16.07
		2008	11.80 ± 1.22	11.50 ± 0.07	9.43 ± 0.75	9.33 ± 0.38	10.10 ± 0.27	8.85 - 14.03
15	Plant height (cm)	2007	220.19 ± 11.88	190.28 ± 2.08	156.62 ± 8.11	168.50 ± 5.17	259.58 ± 5.19	124.76 - 289.20
		2008	249.78 ± 2.95	221.85 ± 2.83	163.35 ± 1.64	175.63 ± 4.01	295.07 ± 2.01	136.92 - 307.28
16	Agronomic performance (score)	2007	1.71 ± 0.09	3.22 ± 0.03	4.43 ± 0.09	1.50 ± 0.15	5.00 ± 0.00	1.66 - 4.92
		2008	1.54 ± 0.13	3.21 ± 0.05	4.47 ± 0.09	1.33 ± 0.14	4.58 ± 0.15	1.01 - 5.09

Table 9. Genotypic and Genotype × Environment variances for spotted stem borer resistance component, agronomic and morphological traits

S.No.	Trait	$\sigma^2_g \pm$ SE			$\sigma^2_{g \times e} \pm$ SE
		2007	2008	Across-season	
1	Stem tunneling (cm)	135.30 ** ± 12.39	454.40 ** ± 48.90	44.70 * ± 21.60	258.00 ** ± 27.70
2	Deadheart incidence (%)	85.80 ** ± 11.00	159.30 ** ± 18.90	40.90 ** ± 11.10	84.70 ** ± 11.80
3	Leaf feeding damage (score)	0.67 ** ± 0.07	0.60 ** ± 0.07	0.26 ** ± 0.06	0.39 ** ± 0.05
4	Recovery resistance (score)	0.73 ** ± 0.08	1.06 ** ± 0.10	0.26 ** ± 0.07	0.64 ** ± 0.07
5	Overall resistance (score)	0.25 ** ± 0.04	0.42 ** ± 0.05	0.17 ** ± 0.03	0.16 ** ± 0.03
6	Glossiness (score)	0.11 ** ± 0.02	- -	- -	- -
7	Seedling vigor (score)	0.16 ** ± 0.02	0.16 ** ± 0.02	0.15 ** ± 0.02	0.01 ± 0.01
8	Basal pigmentation (score)	0.38 ** ± 0.04	0.45 ** ± 0.04	0.41 ** ± 0.04	0.01 ± 0.01
9	Plant color (score)	0.59 ** ± 0.06	0.86 ** ± 0.08	0.65 ** ± 0.06	0.08 ** ± 0.01
10	Testa presence vs absence (score)	0.19 ** ± 0.02	0.23 ** ± 0.02	0.11 ** ± 0.02	0.10 ** ± 0.01
11	Mesocarp thickness (score)	0.27 ** ± 0.03	0.65 ** ± 0.06	0.16 ** ± 0.04	0.30 ** ± 0.03
12	Leaf angle (^{o})	36.27 ** ± 4.74	74.96 ** ± 7.76	29.98 ** ± 4.76	24.91 ** ± 3.67
13	Time to 50% flowering (days)	46.25 ** ± 4.43	45.16 ** ± 4.00	37.66 ** ± 3.78	7.97 ** ± 0.95
14	Number of nodes (nos)	2.58 ** ± 0.24	0.78 ** ± 0.12	1.03 ** ± 0.14	0.64 ** ± 0.09
15	Plant height (cm)	1028.20 ** ± 97.20	1934.90 ** ± 176.40	584.10 ** ± 103.10	896.20 ** ± 86.00
16	Agronomic performance (score)	0.24 ** ± 0.03	0.53 ** ± 0.05	0.27 ** ± 0.03	0.11 ** ± 0.02

*, ** significance at P=0.05 and 0.01 respectively

Table 10. Correlation coefficients among the spotted stem borer resistance component traits for 2007 and 2008 rainy season data sets

Traits	Year	Stem tunneling (cm)	Deadheart incidence (%)	Leaf feeding damage (score)	Recovery resistance (score)	Overall resistance (score)
Stem tunneling (cm)	2007	1.00				
	2008	1.00				
Deadheart incidence (%)	2007	0.02	1.00			
	2008	0.07	1.00			
Leaf feeding damage (score)	2007	0.04	0.43**	1.00		
	2008	0.16*	0.25**	1.00		
Recovery resistance (score)	2007	0.26**	0.18**	0.21**	1.00	
	2008	0.04	0.13*	0.11*	1.00	
Overall resistance (score)	2007	0.06	0.56**	0.27**	0.44**	1.00
	2008	0.09	0.29**	0.11*	0.47**	1.00

*, ** significance at P=0.05 and 0.01 respectively

Table 11. Correlation coefficients among the agronomic and morphological traits observed for 2007 and 2008 rainy season data sets

Traits	Year	GS	SV	BP	PC	TP	MT	LA	TF	NN	PH	AP
Glossiness^{GS} (score)	2007	1.00										
Seedling vigor^{SV} (score)	2007	0.56**	1.00									
	2008		1.00									
Basal pigmentation^{BP} (score)	2007	0.03	0.03	1.00								
	2008		-0.09	1.00								
Plant color^{PC} (score)	2007	0.03	0.04	0.45**	1.00							
	2008		-0.04	0.38**	1.00							
Testa presence vs absence^{TP} (score)	2007	0.02	-0.05	0.11*	0.21**	1.00						
	2008		-0.12*	0.27**	0.16*	1.00						
Mesocarp thickness^{MT} (score)	2007	-0.02	-0.07	-0.04	0.09	0.30**	1.00					
	2008		-0.19**	0.16*	0.03	0.57**	1.00					
Leaf angle^{LA} (^{o})	2007	-0.08	-0.04	0.02	0.05	0.37**	0.43**	1.00				
	2008		-0.06	0.06	0.08	0.43**	0.21**	1.00				
Time to 50% flowering^{TF} (days)	2007	0.08	0.13*	0.06	0.01	0.13*	0.06	0.13*	1.00			
	2008		0.02	-0.02	-0.09	0.00	0.06	-0.02	1.00			
Number of nodes^{NN} (nos)	2007	0.11*	0.13*	0.07	0.08	0.08	0.05	0.07	0.59**	1.00		
	2008		0.03	0.09	0.12*	0.11*	0.11*	-0.06	0.45**	1.00		
Plant height^{PH} (cm)	2007	-0.09	-0.05	-0.20**	-0.14*	0.09	0.02	-0.02	0.07	0.07	1.00	
	2008		-0.09	-0.20**	-0.09	-0.04	0.02	-0.09	-0.11*	-0.11*	1.00	
Agronomic performance^{AP} (score)	2007	0.09	0.01	0.13*	0.17**	-0.04	0.08	-0.04	0.07	0.15*	-0.17**	1.00
	2008		0.02	0.09	0.22**	0.14*	0.08	0.12*	-0.13*	0.12*	-0.21**	1.00

*, ** significance at P=0.05 and 0.01 respectively

Table 12. Correlation coefficients between spotted stem borer resistance, agronomic and morphological traits for 2007 and 2008 rainy season data sets

Traits	Year	Stem tunneling (cm)	Deadheart incidence (%)	Leaf feeding damage (score)	Recovery resistance (score)	Overall resistance (score)
Glossiness (score)	2007	-0.12*	0.04	0.05	0.02	0.10
Seedling vigor (score)	2007	0.01	0.18**	0.03	0.06	0.16*
	2008	-0.02	0.05	0.11*	-0.08	0.02
Basal pigmentation (score)	2007	-0.14*	-0.04	-0.06	-0.10	-0.09
	2008	0.05	-0.10	-0.13*	-0.04	-0.07
Plant color (score)	2007	0.09	-0.09	-0.17**	0.02	-0.02
	2008	-0.05	-0.13*	-0.14*	-0.02	-0.06
Testa presence vs absence (score)	2007	0.14*	-0.10	-0.17**	-0.06	-0.10
	2008	0.12*	-0.06	-0.01	-0.10	-0.04
Mesocarp thickness (score)	2007	0.18**	-0.13*	-0.15*	-0.04	-0.01
	2008	0.10	0.02	-0.01	-0.14*	-0.11*
Leaf angle (^{o})	2007	0.14*	-0.05	-0.22**	-0.08	0.04
	2008	0.09	-0.12*	-0.06	-0.14*	0.02
Time to 50% flowering (days)	2007	-0.02	0.23**	-0.02	-0.10	0.03
	2008	0.02	0.15*	0.06	-0.08	-0.15*
Number of nodes (nos)	2007	-0.10	-0.12*	-0.10	-0.23**	-0.19**
	2008	-0.07	-0.11*	-0.09	-0.26**	-0.29**
Plant height (cm)	2007	0.24**	-0.02	0.07	0.14*	0.08
	2008	0.17**	0.04	0.04	0.15*	0.04
Agronomic performance (score)	2007	-0.10	-0.16*	-0.06	-0.06	-0.01
	2008	-0.05	-0.12*	-0.03	-0.15*	-0.01

*, ** significance at P=0.05 and 0.01 respectively

Table 13. Correlation coefficients estimated for the observed traits using across screening season data set

Traits	*ST*	*DH*	*LF*	*RR*	*OR*	*SV*	*BP*	*PC*	*TP*	*MT*	*LA*	*TF*	*NO*	*PH*	*AP*
Stem tunnelingST (cm)	1.00														
Deadheart incidence^{DH} (%)	0.10	1.00													
Leaf feeding damage ^{LF} (score)	0.16*	0.36**	1.00												
Recovery resistance^{RR} (score)	0.19**	0.28**	0.20**	1.00											
Overall resistance^{OR} (score)	0.14*	0.51**	0.24**	0.59**	1.00										
Seedling vigor^{SV} (score)	-0.03	0.13*	0.11*	-0.04	0.11*	1.00									
Basal pigmentation^{BP} (score)	-0.02	-0.08	-0.15*	-0.07	-0.06	-0.06	1.00								
Plant color^{PC} (score)	0.02	-0.14*	-0.20**	-0.02	-0.04	0.02	0.44**	1.00							
Testa presence vs absence^{TP} (score)	0.17**	-0.13*	-0.14*	-0.04	-0.09	-0.09	0.21**	0.21**	1.00						
Mesocarp thickness^{MT} (score)	0.13*	-0.08	-0.11*	-0.12*	-0.14*	-0.19**	0.10	0.06	0.55**	1.00					
Leaf angle^{LA} (^{o})	0.15*	-0.11*	-0.10	-0.10	0.00	-0.01	0.04	0.07	0.47**	0.37**	1.00				
Time to 50% flowering^{TF} (days)	-0.04	0.19**	0.02	-0.14*	-0.11*	0.11*	0.02	-0.05	0.08	0.08	0.06	1.00			
Number of nodes^{NN} (nos)	-0.12*	-0.10	-0.10	-0.36**	-0.30**	0.12*	0.07	0.10	0.08	0.08	0.02	0.63**	1.00		
Plant height^{PH} (cm)	0.26**	0.03	0.06	0.24**	0.10	-0.10	-0.26**	-0.14*	0.04	0.06	-0.04	-0.01	-0.01	1.00	
Agronomic performance^{AP} (score)	-0.10	-0.22**	-0.11*	-0.15*	-0.06	0.02	0.12*	0.24**	0.09	0.11*	0.08	-0.07	0.19**	-0.26**	1.00

*, ** significance at P=0.05 and 0.01 respectively

Tale 14. Operational heritability estimates for the observed traits in individual and across season data sets

S.No.	Trait	Heritability (h^2_{bs})		
		2007	2008	Across season
1	Stem tunneling (cm)	0.95	0.82	0.17
2	Deadheart incidence (%)	0.70	0.75	0.38
3	Leaf feeding damage (score)	0.80	0.74	0.47
4	Recovery resistance (score)	0.81	0.91	0.40
5	Overall resistance (score)	0.58	0.79	0.53
6	Glossiness (score)	0.54	-	-
7	Seedling vigor (score)	0.60	0.69	0.76
8	Basal pigmentation (score)	0.82	0.89	0.92
9	Plant color (score)	0.83	0.97	0.89
10	Testa presence vs absence (score)	0.90	0.98	0.67
11	Mesocarp thickness (score)	0.70	0.93	0.46
12	Leaf angle (^{o})	0.69	0.85	0.59
13	Time to 50% flowering (days)	0.91	0.98	0.87
14	Number of nodes (nos)	0.92	0.59	0.66
15	Plant height (cm)	0.92	0.95	0.54
16	Agronomic performance (score)	0.71	0.85	0.72

Table 15. Across-season mean values and proportion of transgressive segregants for the observed traits in the RILs

Sl. No.	Traits	Across season mean value			Test of significance		Proportion of transgressive segregants	
		ICSV 745 (P_1)	RIL	PB 15520 (P_2)	P_1 vs RIL	P_2 vs RIL	ICSV 745	PB 15520
1	Stem tunneling (cm)	64.73 ± 3.33	40.23 ± 0.90	29.71 ± 1.38	**	**	0.08	0.18
2	Deadheart incidence (%)	81.71 ± 5.51	67.24 ± 0.66	58.43 ± 0.39	**	**	0.08	0.24
3	Leaf feeding damage (score)	7.68 ± 0.36	6.14 ± 0.05	5.12 ± 0.19	**	**	0.00	0.11
4	Recovery resistance (score)	7.41 ± 0.15	5.57 ± 0.05	3.93 ± 0.06	**	**	0.00	0.02
5	Overall resistance (score)	7.08 ± 0.15	6.22 ± 0.04	5.56 ± 0.04	**	**	0.07	0.10
6	Seedling vigor (score)	2.33 ± 0.16	1.84 ± 0.03	1.61 ± 0.06	**	**	0.16	0.32
7	Basal pigmentation (score)	1.07 ± 0.04	1.74 ± 0.04	2.80 ± 0.05	**	**	0.00	0.00
8	Plant color (score)	0.99 ± 0.01	2.23 ± 0.05	2.99 ± 0.00	**	**	0.00	0.00
9	Testa presence vs absence (score)	1.00 ± 0.01	1.52 ± 0.03	2.00 ± 0.00	**	**	0.00	0.00
10	Mesocarp thickness (score)	1.45 ± 0.20	2.03 ± 0.04	3.00 ± 0.00	**	**	0.00	0.00
11	Leaf angle (^{o})	50.14 ± 2.91	55.24 ± 0.46	71.72 ± 0.96	*ns*	**	0.21	0.03
12	Time to 50% flowering (days)	70.49 ± 0.20	75.84 ± 0.39	89.87 ± 0.69	**	**	0.19	0.04
13	Number of nodes (nos)	11.99 ± 1.08	11.03 ± 0.07	8.71 ± 0.26	*ns*	**	0.16	0.00
14	Plant height (cm)	234.98 ± 5.20	206.06 ± 2.03	159.98 ± 3.41	**	**	0.18	0.08
15	Agronomic performance (score)	1.63 ± 0.11	3.22 ± 0.04	4.45 ± 0.09	**	**	0.00	0.01

** Significance at P=0.05

Table 16. Linkage group-wise marker interval position and chi-square values

Linkage group	Markers	Map interval (cM)	Alleles (%)		Chi-square value	Probability
			ICSV 745	PB 15520		
SBI-01(a)	Xcup01	-	56	29	20.88	0.000 ***
	Xcup06	7.1	60	35	16.15	0.000 ***
	Xcup24	35.5	63	31	27.12	0.000 ***
	Xcup73	15.1	38	39	0.02	0.886
	Xtxp357	23.3	46	39	1.20	0.274
	Xisp10203	3.4	47	43	0.64	0.425
	Xisp10340	3.1	31	41	3.45	0.063
	Xiabtp450	4.4	44	47	0.16	0.691
SBI-01(b)	Xtxp229	-	41	49	1.77	0.183
	Xcup60	5.6	15	33	17.02	0.000 ***
	Xcup44	13.4	43	45	0.11	0.737
	Xtxp340	22.6	28	31	0.54	0.461
	Xtxp248	5.4	41	49	1.75	0.185
	Xtxp316	8.4	34	38	0.45	0.504
SBI-02(a)	Xisep0747	-	47	42	0.65	0.421
	Xtxp025	24.0	41	12	39.47	0.000 ***
	Xtxp304	5.7	37	22	9.76	0.002 **
SBI-02(b)	Xcup74	-	39	51	3.95	0.047 *
	Xtxp298	20.4	35	47	4.33	0.038 *
	Xtxp056.1	32.4	33	47	6.42	0.011 *
	Xisp10259	18.1	33	47	6.42	0.011 *
	Xtxp207	5.2	23	37	8.53	0.004 **
	Xtxp296	3.8	29	38	2.61	0.106
	Xisp10228	9.5	14	42	36.27	0.000 ***
SBI-03	Xtxp228	-	44	45	0.07	0.789
	Xcup61	0.2	45	45	0.00	0.947
	Xisp10331	7.7	48	40	1.80	0.179
	Xisp10254	7.8	49	40	2.16	0.142
	Xisp10207	6.9	46	43	0.22	0.639
	Xtxp009	0.0	49	32	8.28	0.004 **
	Xisp10277	3.7	52	39	4.49	0.034
	Xtxp033	43.5	47	35	4.41	0.036
	Xisp10307	20.7	49	35	5.45	0.020 *
	Xisp10231	2.7	51	31	12.14	0.000 ***
	Xcup38	30.7	41	31	3.16	0.075
	Xisp10332	47.5	41	36	1.01	0.315
	Xisp10361	2.8	51	34	8.32	0.004 **
	Xtxp034	7.1	40	27	6.02	0.014 *
	Xcup14	20.4	43	41	0.12	0.732
SBI-04	Xisep0224	-	51	39	4.27	0.039 *
	Xisp10230	30.3	56	27	25.50	0.000 ***
	Xiabtp364	41.1	52	38	5.12	0.024 *
	Xtxp041	58.8	47	32	7.22	0.007 **
	Xtxp327	4.0	49	40	2.18	0.140
	Xisp10229	12.5	49	41	1.77	0.183

Contd.,

Table 16 (Contd.,). Linkage group-wise marker interval position and chi-square values

Linkage group	Markers	Map interval (cM)	Alleles (%)		Chi-square value	Probability	
			ICSV 745	PB 15520			
SBI-05	Xisp10257	-	41	40	0.08	0.779	
	Xtxp225	4.4	40	43	0.31	0.579	
	Xisp10350	17.2	25	37	5.43	0.020	*
	Xtxp262$_{(kaf2)}$	11.0	18	54	45.00	0.000	***
SBI-06	Xisep0444	-	53	39	5.03	0.025	*
	Xisp10264	68.3	21	46	22.88	0.000	***
	Xtxp057	17.2	39	50	3.53	0.060	
SBI-07	Xisep0131	-	51	22	29.12	0.000	***
	Xisp10233	25.8	45	36	2.82	0.093	
	Xtxp312	0.1	45	45	0.00	0.947	
	Xtxp227	28.2	38	51	4.84	0.028	*
	Xisp10206	9.9	39	41	0.12	0.726	
	Xgap342	0.6	41	49	1.96	0.162	
	Xisep0829	28.8	35	57	14.06	0.000	***
	Xtxp295	15.5	40	49	2.57	0.109	
	XSbAGB02	9.8	43	33	3.52	0.061	
	Xcup57	5.4	44	49	0.52	0.471	
	Xisep0704	8.4	42	51	2.46	0.117	
SBI-08	Xcup47	-	32	53	12.64	0.000	***
	Xiabtp349	27.0	44	43	0.04	0.839	
	Xtxp210	10.2	43	42	0.02	0.891	
	Xtxp292	0.5	33	49	8.20	0.004	*
	Xisp10279	15.1	38	37	0.05	0.826	
	Xtxp018	5.3	46	33	4.83	0.028	*
	Xtxp250	1.9	47	43	0.36	0.550	
	Xisp10333	33.7	45	43	0.07	0.788	
SBI-09	Xtxp289	-	40	39	0.05	0.831	
	Xisep0506	34.8	42	47	0.75	0.386	
	Xgap015	23.1	41	39	0.18	0.671	
	Xisp10358	0.0	40	42	0.12	0.728	
	Xtxp010	17.2	40	45	0.79	0.373	
	Xiabtp475	31.0	49	42	1.27	0.259	
	Xgap032	18.1	44	47	0.16	0.691	
	Xgap206	33.9	49	39	2.59	0.107	
SBI-10	Xcup50	-	40	50	2.78	0.096	
	Xcup49	13.9	45	28	9.69	0.002	**
	Xisp10359	69.3	21	29	3.53	0.060	
	Xisp10321	14.9	51	42	2.07	0.150	
	Xtxp331	3.7	57	34	14.31	0.000	***
	Xtxp270	0.0	55	33	14.70	0.000	***
	Xtxp217	2.9	48	39	2.64	0.104	
	Xisp10263	5.3	57	35	14.06	0.000	***
	Xtxp320.2	16.3	60	31	23.48	0.000	***
	Xtxp141	37.7	53	38	6.33	0.012	*
	Xisp10272	8.2	60	40	10.00	0.002	**

Table 17. Distribution and distance coverage of 90 SSR markers across the 10 sorghum linkage groups

Linkage group	Number of markers	% of total markers	Total distance (cM)	Average distance (cM)
SBI-01a	8	8.89	91.9	11.5
SBI-01b	6	6.67	55.4	9.2
SBI-02a	3	3.33	29.7	9.9
SBI-02b	7	7.78	89.4	12.8
SBI-03	15	16.67	201.7	13.4
SBI-04	6	6.67	146.7	24.5
SBI-05	4	4.44	32.6	8.2
SBI-06	3	3.33	85.5	28.5
SBI-07	11	12.22	132.5	12.0
SBI-08	8	8.89	93.7	11.7
SBI-09	8	8.89	158.1	19.8
SBI-10	11	12.22	172.2	15.7
Total	90		1289.4	14.3

Table 18. QTLs identified for spotted stem borer resistance component traits using the (ICSV 745 x PB 15520-1) - based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval			LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
										Interaction component	Effect	Part R^2 (%)	
Stem tunneling													
2007 rainy	SBI-02b	78	Xtxp207	-	Xtxp296	2.8	5.9	-3.0	5.6	-	-	-	22.7
	SBI-03	122	Xisp10231	-	Xcup38	3.7	6.6	-2.1	2.4	-	-	-	
	SBI-07	128	Xcup57	-	Xisep0704	2.6	4.8	-4.0	9.4	-	-	-	
	SBI-08	38	Xtxp292	-	Xisp10279	10.4	17.5	-5.1	15.6	-	-	-	
2008 rainy	SBI-01a	2	Xcup01	-	Xcup06	2.7	5.6	4.4	3.8	a2*a3	6.7	6.6	40.0
	SBI-01a	56	Xcup24	-	Xcup73	9.9	16.8	-9.8	15.6	a2*a4	5.8	5.4	
	SBI-07	58	Xtxp227	-	Xisp10206	5.5	9.7	-6.5	7.0	a3*a4	7.4	8.0	
	SBI-07	112	Xtxp295	-	XSbAGB02	5.1	9	-5.1	5.0	a3*a5	3.9	2.6	
	SBI-10	108	Xtxp217	-	Xisp10263	3.8	6.8	-3.7	3.0	-	-	-	
Dead heart incidence													
2007 rainy	SBI-01a	90	Xisp10340	-	Xiabtp450	3.4	6.4	2.5	5.0	a1*a3	2.2	2.8	33.9
	SBI-03	0	Xtxp228	-	Xisp10331	2.6	4.8	-2.8	6.9	-	-	-	
	SBI-07	98	Xisep0829	-	Xtxp295	15.1	24.8	-7.0	27.7	-	-	-	
	SBI-08	34	Xiabtp349	-	Xtxp210	3.6	6.6	2.1	3.2	-	-	-	
										-	-	-	
2008 rainy	SBI-07	100	Xisep0829	-	Xtxp295	8.2	14.2	-5.9	10.0	-	-	-	14.6
	SBI-10	124	Xisp10263	-	Xtxp320.2	3.1	5.6	-4.2	6.3	-	-	-	

Contd.,

Table 18 (Contd.,). QTLs identified for spotted stem borer resistance component traits using the (ICSV 745 x PB 15520-1) - based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval	LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
								Interaction component	Effect	Part R^2 (%)	
Leaf feeding damage											
2007 rainy	SBI-02a	6	Xisep0747 - Xtxp025	2.6	6.1	-0.1	1.6	a1*a2	-0.3	4.1	12.1
	SBI-07	108	Xisep0829 - Xtxp295	4.0	7.2	-0.3	9.2	-	-	-	
2008 rainy	SBI-01b	46	Xtxp340 - Xtxp248	4.1	7.8	-0.3	6.8	-	-	-	10.9
	SBI-07	64	Xtxp227 - Xisp10206	4.1	7.4	-0.3	7.1	-	-	-	
Recovery resistance											
2007 rainy	SBI-01b	44	Xtxp340 - Xtxp248	2.7	5.2	-0.2	3.9	-	-	-	28.0
	SBI-02b	50	Xtxp298 - Xtxp056.1	9.6	16.9	-0.5	16.7	-	-	-	
	SBI-07	118	Xtxp295 - XSbAGB02	9.3	16.2	-0.3	12.1	-	-	-	
2008 rainy	SBI-03	24	Xisp10207 - Xisp10277	10.1	17	-0.4	12.7	-	-	-	37.7
	SBI-03	94	Xisp10231 - Xcup38	4.4	7.9	-0.3	7.1	-	-	-	
	SBI-07	94	Xisep0829 - Xtxp295	3.6	6.5	-0.2	4.8	-	-	-	
	SBI-07	124	XSbAGB02 - Xcup57	16.7	26.7	-0.5	18.5	-	-	-	
Overall resistance											
2007 rainy	SBI-07	96	Xisep0829 - Xtxp295	8.3	14.2	-0.3	17.1	-	-	-	15.5
2008 rainy	SBI-03	180	Xisp10361 - Xtxp034	3.2	5.9	0.2	3.9	-	-	-	11.5
	SBI-07	98	Xisep0829 - Xtxp295	4.9	8.7	-0.2	7.9	-	-	-	

Table 19. QTLs identified for spotted stem borer resistance component traits using the (ICSV 745 x PB 15520-1) - based RIL population for across-season data set

Trait / Linkage group	Position cM	Marker interval		LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Significance of Q×E	Adj R^2 (%)
								Interaction component	Effects	Part R^2 (%)		
Stem tunneling												
SBI-01a	56	Xcup24	- Xcup73	10.0	16.9	-5.8	16.3	a1*a3	3.4	4.7	**	36.4
SBI-04	144	Xtxp327	- Xisp10229	3.1	5.6	3.4	6.0	a3*a4	3.8	6.1	*ns*	
SBI-07	58	Xtxp227	- Xisp10206	4.1	7.3	-3.6	6.8	-	-	-	**	
SBI-07	112	Xtxp295	- XSbAGB02	5.8	10.1	-4.1	9.2	-	-	-	*	
SBI-10	104	Xtxp270	- Xtxp217	3.4	6.1	-2.0	2.8	-	-	-	*	
Deadheart incidence												
SBI-07	100	Xisep0829	- Xtxp295	19.0	29.9	-6.4	23.7	-	-	-	*ns*	23.0
Leaf feeding damage												
SBI-02a	6	Xisep0747	- Xtxp025	3.8	8.6	-0.2	3.4	a1*a3	-0.2	2.6	*ns*	17.3
SBI-07	64	Xtxp227	- Xisp10206	3.5	6.3	-0.2	6.2	-	-	-	*ns*	
SBI-07	108	Xisep0829	- Xtxp295	2.7	4.9	-0.2	5.1	-	-	-	**	
Recovery resistance												
SBI-03	20	Xisp10254	- Xtxp009	4.5	8.1	-0.2	5.8	-	-	-	**	35.4
SBI-03	92	Xisp10307	- Xisp10231	3.5	6.4	-0.2	6.8	-	-	-	**	
SBI-07	94	Xisep0829	- Xtxp295	2.6	4.7	-0.2	5.5	-	-	-	*ns*	
SBI-07	122	XSbAGB02	- Xcup57	14.3	23.5	-0.4	20.2	-	-	-	**	
Overall resistance												
SBI-03	180	Xisp10361	- Xtxp034	3.3	6.1	0.1	3.8	-	-	-	*ns*	20.1
SBI-07	96	Xisep0829	- Xtxp295	10.7	18.1	-0.3	16.6	-	-	-	*ns*	

Table 20. QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1) - based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval			LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
										Interaction component	Effect	Part R^2 (%)	
Glossiness													
2007 rainy	SBI-01a	44	Xcup24	-	Xcup73	3.6	6.5	0.1	6.1	-	-	-	6.9
	SBI-09	138	Xgap032	-	Xgap206	2.8	5.0	-0.1	3.2	-	-	-	
Seedling vigor													
2007 rainy	SBI-01a	2	Xcup01	-	Xcup06	2.53	5.2	-0.1	4.5	a2*a5	-0.1	2.0	26.9
	SBI-01a	42	Xcup06	-	Xcup24	5.7	10	0.1	6.6	a3*a5	0.1	2.6	
	SBI-01a	90	Xisp10340	-	Xiabtp450	3.03	5.6	0.1	5.8	-	-	-	
	SBI-01b	18	Xcup60	-	Xcup44	3.42	6.5	-0.1	2.2	-	-	-	
	SBI-03	168	Xcup38	-	Xisp10332	3.89	7	0.1	3.4	-	-	-	
	SBI-09	110	Xiabtp475	-	Xgap032	4.38	7.8	-0.2	12.4	-	-	-	
2008 rainy	SBI-01a	4	Xcup01	-	Xcup06	4.12	8.3	-0.2	8.6	-	-	-	18.9
	SBI-01a	44	Xcup24	-	Xcup73	2.69	4.9	0.1	1.7	-	-	-	
	SBI-03	92	Xisp10307	-	Xisp10231	3.74	6.7	0.1	2.8	-	-	-	
	SBI-03	172	Xisp10332	-	Xisp10361	2.81	5.1	0.1	3.6	-	-	-	
	SBI-04	146	Xtxp327	-	Xisp10229	2.52	4.6	-0.1	5.0	-	-	-	
Basal pigmentation													
2007 rainy	SBI-06	74	Xisp10264	-	Xtxp057	25.13	39.3	0.6	39.8	-	-	-	39.3
2008 rainy	SBI-01b	42	Xtxp340	-	Xtxp248	3.27	6.3	0.1	1.7	a2*a3	0.2	2.8	42.8
	SBI-04	138	Xtxp327	-	Xisp10229	2.52	4.7	0.1	3.1	-	-	-	
	SBI-06	74	Xisp10264	-	Xtxp057	28.67	43.7	0.6	40.7	-	-	-	

Contd.,

Table 20 (Contd.,). QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1)-based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval	LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
								Interaction component	Effect	Part R^2 (%)	
Plant color											
2007 rainy	SBI-06	82	Xisp10264 - Xtxp057	31.2	46.2	0.7	38.3	-	-	-	41.1
	SBI-07	94	Xisep0829 - Xtxp295	2.8	5.1	0.1	3.8	-	-	-	
2008 rainy	SBI-06	82	Xisp10264 - Xtxp057	30.2	45.1	0.7	39.3	-	-	-	38.8
Testa (presence vs absence)											
2007 rainy	SBI-04	146	Xtxp327 - Xisp10229	15.9	26.2	0.2	25.2	-	-	-	24.6
2008 rainy	SBI-04	142	Xtxp327 - Xisp10229	54.4	65.3	0.5	64.1	-	-	-	63.8
Mesocarp thickness											
2007 rainy	SBI-01b	40	Xcup44 - Xtxp340	3.0	5.8	-0.1	2.2	-	-	-	26.2
	SBI-04	144	Xtxp327 - Xisp10229	16.3	26.6	0.4	26.4	-	-	-	
2008 rainy	SBI-01a	86	Xisp10203 - Xisp10359	3.9	7.1	-0.2	5.2	-	-	-	24.2
	SBI-04	146	Xtxp327 - Xisp10229	9.9	16.8	0.4	23.6	-	-	-	
Leaf angle											
2007 rainy	SBI-04	146	Xtxp327 - Xisp10229	11.9	20.0	3.5	18.9	-	-	-	18.2
2008 rainy	SBI-04	146	Xtxp327 - Xisp10229	27.2	40.0	6.4	36.9	-	-	-	36.4

Contd.,

Table 20 (Contd.,). QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1)-based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval			LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
										Interaction component	Effect	Part R^2 (%)	
Time to flowering													
2007 rainy	SBI-01a	20	Xcup06	-	Xcup24	2.9	5.2	-2.7	9.2	a1*a6	-1.2	2.0	47.9
	SBI-01a	82	Xtxp357	-	Xisp10203	7.4	12.8	2.1	10.6	a2*a5	0.9	2.1	
	SBI-01b	36	Xcup44	-	Xtxp340	5.8	10.7	-1.0	1.9	-	-	-	
	SBI-04	32	Xisp10230	-	Xiabtp364	10.2	17.2	2.4	10.8	-	-	-	
	SBI-06	4	Xisep0444	-	Xisp10264	21.7	33.9	4.1	26.0	-	-	-	
	SBI-10	102	Xtxp270	-	Xtxp217	3.0	5.4	2.2	11.3	-	-	-	
2008 rainy	SBI-01a	16	Xcup06	-	Xcup24	4.1	7.3	-1.8	5.2	-	-	-	34.4
	SBI-01a	90	Xisp10340	-	Xiabtp450	4.0	7.4	1.6	5.9	-	-	-	
	SBI-01b	38	Xcup44	-	Xtxp340	3.3	6.3	-1.0	2.0	a3*a4	1.6	2.9	
	SBI-04	24	Xisep0224	-	Xisp10230	7.9	14.1	2.3	7.7	-	-	-	
	SBI-06	12	Xisep0444	-	Xisp10264	11.5	19.9	3.3	11.3	-	-	-	
	SBI-10	102	Xtxp270	-	Xtxp217	6.2	11.0	1.8	8.0	-	-	-	
Number of nodes													
2007 rainy	SBI-04	18	Xisep0224	-	Xisp10230	4.76	8.7	0.5	4.4	-	-	-	26.4
	SBI-06	0	Xisep0444	-	Xisp10264	13.16	22.2	0.7	17.2	-	-	-	
	SBI-07	92	Xgap342	-	Xisep0829	2.77	5	0.5	7.3	-	-	-	
	SBI-10	106	Xtxp217	-	Xisp10263	6.81	11.9	0.4	6.9	-	-	-	
2008 rainy	SBI-06	0	Xisep0444	-	Xisp10264	4.2	7.8	0.2	4.8	a1*a3	-0.1	1.9	20.5
	SBI-07	92	Xgap342	-	Xisep0829	4.78	8.6	0.3	8.8	a2*a3	0.2	2.4	
	SBI-10	102	Xtxp270	-	Xtxp217	6.1	10.9	0.3	5.9	-	-	-	

Contd.,

Table 20 (Contd.,). QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1)-based RIL population for 2007 and 2008 rainy season data sets

Trait / Season	Linkage group	Position (cM)	Marker interval			LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Adj. R^2 (%)
										Interaction component	Effect	Part R^2 (%)	
Plant height													
2007 rainy	SBI-01a	50	Xcup24	-	Xcup73	4.1	7.3	-11.0	8.8	a2*a3	11.8	7.4	33.1
	SBI-06	72	Xisp10264	-	Xtxp057	6.6	12.3	-15.2	13.5	-	-	-	
	SBI-07	110	Xtxp295	-	XSbAGB02	9.3	15.8	-14.3	17.5	-	-	-	
2008 rainy	SBI-01a	72	Xcup73	-	Xtxp357	4.6	8.2	-11.8	5.7	a1*a2	-9.1	2.0	42.4
	SBI-06	66	Xisep0444	-	Xisp10264	5.0	9.1	-15.2	10.0	-	-	-	
	SBI-07	118	Xtxp295	-	XSbAGB02	21.3	32.7	-23.5	27.2	-	-	-	
Agronomic performance													
2007 rainy	SBI-01a	0	Xcup01	-	Xcup06	4.58	9.3	0.1	6.6	a1*a6	0.1	2.0	31.6
	SBI-01a	54	Xcup24	-	Xcup73	3.56	6.5	0.1	3.1	-	-	-	
	SBI-02b	10	Xcup74	-	Xtxp298	3.56	6.6	-0.2	4.5	-	-	-	
	SBI-02b	88	Xtxp296	-	Xisp10228	3.93	12.2	-0.1	1.9	-	-	-	
	SBI-04	40	Xisp10230	-	Xiabtp364	3.2	5.9	0.1	2.2	-	-	-	
	SBI-04	146	Xtxp327	-	Xisp10229	2.93	5.4	0.1	3.9	-	-	-	
	SBI-07	96	Xisep0829	-	Xtxp295	7.01	12.4	0.3	14.0	-	-	-	
2008 rainy	SBI-01a	20	Xcup06	-	Xcup24	11.6	19.5	0.4	11.9	-	-	-	39.7
	SBI-04	146	Xtxp327	-	Xisp10229	8.89	15.4	0.2	9.0	-	-	-	
	SBI-07	94	Xisep0829	-	Xtxp295	12.28	20.5	0.3	16.7	-	-	-	
	SBI-07	120	XSbAGB02	-	Xcup57	6.53	11.5	0.1	2.9	-	-	-	
	SBI-10	14	Xcup49	-	Xisp10359	3.62	6.6	-0.1	2.4	-	-	-	

Table 21. QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1) - based RIL population for across-season data set

Trait / Linkage group	Position cM	Marker interval		LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Significance of Q×E	Adj R^2 (%)
								Interaction component	Effects	Part R^2 (%)		
Seedling vigor												
SBI-01a	4	Xcup01	- Xcup06	3.6	7.3	-0.1	7.6	-	-	-	*ns*	19.2
SBI-01a	44	Xcup24	- Xcup73	5.1	9.0	0.1	6.0	-	-	-	*ns*	
SBI-01b	18	Xcup60	- Xcup44	3.0	5.6	-0.1	1.8	-	-	-	*ns*	
SBI-03	172	Xcup38	- Xisp10332	4.5	8.0	0.1	7.9	-	-	-	*ns*	
SBI-04	146	Xtxp327	- Xisp10229	2.6	4.8	-0.1	3.4	-	-	-		
Basal pigmentation												
SBI-06	74	Xisp10264	- Xtxp057	27.8	42.6	0.6	42.5	-	-	-	*ns*	42.1
Plant color												
SBI-06	82	Xisp10264	- Xtxp057	34.8	49.8	0.7	42.4	-	-	-	*ns*	44.5
SBI-07	94	Xisep0829	- Xisep0829	2.6	4.7	0.1	3.8	-	-	-	*ns*	
Testa presence vs absence												
SBI-04	144	Xtxp327	- Xisp10229	44.3	56.8	0.4	55.6	-	-	-	*ns*	55.2
Mesocarp thickness												
SBI-01a	86	Xisp10203	- Xisp10359	3.1	5.7	-0.1	4.2	-	-	-	*	35.8
SBI-04	146	Xtxp327	- Xisp10229	15	24.5	0.4	36.3	-	-	-	*ns*	
Leaf angle												
SBI-04	146	Xtxp327	- Xisp10229	27.4	40.1	4.9	39.2	-	-	-	*ns*	38.7

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Table 21 (Contd.,). QTLs identified for agronomic and morphological traits using the (ICSV 745 x PB 15520-1) - based RIL population for across-season data sets

Trait / Linkage group	Position cM	Marker interval		LOD	R^2 (%)	Additive effect	Part R^2 (%)	Epistatic effect			Significance of Q×E	Adj R^2 (%)
								Interaction component	Effects	Part R^2 (%)		
Time to 50% flowering												
SBI-01a	82	Xtxp357	- Xisp10203	7.7	13.4	1.9	10.7	a2*a3	1.30	2.9	*ns*	44.0
SBI-01b	36	Xcup44	- Xtxp340	5.2	9.5	-1.2	2.9	-	-	-	*ns*	
SBI-04	32	Xisp10230	- Xiabtp364	12.5	20.7	2.4	13.2	-	-	-	*ns*	
SBI-06	4	Xisep0444	- Xisp10264	20.1	31.7	3.5	25.1	-	-	-	**	
SBI-10	102	Xtxp270	- Xtxp217	9.5	16.2	2.1	13.7	-	-	-	*ns*	
Number of nodes												
SBI-04	16	Xisep0224	- Xisp10230	4.2	7.8	0.4	5.4	-	-	-	*ns*	31.4
SBI-06	0	Xisep0444	- Xisp10264	11.8	20.4	0.5	16.6	-	-	-	**	
SBI-07	90	Xgap342	- Xisep0829	5.9	10.6	0.4	10.6	-	-	-	*ns*	
SBI-10	106	Xtxp217	- Xisp10263	8.2	14.3	0.4	8.8	-	-	-	*ns*	
Plant height												
SBI-01a	54	Xcup24	- Xcup73	6.2	10.9	-9.3	8.6	-	-	-	*ns*	42.1
SBI-06	68	Xisep0444	- Xisp10264	10.3	17.7	-13.2	16.0	-	-	-	*ns*	
SBI-07	114	Xtxp295	- XSbAGB02	22.3	33.8	-19.6	30.6	-	-	-	**	
Agronomic performance												
SBI-01a	16	Xcup06	- Xcup24	7.6	13.2	0.2	8.3	a2*a3	0.1	2.5	*ns*	41.7
SBI-01a	52	Xcup24	- Xcup73	2.6	4.7	0.0	0.4	-	-	-	*ns*	
SBI-02b	8	Xcup74	- Xtxp298	2.6	4.7	0.0	1.5	-	-	-	*ns*	
SBI-02b	86	Xtxp296	- Xisp10228	2.5	7.9	-0.1	1.2	-	-	-	*ns*	
SBI-04	146	Xtxp327	- Xisp10229	6.5	11.5	0.2	7.0	-	-	-	**	
SBI-07	94	Xisep0829	- Xtxp295	13.8	22.6	0.3	16.4	-	-	-	**	
SBI-07	120	XSbAGB02	- Xcup57	3.5	6.4	0.1	2.9	-	-	-	**	

Table 22. Weather parameters recorded during 2007 and 2008 rainy seasons at ICRISAT-Patancheru

Month	Total Rainfall (mm)		Average Rainfall (mm/day)		Evaporation (mm/day)		Maximum Temperature (^{o}c/day)		Minimum Temperature (^{o}c/day)		Relative Humidity at 07:17 hrs (%)		Relative Humidity at 14:17hrs (%)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
June	157.60	75.60	5.25	2.52	6.11	8.61	33.25	33.78	23.61	23.47	85.17	79.83	56.30	47.73
July	61.10	114.30	1.97	3.69	5.55	6.44	31.40	32.55	23.19	22.90	86.19	83.74	59.13	60.87
August	222.90	382.10	7.19	12.33	4.17	3.65	29.95	29.18	22.07	21.80	91.58	92.42	66.87	70.97
September	148.40	184.40	4.95	6.15	3.18	3.70	29.27	29.56	21.97	21.03	94.33	93.87	71.73	65.27
October	22.60	85.40	0.73	2.75	4.08	4.48	30.35	31.01	18.27	18.79	92.55	91.29	49.00	48.26
November	27.60	26.60	0.92	0.89	4.56	3.74	29.48	29.62	13.12	16.10	91.30	90.47	39.10	45.53
Across months	**640.20**	**868.40**	**3.50**	**4.75**	**4.61**	**5.10**	**30.61**	**30.95**	**20.39**	**20.69**	**90.19**	**88.61**	**57.04**	**56.50**

Table 23. RILs ranked for spotted stem borer resistance component traits based on genotypic and phenotypic values

Stem tunneling (cm)			Deadheart incidence (%)			Leaf feeding (score)			Recovery resistance (score)			Overall resistance (score)		
RIL No.	Mean	Rank	RIL No.	Mean	Rank	RIL No.	Mean	Rank	RIL No.	Mean	Rank	RIL No.	Mean	Rank
135	19.5	1	236	37.8	1	239	4.6	1	188	4.0	1	244	4.9	1
108	24.2	2	241	45.0	1	180	4.7	1	67	4.4	1	196	5.1	1
24	25.4	2	19	45.4	1	110	5.1	1	30	3.7	2	1	5.3	1
93	24.5	3	23	46.0	1	16	4.5	2	179	3.6	3	217	5.3	1
212	25.2	3	35	46.7	1	137	4.1	3	5	4.3	3	253	5.3	1
47	26.4	4	53	48.0	1	69	4.9	3	214	4.4	3	53	5.4	1
109	19.7	5	227	49.4	1	108	5.1	3	250	4.3	4	246	5.5	1
92	22.2	5	201	49.7	1	35	4.5	4	99	4.4	4	236	5.5	1
144	23.1	5	45	50.4	1	202	4.5	4	201	4.5	4	221	5.6	1
231	23.1	5	221	51.2	1	214	4.6	4	168	4.2	5	216	5.1	2
78	24.4	5	239	52.8	1	19	4.6	4	185	4.4	5	93	5.5	2
17	25.4	5	251	52.8	1	88	4.7	4	253	4.5	5	122	5.3	3
64	25.4	5	178	52.9	1	68	4.8	4	193	4.1	6	137	5.3	3
254	25.2	6	163	53.2	1	247	4.9	4	190	4.3	6	261	5.4	3
73	23.0	7	182	53.5	1	191	5.0	4	245	4.4	6	199	5.4	3
11	24.7	7	137	54.5	1	188	5.0	4	196	4.5	6	265	5.5	3
40	25.0	8	69	54.6	1	236	5.1	4	260	3.5	7	34	5.5	3
141	18.7	9	157	55.5	1	212	4.1	5	98	3.8	7	171	5.5	3
103	23.1	9	188	49.0	2	223	4.7	5	205	4.2	7	241	5.5	3
62	26.0	9	185	53.3	2	205	4.9	6	223	4.0	8	250	5.6	3
169	15.9	10	204	46.8	3	192	5.0	6	261	4.2	8	185	4.8	4
222	18.8	10	197	51.2	3	62	5.0	7	158	4.2	8	251	5.4	4
71	25.0	10	34	37.5	4	84	5.1	8	135	4.3	8	48	5.6	4
220	26.6	10	6	43.7	4	115	4.8	9	171	4.5	8	67	5.6	4
52	25.4	11	90	44.8	4	227	5.0	9	169	3.6	9	245	5.4	5
152	26.9	11	202	45.5	4	197	3.9	10	213	3.9	9	97	5.5	5
173	19.8	12	91	52.9	4	6	4.7	10	91	4.5	9	188	4.6	6
194	23.0	13	213	55.2	4	63	4.2	11	39	4.5	10	51	5.0	7
263	20.6	14	258	50.98	5	263	4.7	11	191	4.5	11	160	5.3	8

Table 24. Comparative position of maize genomic regions (associated with stem borer resistance traits) with sorghum genome

Markers / genes	Type	Maize genome					Sorghum genome			
		Bin	BAC Clone	E-value	Max Score	Associated trait	Chromosome	Position (bp)	Max score	E-value
bnl5.62[1]	RFLP	1.01	AC155361	0.00	936	ECB Tunneling	1	72440158 - 72440811	206.7	2.2E-51
umc164	RFLP	1.01	AC155517	0.00	926	-	1	72083318 - 72084009	418.8	8E-115
php20537	RFLP	1.01	AC155469	0.00	579	-	1	71376574 - 71376667	123.4	4.2E-26
umc157[1]	RFLP	1.02	AC177915	0.00	2424	ECB Tunneling	1	69588737 - 69589110	93.7	5.8E-17
umc76	RFLP	1.03	AC204265	0.00	1080	-	1	54022760 - 65402334	89.7	5.1E-16
umc29	RFLP	1.04	AC209839	0.00	928	ECB Tunneling	1	60092082 - 60092533	448.5	3.2E-124
npi262[4]	RFLP	1.04	AC207320	0.00	628	SCB and SWCB Leaf feeding	1	53685573 - 53685671	133.3	2.4E-29
umc58[2]	RFLP	1.06	AC209698	0.00	813	SCB and SWCB Leaf feeding	7	61170138 - 61170643	89.7	3.4E-16
umc33[1,3]	RFLP	1.07	AC215279	0.00	878	ECB Tunneling	7	55255132 - 55255448	192.8	3.2E-47
umc128[1]	RFLP	1.08	AC208567	0.00	878	ECB Tunneling	1	19135447 - 19135567	89.7	5.4E-16
bnl8.10	RFLP	1.08	AC199774	1E-112	410	-	1	12950890 - 12951103	139.3	1.7E-31
csu164	RFLP	1.09	AC191429	0.00	837	-	1	12385120 - 12385556	349.4	2.2E-94
umc140	RFLP	1.09	AC186519	1E-130	470	-	1	11778593 - 11778723	58	1.2E-06
umc161[2,6]	RFLP	1.11	AC234189	0.00	1417	SWCB and ECB Leaf feeding	1	5447964 - 5448716	763.7	0.00
bnl6.32[2,6]	RFLP	1.12	AC207546	0.00	1015	SCB and ECB Leaf feeding	1	1277700 - 1278144	81.8	8.8E-14

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Table 24 (Contd.,). Comparative position of maize genomic regions (associated with stem borer resistance traits) with sorghum genome

Markers / genes	Type	Maize genome					Sorghum genome				
		Bin	BAC Clone	E-value	Max Score	Associated trait	Chromosome	Position (bp)		Max score	E-value
npi239	RFLP	2.01/2.02	AC212718	0.00	844	-	6	61116780 -	61117157	99.6	3.3E-19
umc6	RFLP	2.03	AC194000	0.00	704	-	6	55441226 -	55441659	87.7	1.7E-15
umc34	RFLP	2.04	AC213314	0.00	916	-	6	51947508 -	51947573	101.6	1.8E-19
umc259	RFLP	2.05	AC192270	2E-70	272	-	6	50746384 -	50746843	305.8	3.7E-81
umc88	RFLP	2.06	AC200756	0.00	894	-	2	55728201 -	55728427	664.6	0.00
umc98	RFLP	2.06	AC190873	0.00	2064	-	2	57663481 -	57663944	1118.5	0.00
umc4[7]	RFLP	2.06	AC194048	0.00	670	ECB Tunneling	2	69126512 -	69126758	303.8	8.8E-81
umc137[1]	RFLP	2.07/2.08	AC217052	1E-109	400	ECB Tunneling	2	72699403 -	72699475	81.8	4E-14
bnl17.14[6]	RFLP	2.1	AC225392	1E-154	549	ECB Leaf feeding	2	2391889 -	2399794	147.2	1.2E-33
bnl8.15	RFLP	3.01	AC194194	0.00	765	-	3	698934 -	699069	174.9	7.7E-42
csu32	RFLP	3.02	AC212282	1E-128	464	-	3	1956440 -	1956776	200.7	1.1E-49
csu230	RFLP	3.02	AC209718	0.00	2714	-	3	2812116 -	2814836	2099.8	0.00
umc50	RFLP	3.04	AC191375	1E-119	432	-	3	5470227 -	5470308	85.7	5.3E-15
umc92	RFLP	3.04	AC190892	1E-104	383	-	3	6591698 -	6591742	60	1.6E-07
bnl8.01	RFLP	3.06	AC212282	1E-128	464	-	3	69113548 -	69114020	105.6	6E-21
umc165	RFLP	3.06	AC206939	1E-102	377	-	3	68609715 -	68609857	188.8	2E-46
bnl6.16	RFLP	3.07	AC185127	0.00	890	-	3	64470837 -	64471226	404.9	4.6E-111
npi457	RFLP	3.09	AC191045	1E-165	587	-	3	53578998 -	53579302	220.5	9.4E-56
umc63[2,3]	RFLP	3.09	AC209091	0.00	1225	Leaf feeding SWCB and ECB tunneling	3	57204810 -	57222529	724.1	0.00

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Table 24 (Contd.,). Comparative position of maize genomic regions (associated with stem borer resistance traits) with sorghum genome

Markers / genes	Type	Maize genome					Sorghum genome			
		Bin	BAC Clone	E-value	Max Score	Associated trait	Chromosome	Position (bp)	Max score	E-value
umc87	RFLP	4.03	AC185498	0.00	761	-	5	12078741 - 12079229	218.6	5.6E-55
umc31[6]	RFLP	4.03	AC185498	0.00	749	Leaf feeding ECB	5	12078741 - 12079229	218.6	5.5E-55
bnl10.05	RFLP	4.08	AC198325	0.00	827	-	5	1122718 - 1122893	109.5	3.8E-22
bnl7.65	RFLP	4.08	AC197758	1E-115	420	-	5	1025293 - 1025455	63.9	1.8E-08
npi410	RFLP	4.08	AC191407	0.00	644	-	5	51133274 - 51133605	301.8	3.5E-80
umc52	RFLP	4.09	AC185269	0.00	1191	-	5	6193008 - 6193819	406.9	2.0E-111
bnl6.25	RFLP	5.01	AC193957	6E-80	301	-	1	3539021 - 3539177	75.8	1.6E-12
umc90	RFLP	5.02	AC210063	0.00	1499	-	1	7554121 - 7555593	309.7	5.1E-82
umc72	RFLP	5.02	AC195684	0.00	795	-	1	7630909 - 7631274	492.1	2.4E-137
bnl7.43	RFLP	5.03	AC211207	0.00	777	-	1	2033890 - 2034373	282	3.7E-74
bnl10.06	RFLP	5.03	AC211207	0.00	908	-	1	2034086 - 2034394	331.5	5.4E-89
bnl5.71[2,6]	RFLP	5.04	AC200869	0.00	973	Leaf feeding ECB and SCB	4	59530041 - 59530268	720.1	0.00
bnl5.40[4]	RFLP	5.05	AC213883	1E-147	527	Leaf feeding SCB	4	63300261 - 63300361	103.6	1.5E-20
umc51[4]	RFLP	5.06	AC208793	1E-148	529	Leaf feeding SCB and SWCB	4	61438859 - 61439448	85.7	5.5E-15
umc126[2,4,6]	RFLP	5.06	AC207286	5E-19	101	Leaf feeding by ECB, SCB and SWCB and ECB tunneling	4	63150965 - 63152359	91.7	1.2E-16
umc156	RFLP	5.06	AC207286	1E-179	634	-	4	63147408 - 63147856	143.2	2.9E-32
umc104[2]	RFLP	5.08	AC207417	0.00	1287	Leaf feeding SWCB	4	66347120 - 66347849	601.1	6.0E-170
bnl7.49	RFLP	5.08	AC203533	1E-52	212	-	4	66675234 - 66675437	198.7	5.1E-49
php10017[6]	RFLP	5.09	AC210997	0.00	989	Leaf feeding ECB	4	66716271 - 66716601	115.5	6.5E-24

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Table 24 (Contd.,). Comparative position of maize genomic regions (associated with stem borer resistance traits) with sorghum genome

Markers / genes	Type	Maize genome					Sorghum genome			
		Bin	BAC Clone	E-value	Max Score	Associated trait	Chromosome	Position (bp)	Max score	E-value
umc59[7]	RFLP	6.01	AC215866	1E-150	537	ECB Tunneling	10	59295228 - 59295314	101.6	9.2E-20
mir2[8]	cDNA	6.02	AC207260	0.00	817	Maize lepidopteran insect resistance	10	57831160 - 57881877	174.9	2.8E-41
mir1[8]	cDNA	6.02	AC207260	0.00	888	Maize lepidopteran insect resistance	10	57831159 - 57881988	331.5	1.7E-88
umc65[2]	RFLP	6.04	AC212031	0.00	757	Leaf feeding ECB and SWCB	10	49966523 - 49966633	129.3	5.7E-28
umc21[2]	RFLP	6.05	AC197533	0.00	1639	Leaf feeding SWCB	10	17397960 - 17398572	500	2.3E-139
umc46	RFLP	6.05	AC212361	0.00	676	-	9	48213725 - 48213920	151.2	9.0E-35
bnl15.45	RFLP	6.05	AC207532	6E-71	272	-	9	40923968 - 40924085	125.4	2.1E-27
bnl7.25	RFLP	6.05	AC207532	1E-151	539	-	9	40924085 - 40923915	182.9	2.3E-44
bnl3.03	RFLP	6.06	AC191116	1E-140	504	-	9	13766653 - 13766726	89.7	2.7E-16
umc38[4]	RFLP	6.06	AC196258	0.00	684	Leaf feeding SWCB	9	53007564 - 53009285	188.8	1.1E-45
asg8[7]	RFLP	7.01	AC215675	1E-154	549	ECB Tunneling	2	2815509 - 2815786	464.4	3.2E-129
umc136	RFLP	7.02	AC198589	1E-110	402	-	2	60696919 - 60697278	77.8	9.3E-13
umc116[4]	RFLP	7.02	AC202390	1E-110	404	Leaf feeding SWCB	2	61990096 - 61990129	52	7.1E-05
bnl8.37	RFLP	7.04	AC197343	0.00	890	-	2	72075652 - 72075983	327.6	8.9E-88
bnl14.07[4]	RFLP	7.04	AC192356	0.00	642	Leaf feeding SCB	2	72203933 - 72204364	155.1	6.7E-36
umc80	RFLP	7.04	AC220975	0.00	1522	-	2	74279584 - 74280141	670.5	0.00
bnl8.44[7]	RFLP	7.05	AC210839	0.00	1011	ECB Tunneling	2	76783833 - 76784328	710.2	0.00

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Table 24 (Contd.,). Comparative position of maize genomic regions (associated with stem borer resistance traits) with sorghum genome

Markers / genes	Type	Maize genome					Sorghum genome			
		Bin	BAC Clone	E-value	Max Score	Associated trait	Chromosome	Position (bp)	Max score	E-value
npi110[4]	RFLP	8.02	AC195361	0.00	670	Leaf feeding SWCB	3	8278363 - 8278764	293.9	1.1E-77
umc124[6]	RFLP	8.03	AC199064	0.00	1164	ECB Tunneling	3	61349017 - 61349176	519.9	2.7E-145
asg24	RFLP	8.03	AC187392	9E-75	285	-	3	3637914 - 3638142	264.1	7.9E-69
asg1b	RFLP	8.06	AC195828	1E-109	398	-	3	71086547 - 71086773	299.8	8.6E-80
umc70	RFLP	8.06	AC198923	1E-171	607	-	3	72460216 - 72460442	67.9	9.0E-10
npi268[6]	RFLP	8.07	AC198987	1E-106	392	ECB leaf feeding	3	68945099 - 68945132	67.9	1.8E-09
npi112	RFLP	8.08	AC197068	0.00	862	-	3	63745332 - 63745753	426.7	1.1E-117
umc7[5]	RFLP	8.08	AC194405	0.00	977	ECB Tunneling	3	65265054 - 65265550	850.9	0.00
umc109[7]	RFLP	9.01	AC213035	0.00	682	ECB Tunneling	10	8526377 - 8526771	188.8	8.3E-46
umc105	RFLP	9.02	AC206266	1E-155	551	-	10	3597199 - 3597529	79.8	2.1E-13
umc20	RFLP	9.03	AC217935	2E-60	238	-	10	54139368 - 54139742	167	1.6E-39
umc153[2]	RFLP	9.03	AC210292	0.00	783	Leaf feeding SCB and SWCB	10	54181643 - 54182312	155.1	6.2E-36
umc95[2,6]	RFLP	9.05	AC210799	0.00	1308	Leaf feeding SCB and SWCB, and ECB tunnel length	1	57672205 - 57672356	79.8	4.5E-13
csu93[4]	RFLP	9.06	AC221009	1E-154	551	Leaf feeding SCB and SWCB	1	65449007 - 65449156	131.3	1.4E-28
npi97	RFLP	9.07	AC194032	5E-80	303	-	1	70944395 - 70944844	406.9	1.0E-111
bnl3.04	RFLP	10.01	AC190635	1E-104	385	-	8	1934479 - 1934592	163	2.9E-38
csu25	RFLP	10.01	AC198744	0.00	1013	-	8	2360403 - 2361327	1233.5	0.00
php20075	RFLP	10.01	AC190635	0.00	1229	-	8	1865208 - 1868970	299.8	5.3E-79
umc130	RFLP	10.03	AC198647	0.00	1233	-	8	38460793 - 38461208	347.4	1.2E-93

[1] Schon *et al.* (1993), [2] Bohn *et al.* (1997); [3] Bohn *et al.* (2000), [4]Groh *et al.* (1998), [5] Cardinal *et al.* (2001), [6] Jampatong *et al.* (2002), [7]Krakowsky et al. (2004).
[8] Maize insect resistant genes: Pechan *et al.* (1999), ECB-European corn borer; SCB- Sugarcane borer, SWCB-South western cornborer

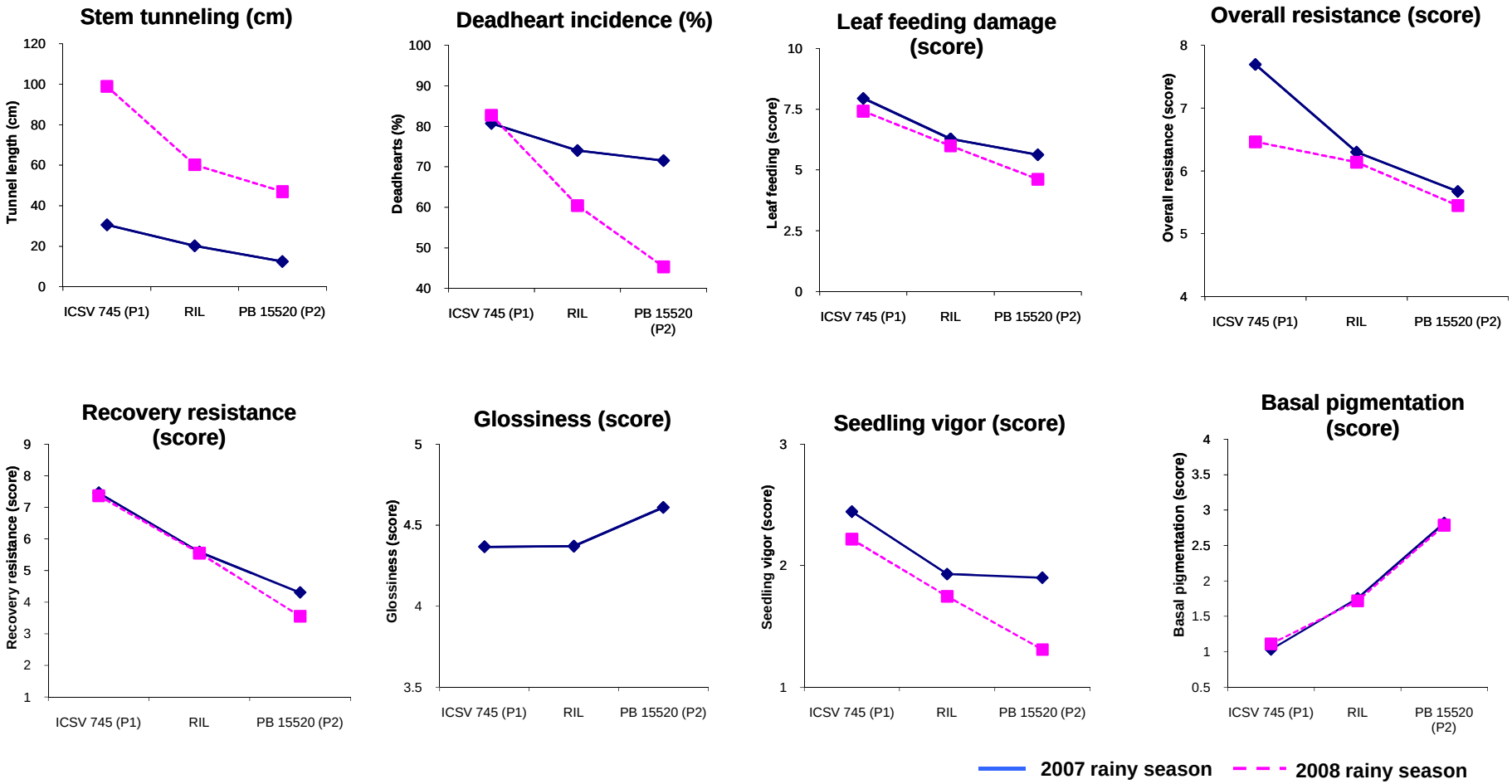


Figure 1. Mean performance of parents and RILs for the observed traits in 2007 and 2008 rainy seasons

Contd.,

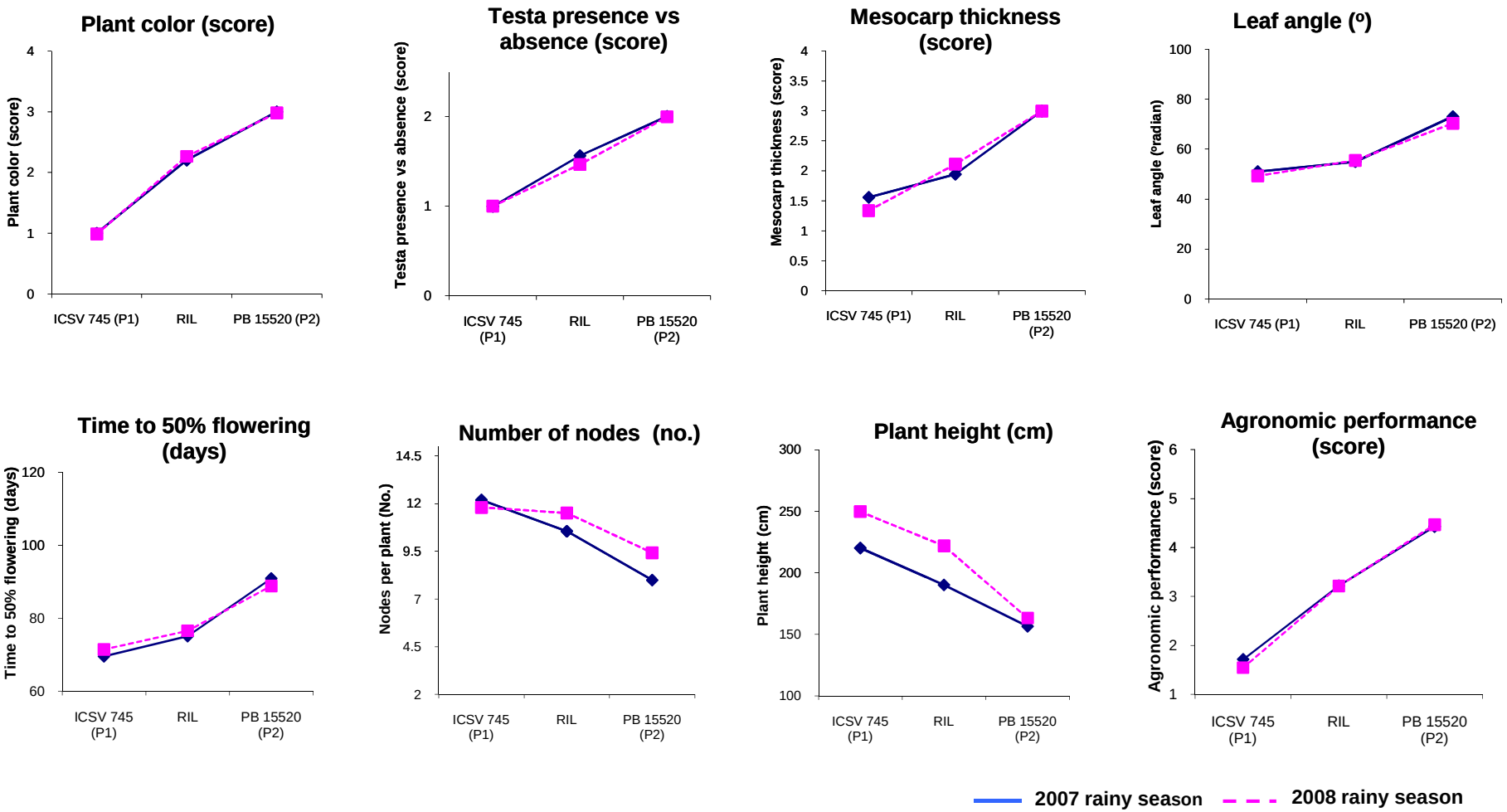


Figure 1 (Contd.,). Mean performance of parents and RILs for the observed traits in 2007 and 2008 rainy seasons

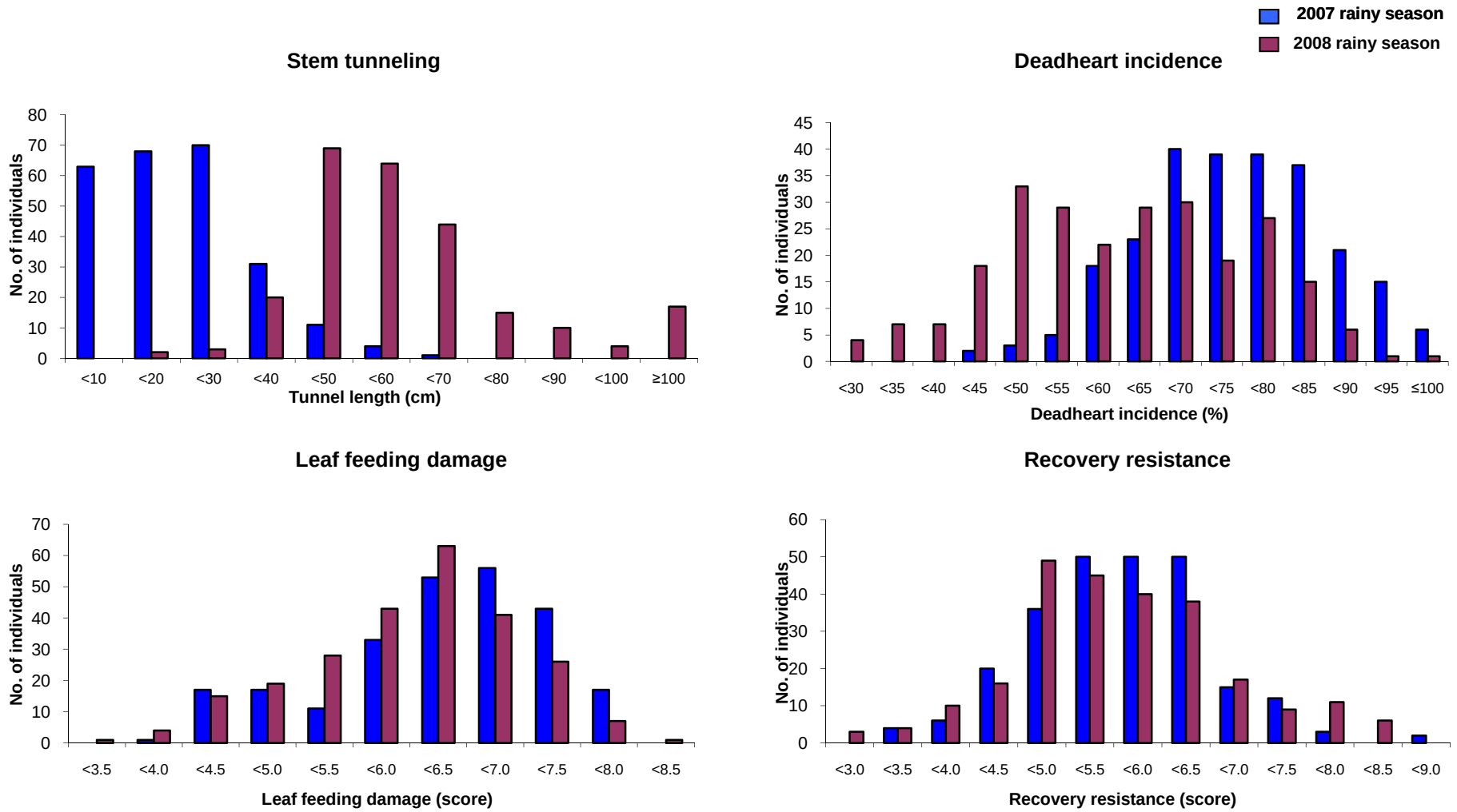


Figure 2. Frequency distribution of RILs for the traits stem tunneling, deadheart incidence, leaf feeding damage and recovery resistance observed in 2007 and 2008 rainy seasons

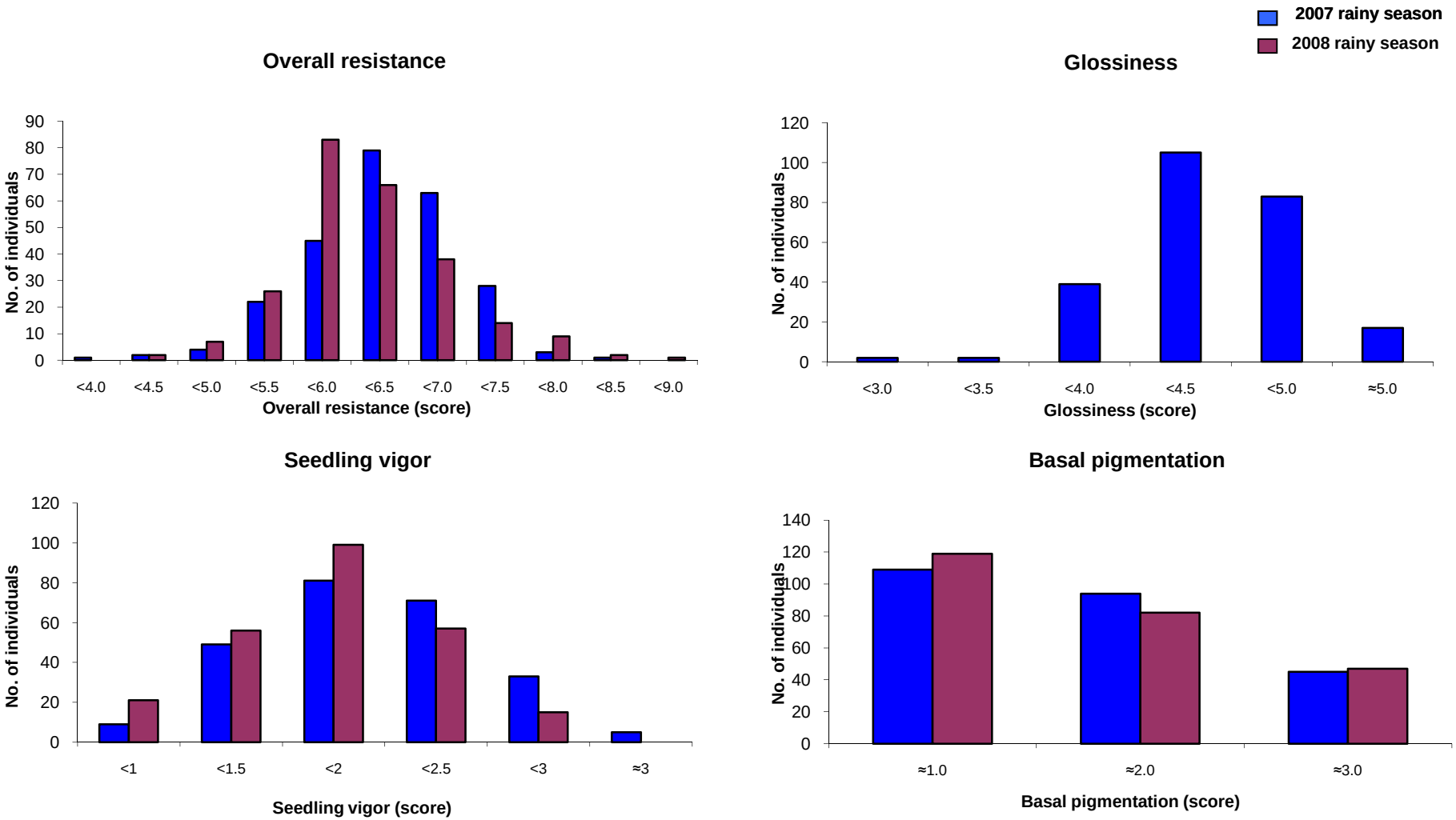


Figure 3. Frequency distribution of RILs for the traits overall resistance, glossiness, seedling vigor and basal pigmentation observed in 2007 and 2008 rainy seasons

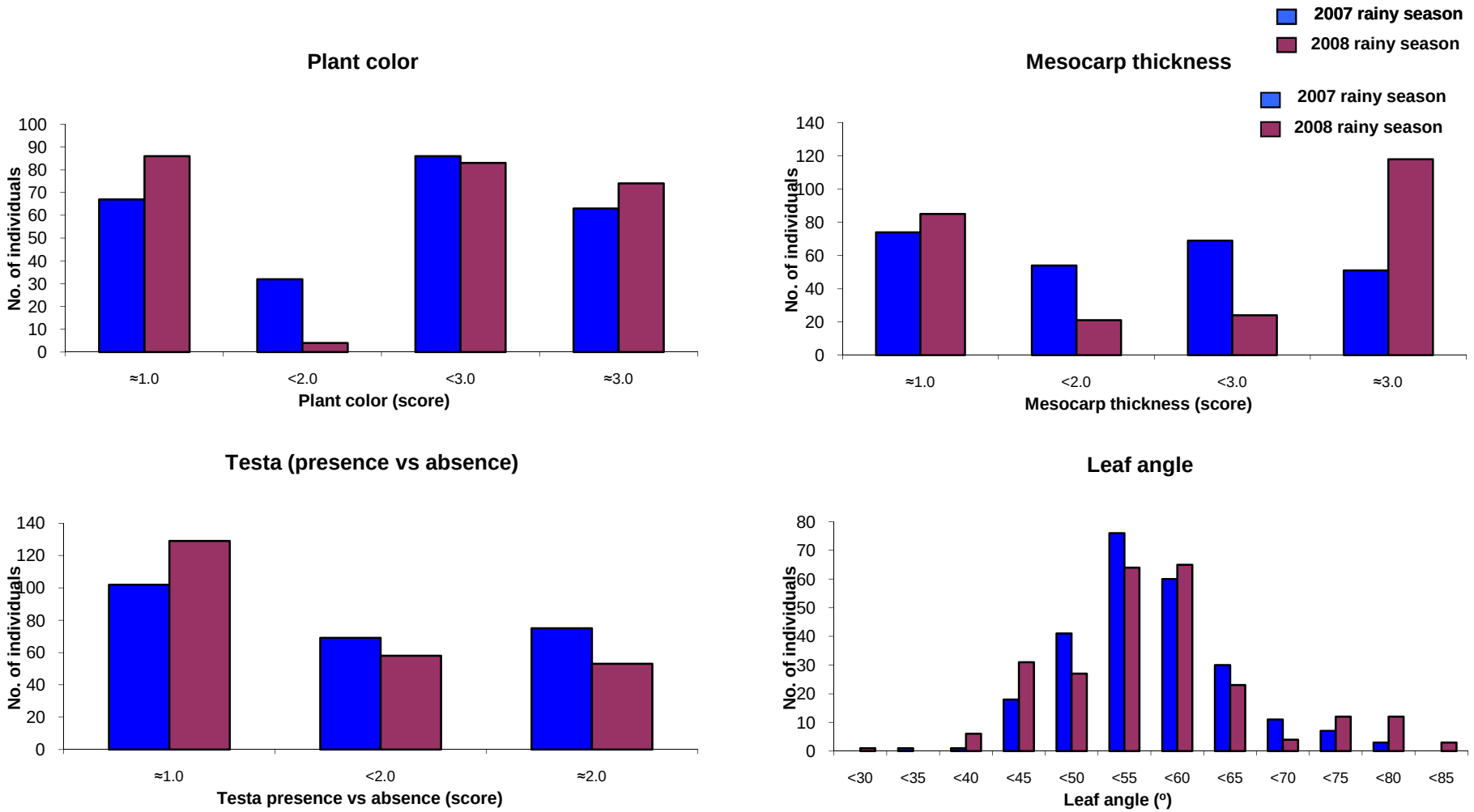


Figure 4. Frequency distribution of RILs for the traits plant color, testa presence vs absence, mesocarp thickness and leaf angle observed in 2007 and 2008 rainy seasons

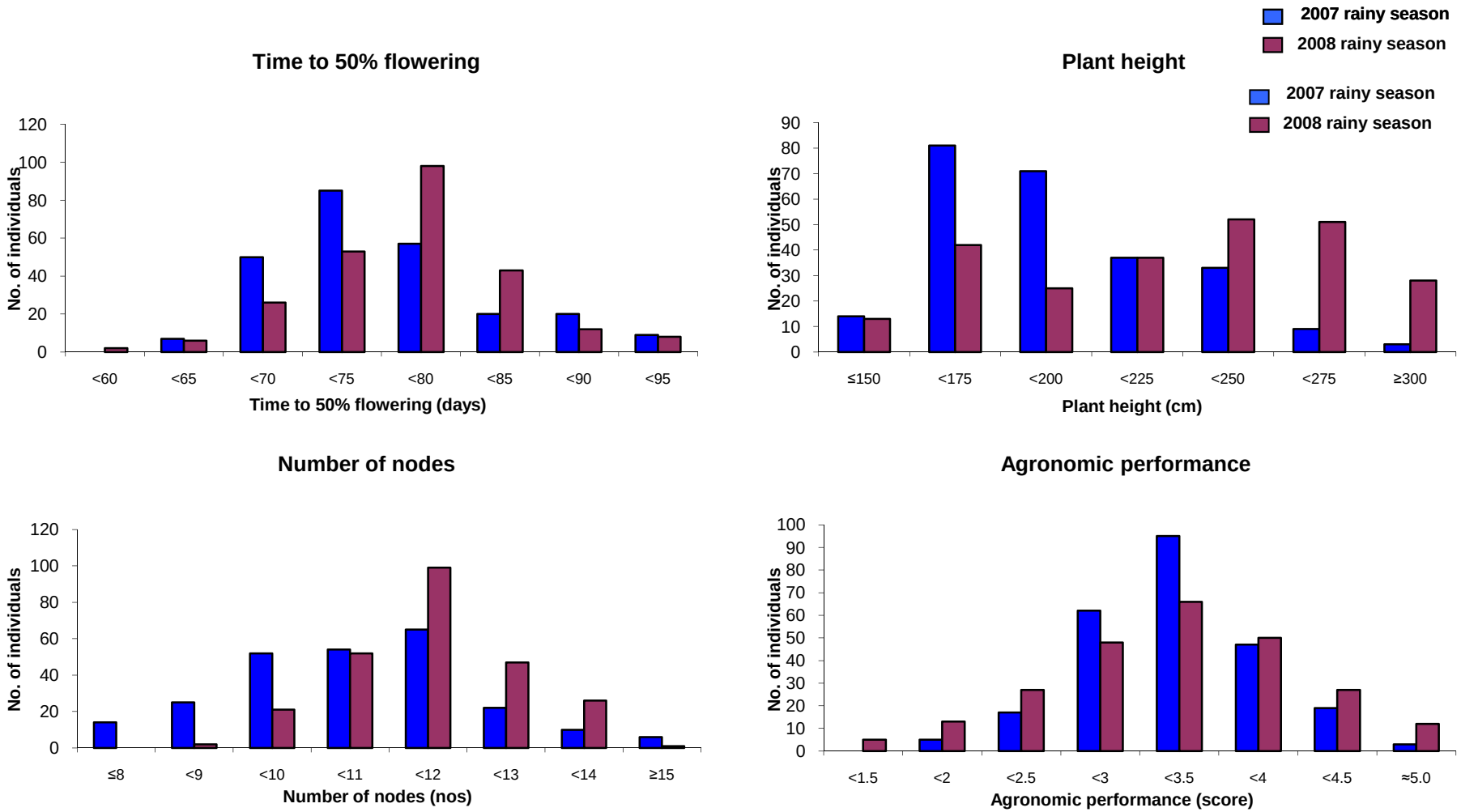


Figure 5. Frequency distribution of RILs for the traits time to 50% flowering, number of nodes, plant height and agronomic performance observed in 2007 and 2008 rainy seasons

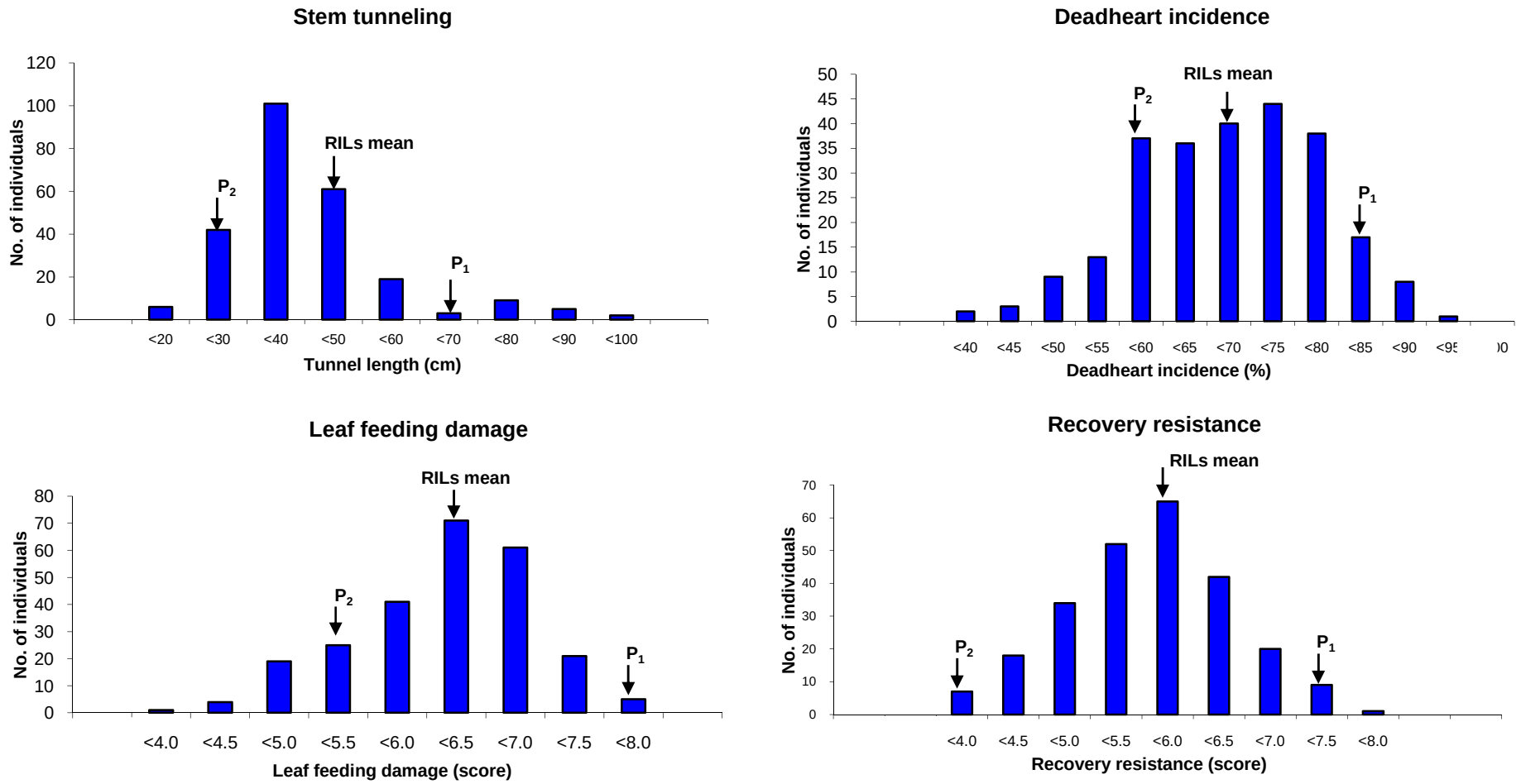


Figure 6. Frequency distribution of RILs for the traits stem tunneling, deadheart incidence, leaf feeding and recovery resistance using across season data sets

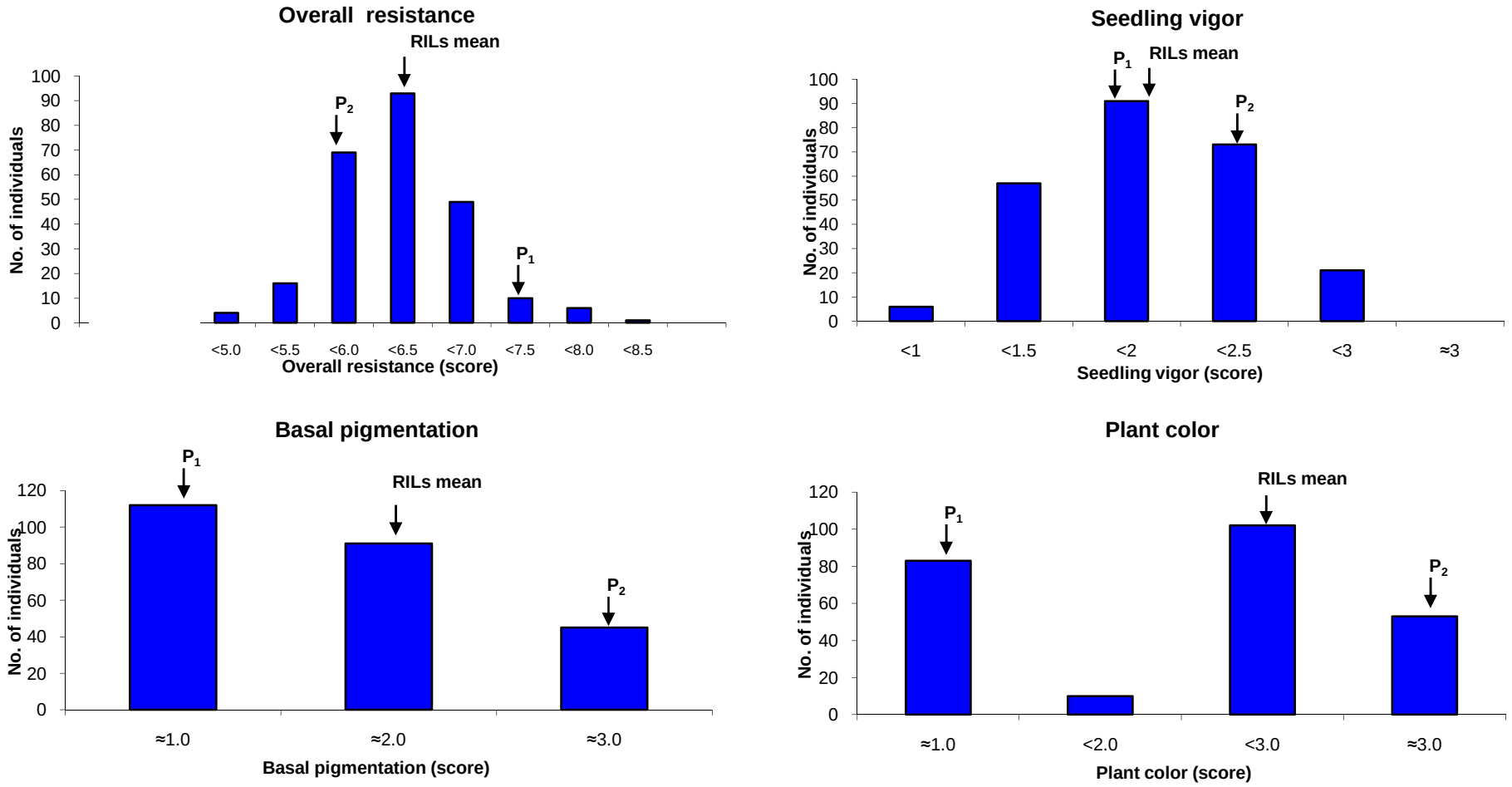


Figure 7. Frequency distribution of RILs for the traits overall resistance, seedling vigor, basal pigmentation and plant color using across season data sets

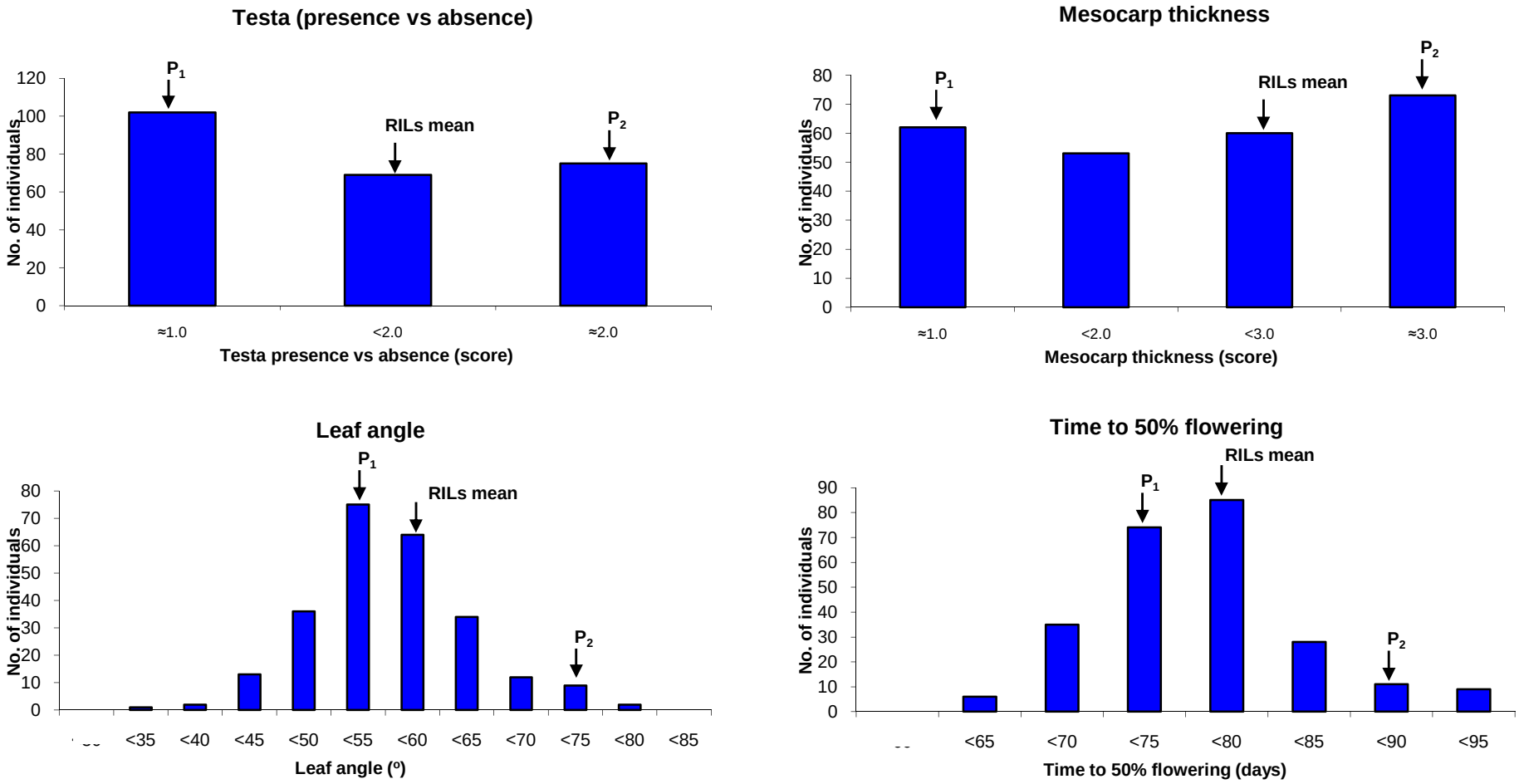


Figure 8. Frequency distribution of RILs for the traits testa presence vs absence, mesocarp thickness, leaf angle and time to 50% flowering using across season data

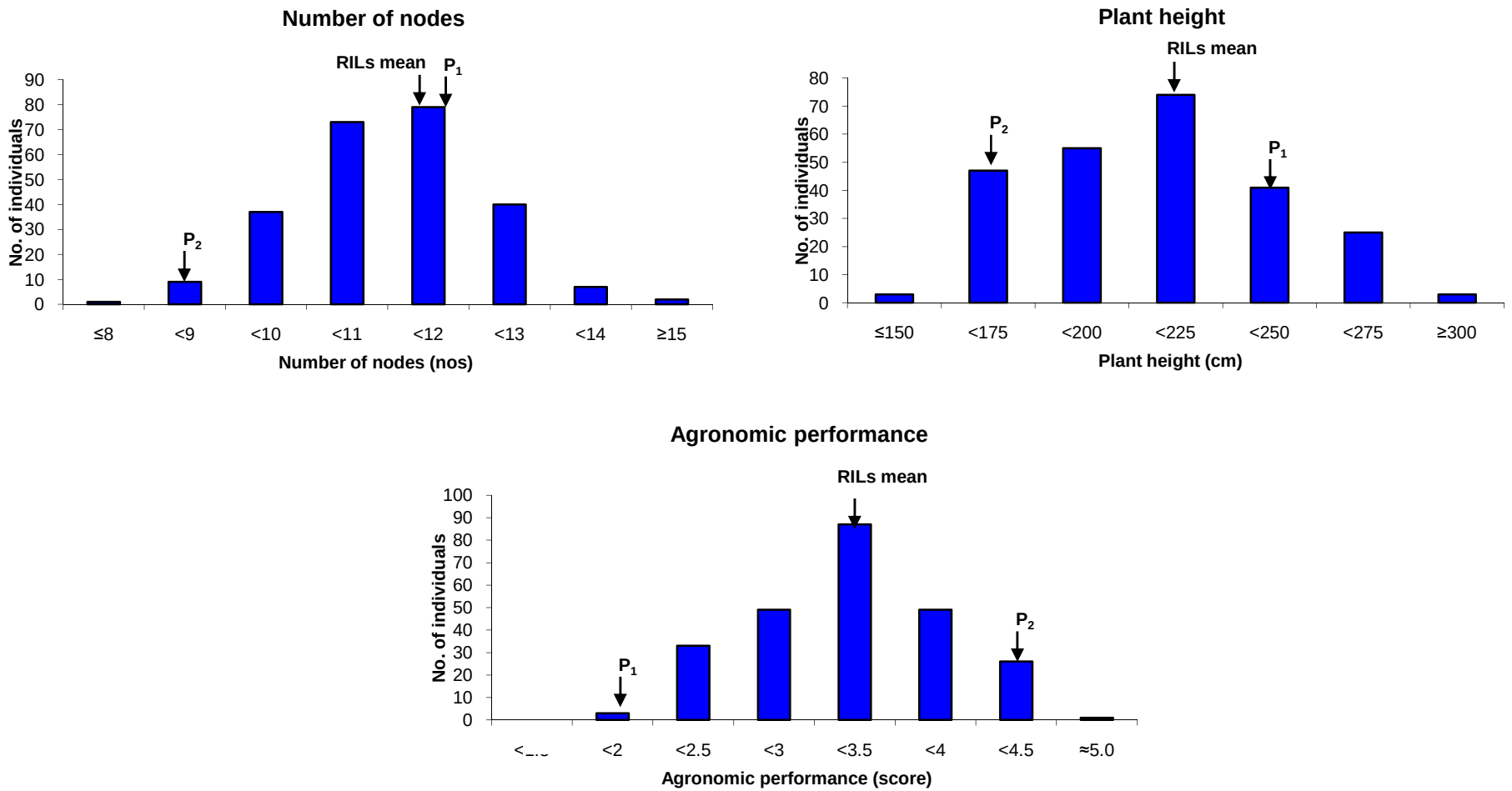


Figure 9. Frequency distribution of RILs for the traits number of nodes, plant height and agronomic performance using across season data sets

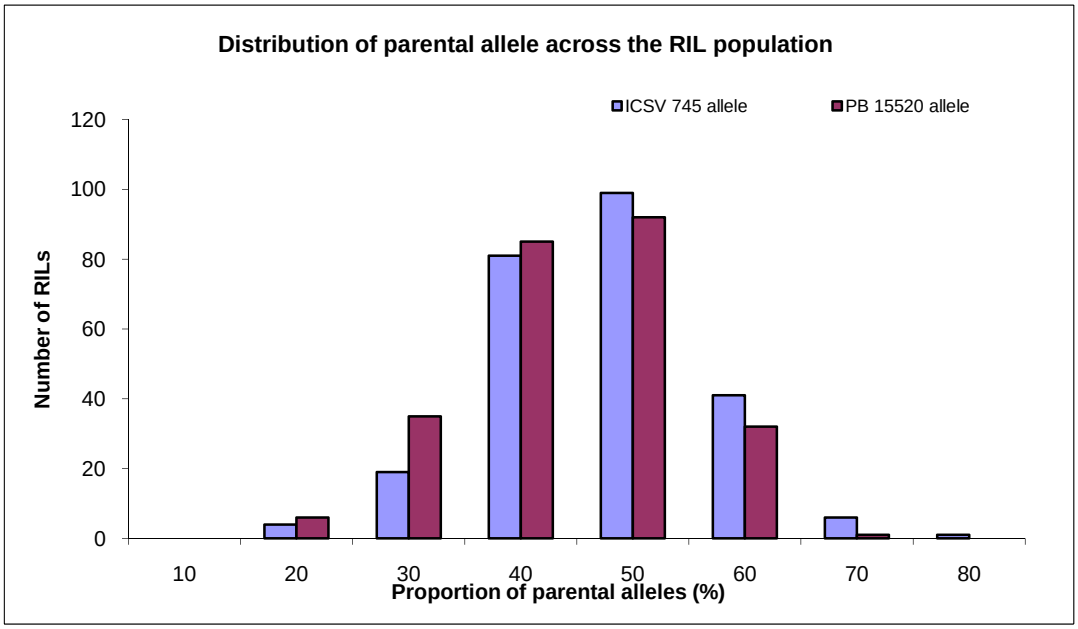


Figure 10. Distribution of parental alleles across the sorghum stem borer resistance RIL mapping population derived from parents ICSV 745 and PB 15520

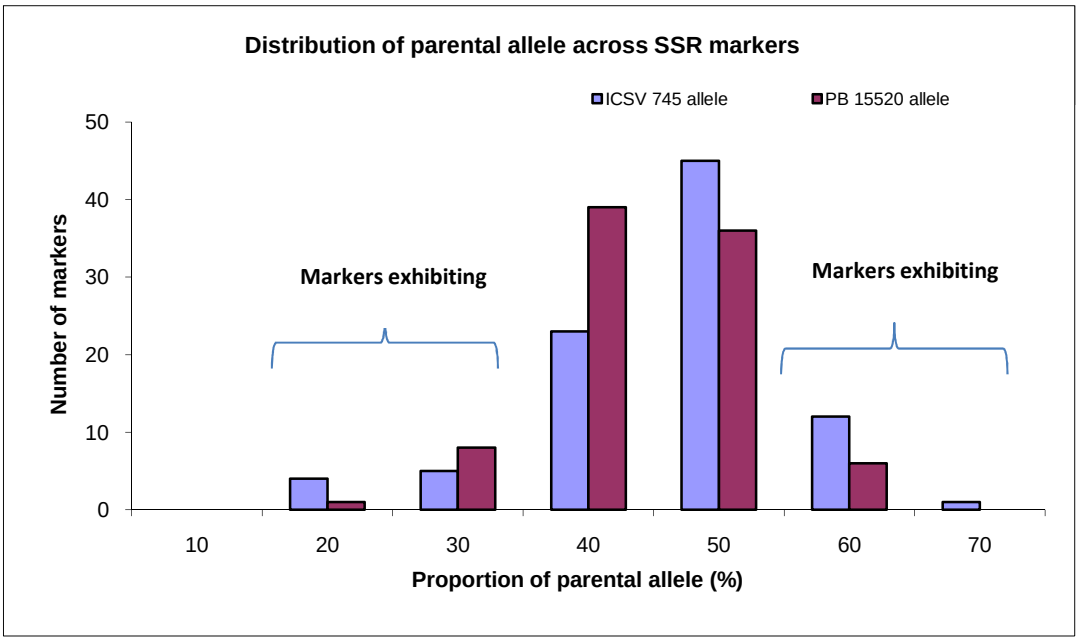


Figure 11. Distribution of parental alleles across 90 SSR markers in the sorghum RIL mapping population derived from parents ICSV 745 and PB 15520

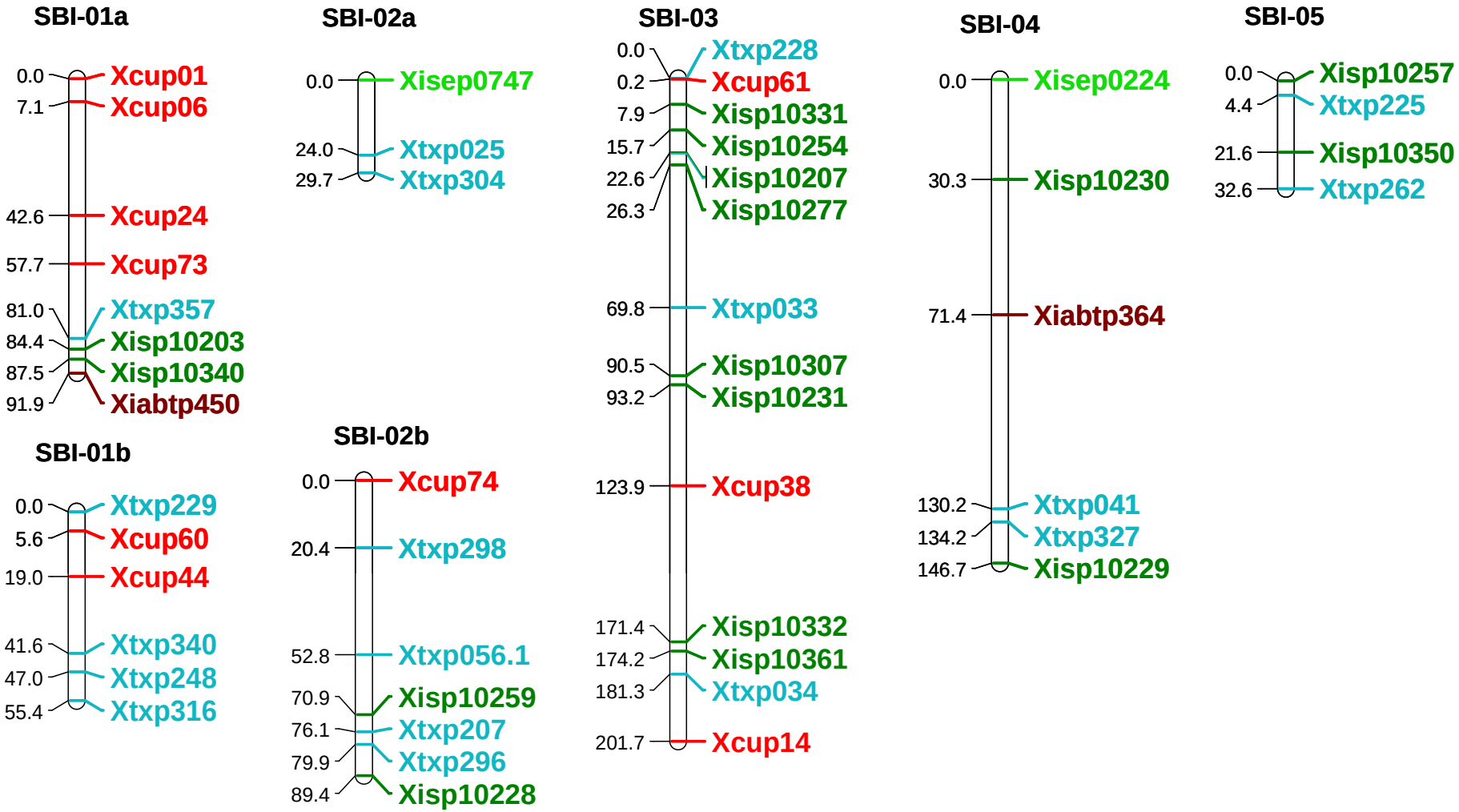


Figure 12. Linkage map of sorghum (ICSV 745 × PB 15520)- based RIL population constructed using 90 SSR markers

contd.,

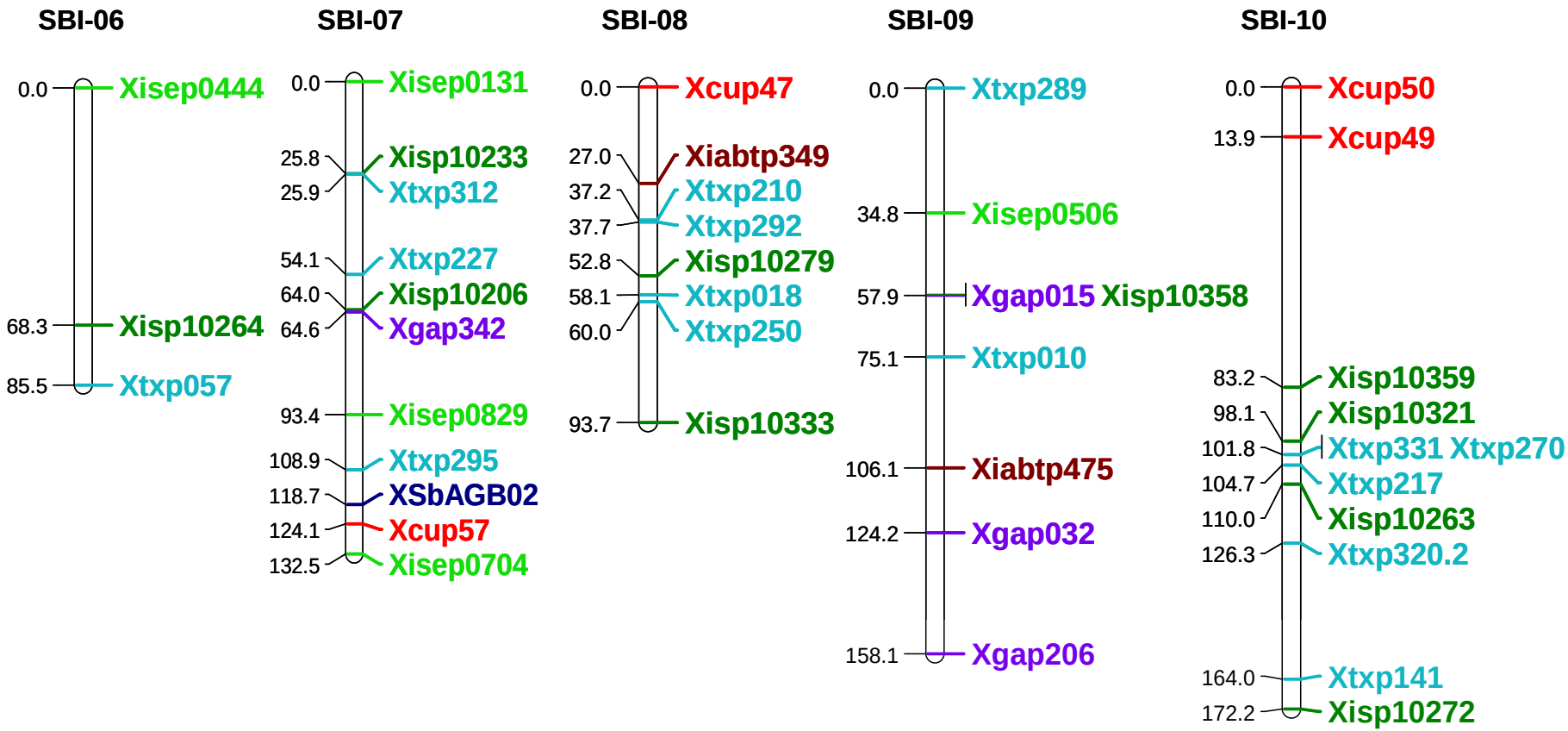


Figure 12 (Contd.,). Linkage map of sorghum (ICSV 745 × PB 15520)- based RIL population constructed using 90 SSR markers

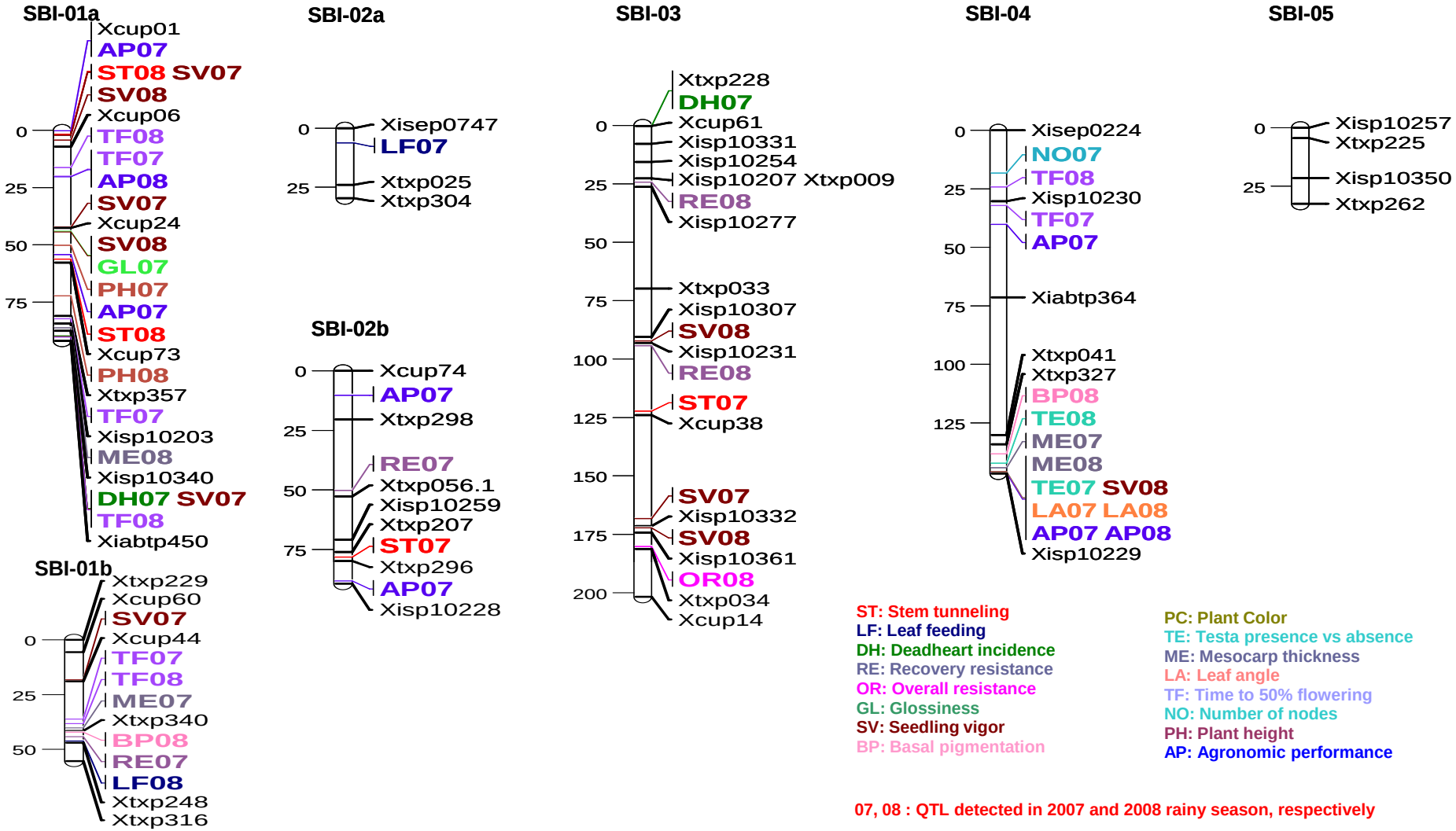


Figure 13. Linkage map of sorghum (ICSV 745 × PB 15520) - based RIL population depicting the position of QTLs detected using individual season data sets

contd.,

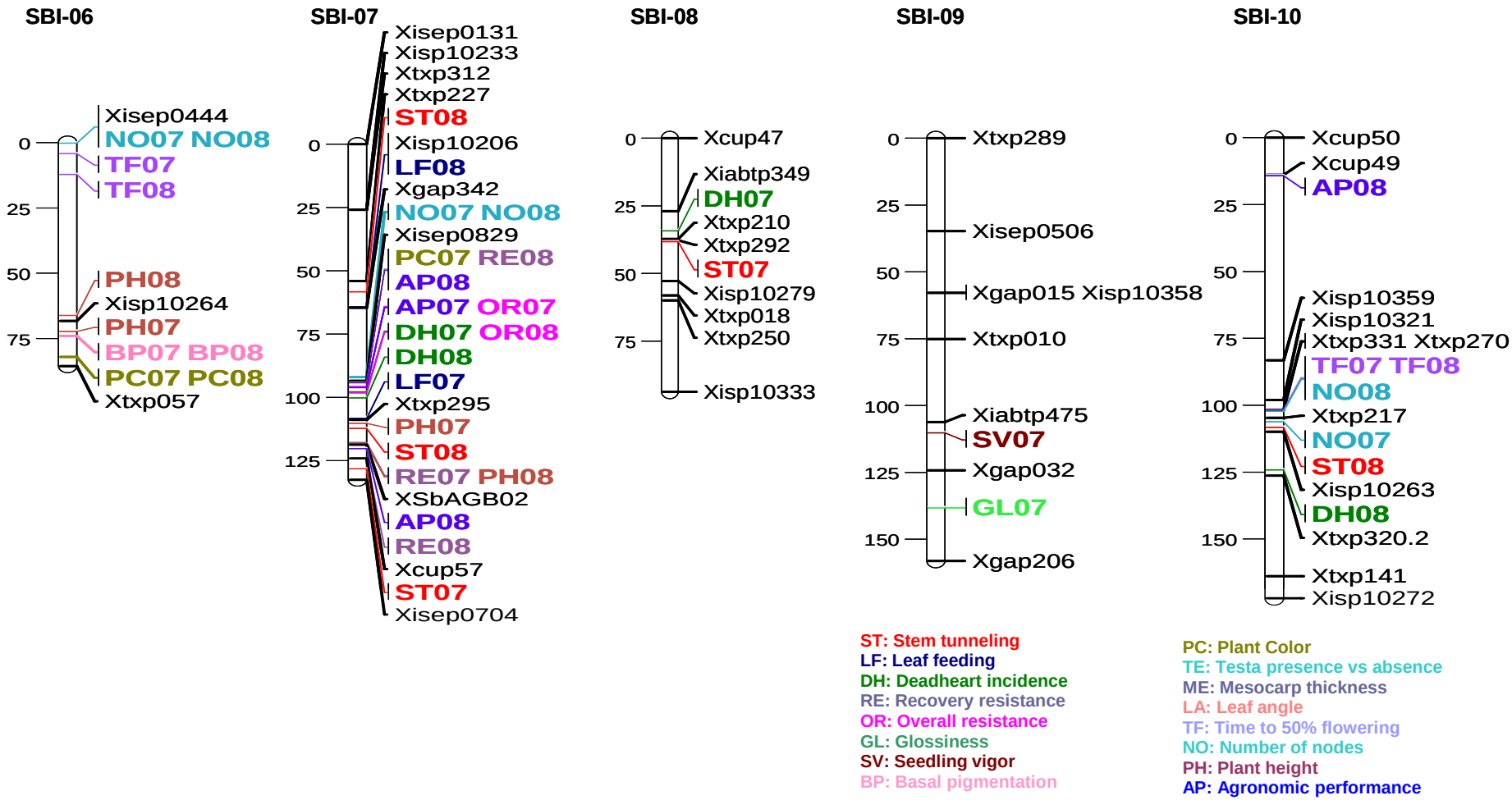


Figure 13 (Contd.,). Linkage map of sorghum (ICSV 745 × PB 15520) - based RIL population depicting the position of QTLs detected using individual season data sets

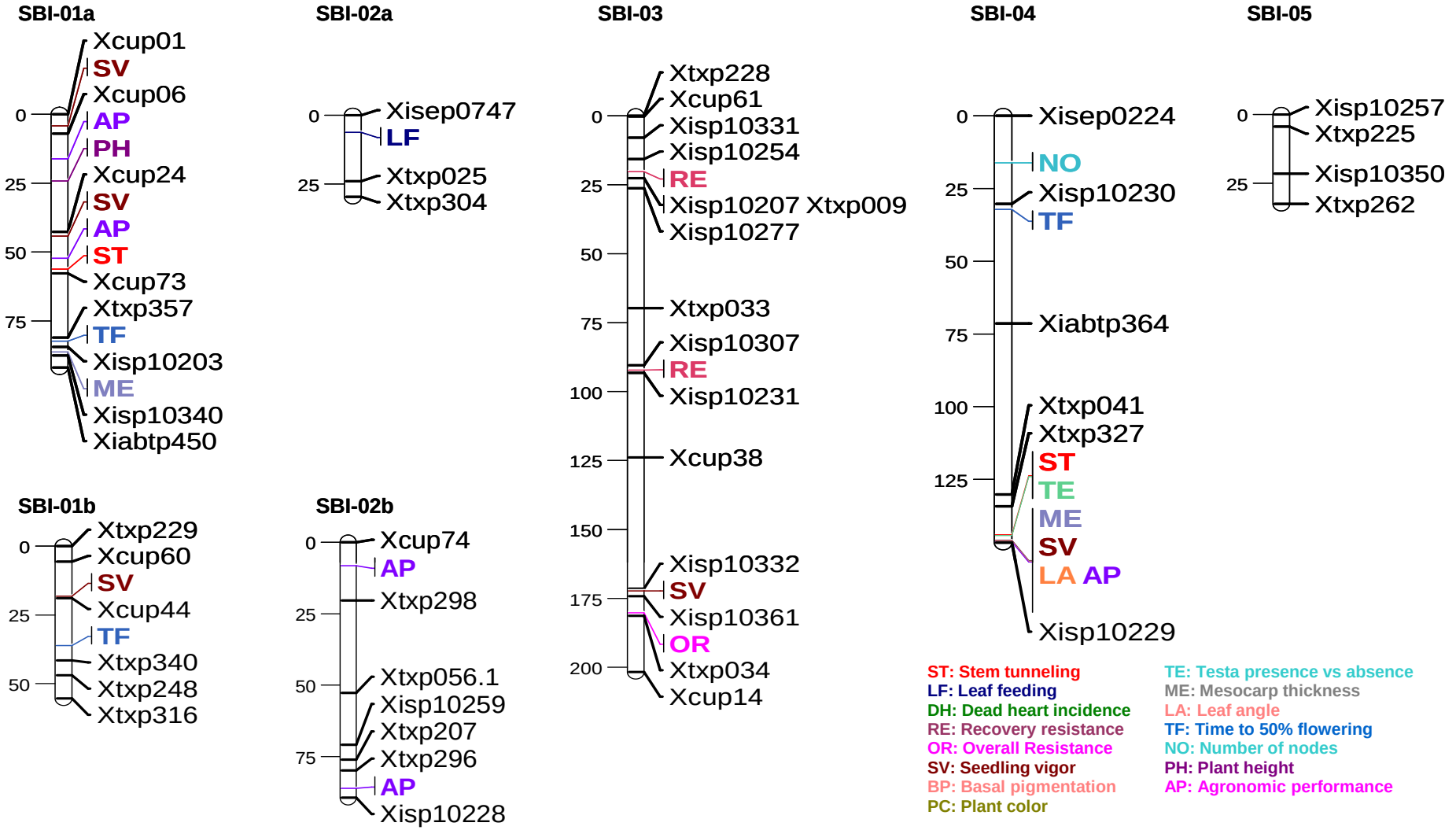


Figure 14. Linkage map of sorghum (ICSV 745 × PB 15520) - based RIL population depicting the position of QTLs detected using across-season data set

contd.,

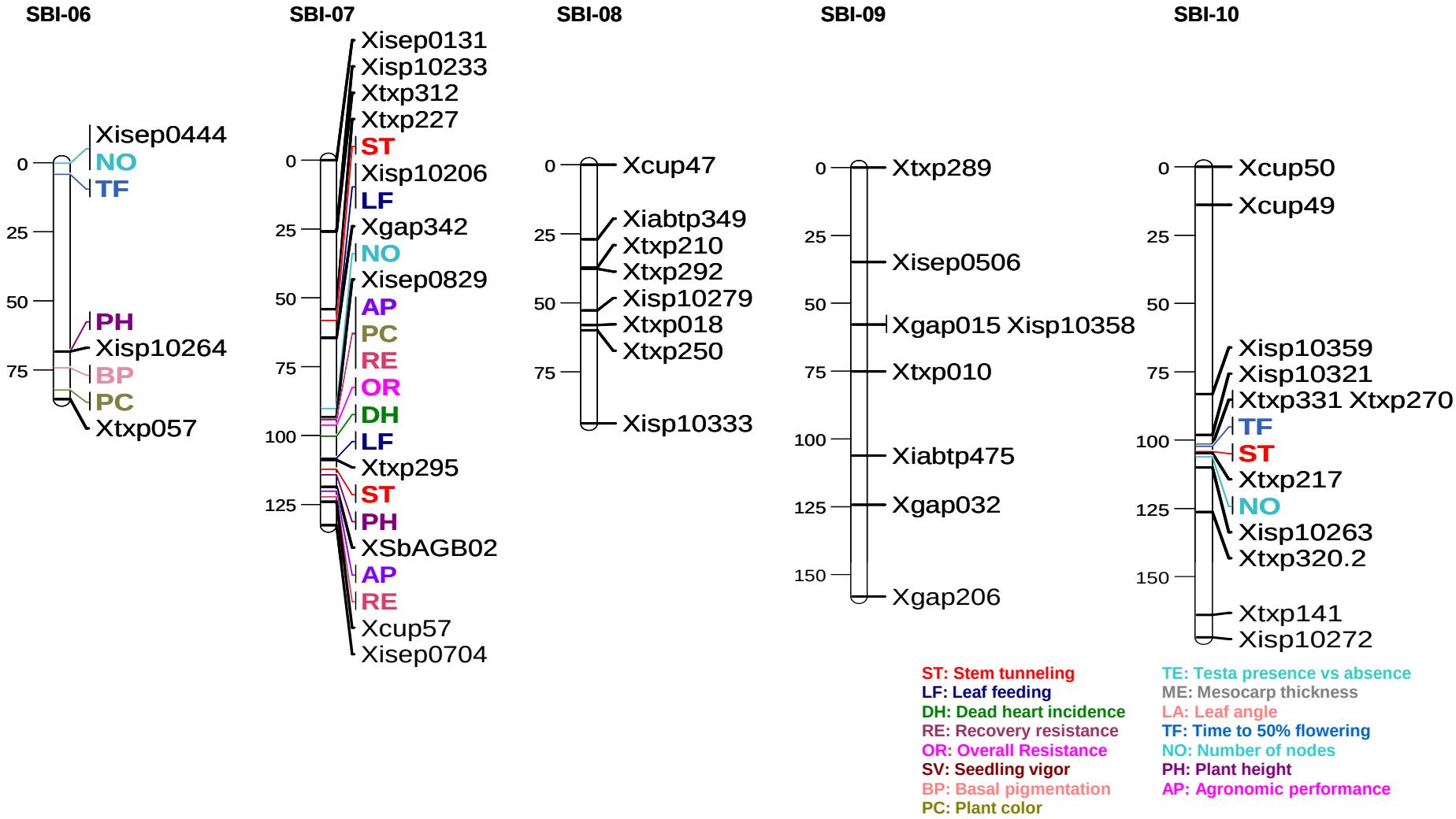


Figure 14 (Contd.,). Linkage map of sorghum (ICSV 745 × PB 15520) - based RIL population depicting the position of QTLs detected using across-season data set

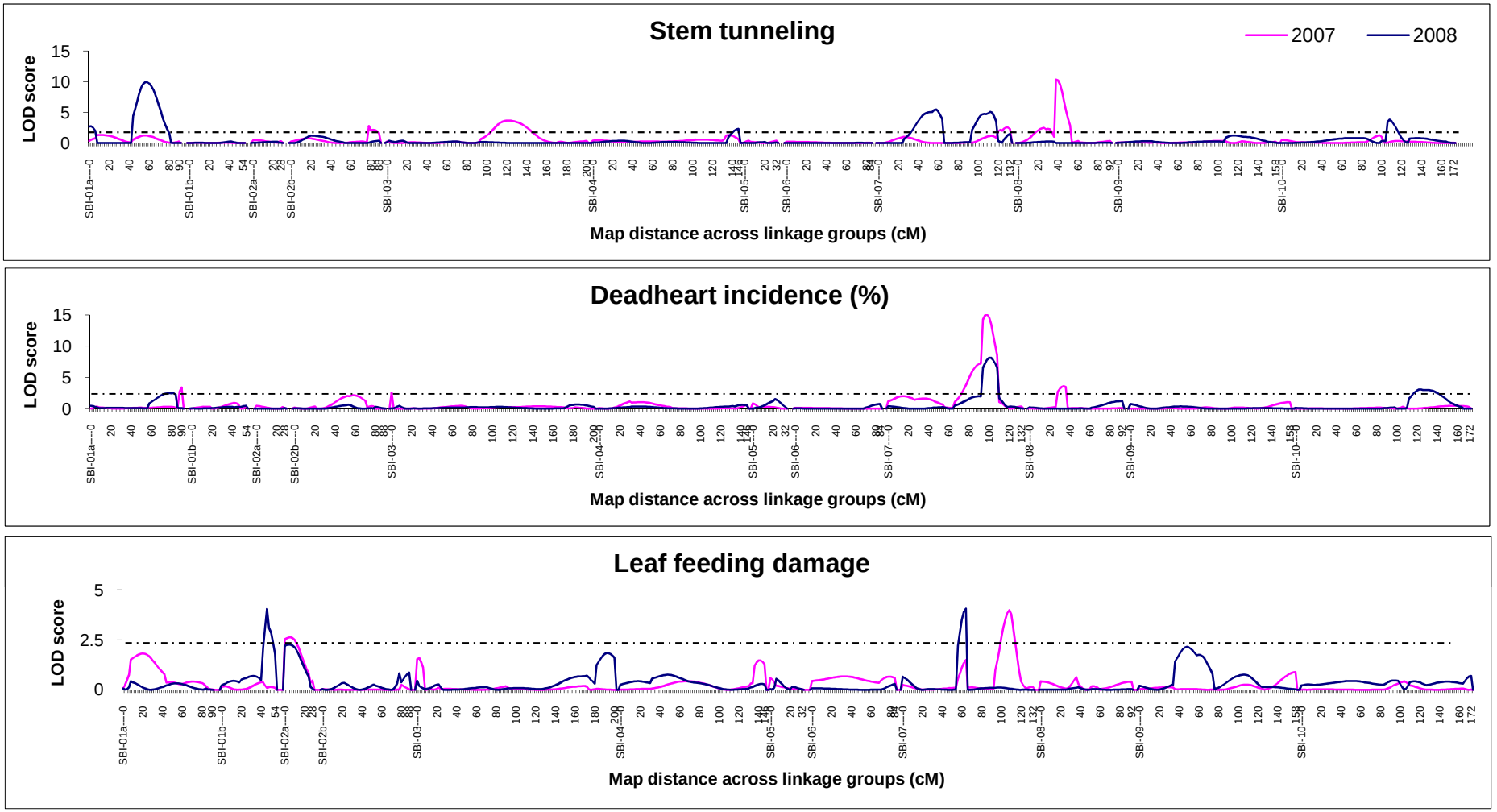


Figure 15. QTL LOD peaks detected for stem tunneling, dead heart incidence and leaf feeding using 2007 and 2008 rainy season data sets

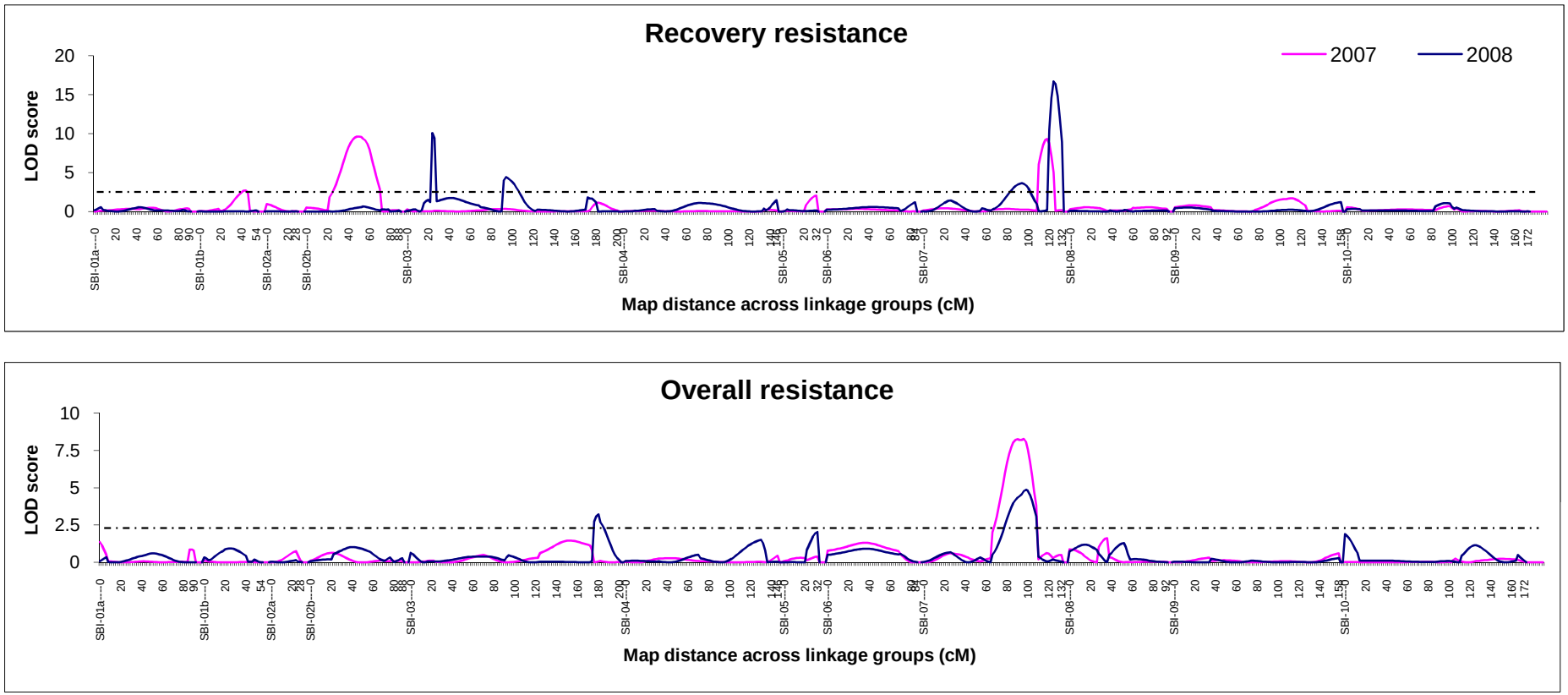


Figure 16. QTL LOD peaks detected for recovery resistance and overall resistance using 2007 and 2008 rainy season data sets

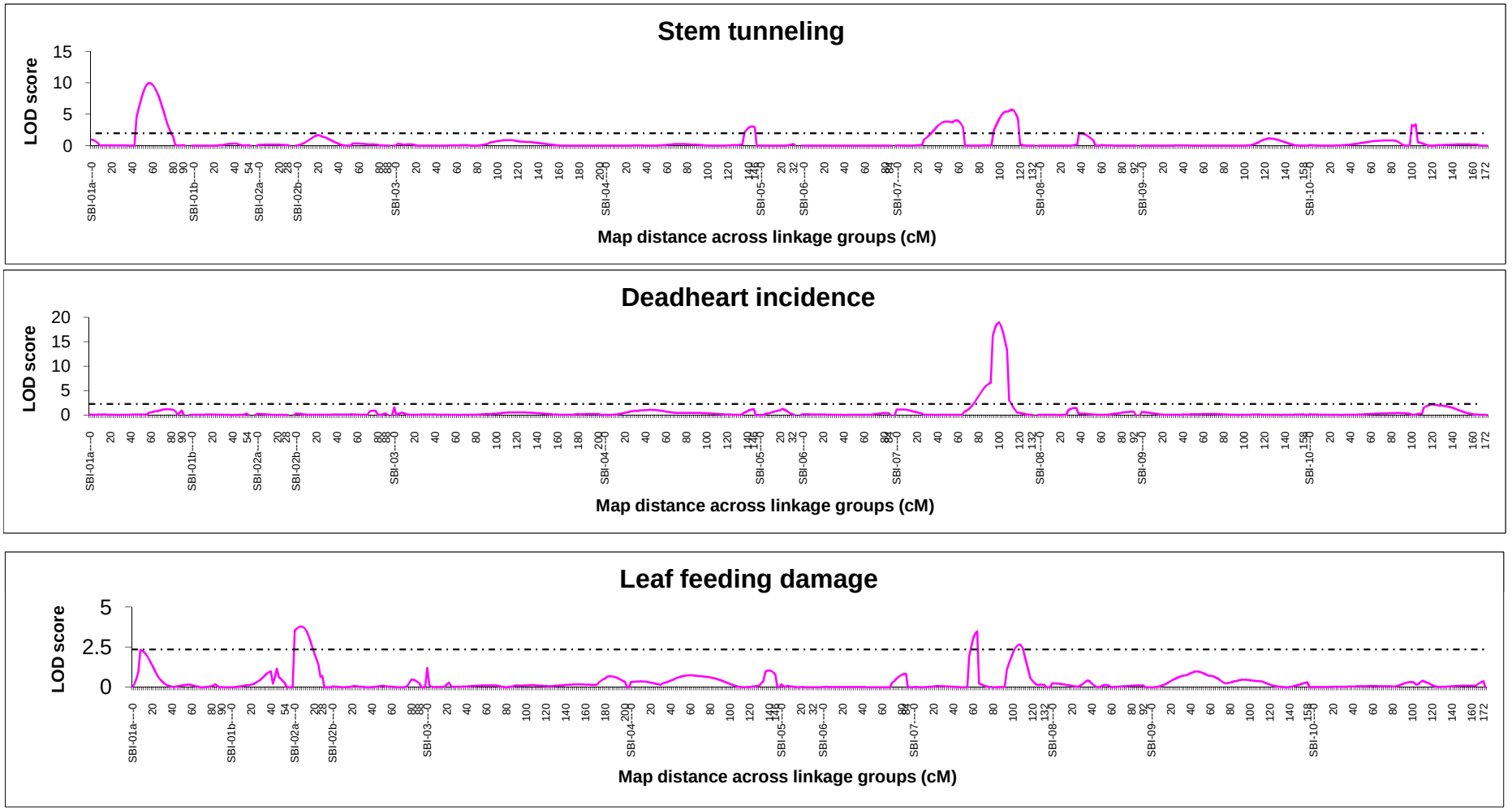
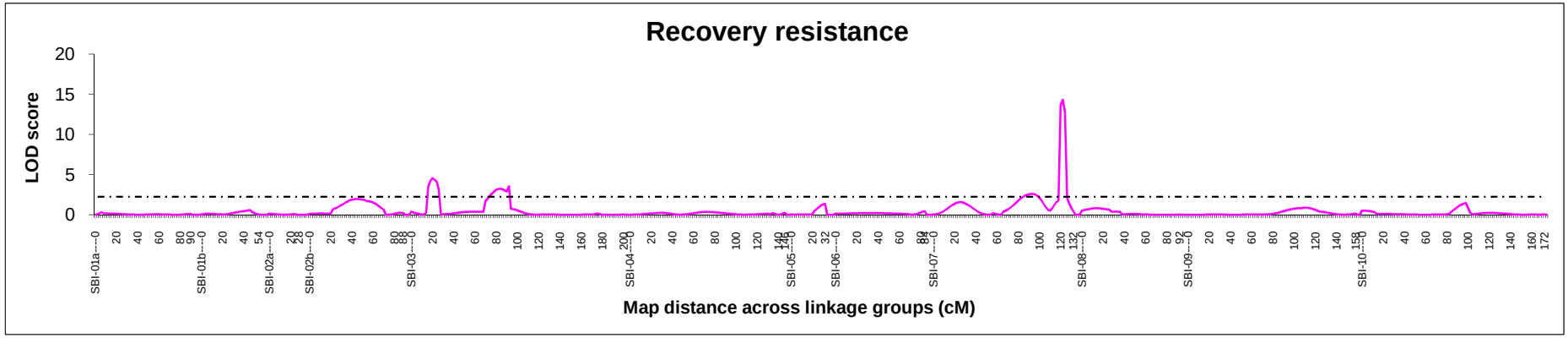


Figure 17. QTL LOD peaks detected for stem tunneling, dead heart incidence and leaf feeding using across-season data set



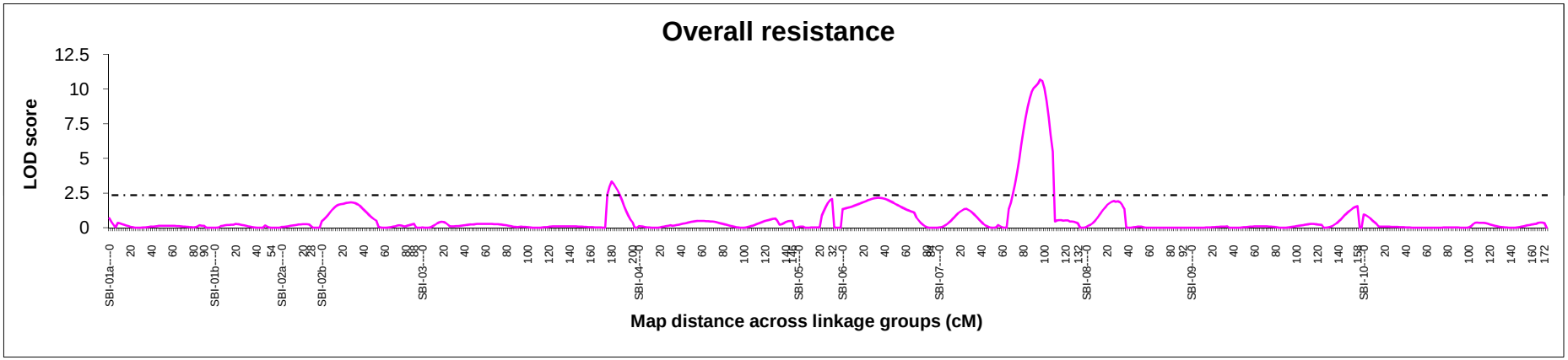


Figure 18. QTL LOD peaks detected for recovery resistance and overall resistance using across-season data set

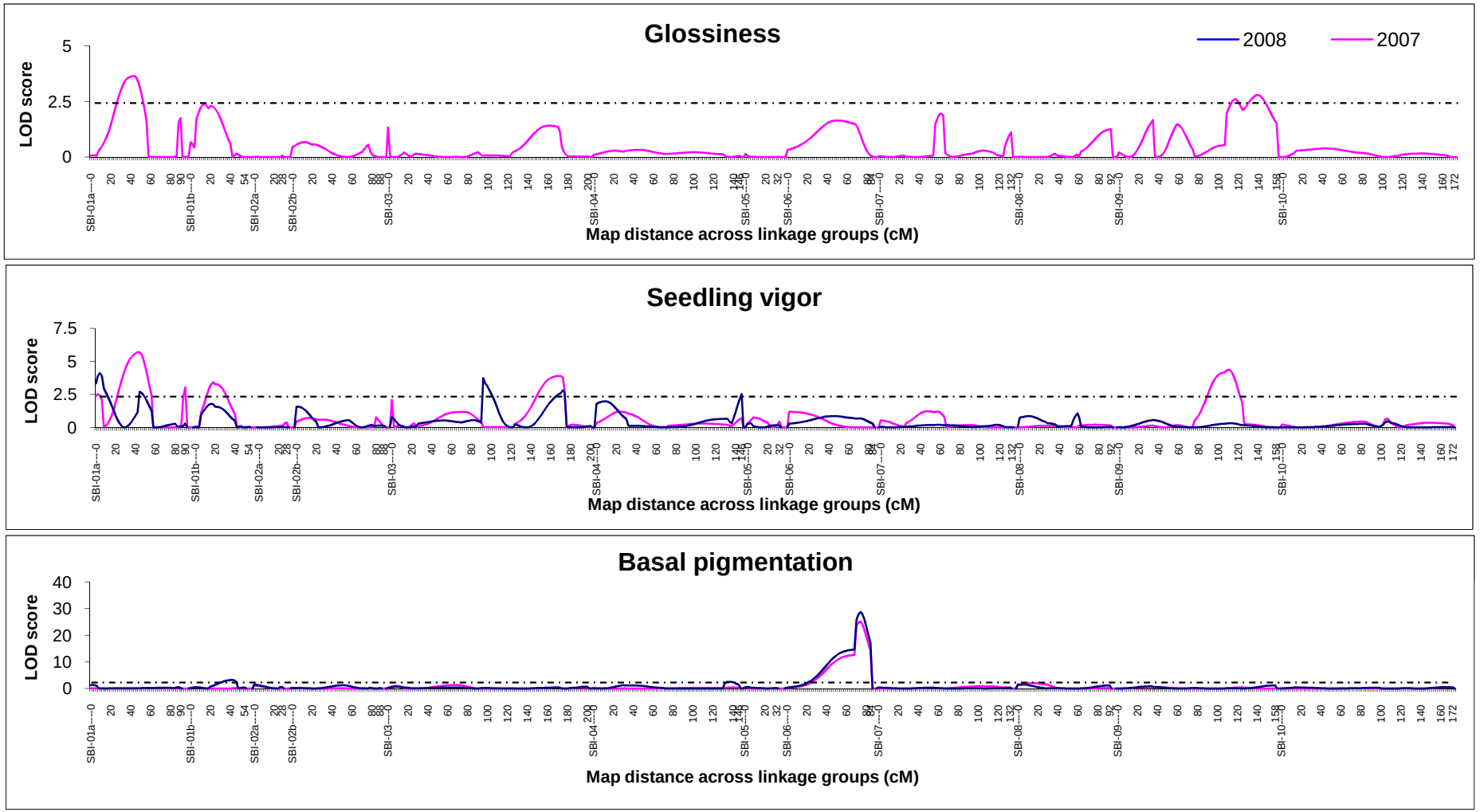


Figure 19. QTL LOD peaks detected for glossiness, seedling vigor and basal pigmentation using 2007 and 2008 rainy season data sets

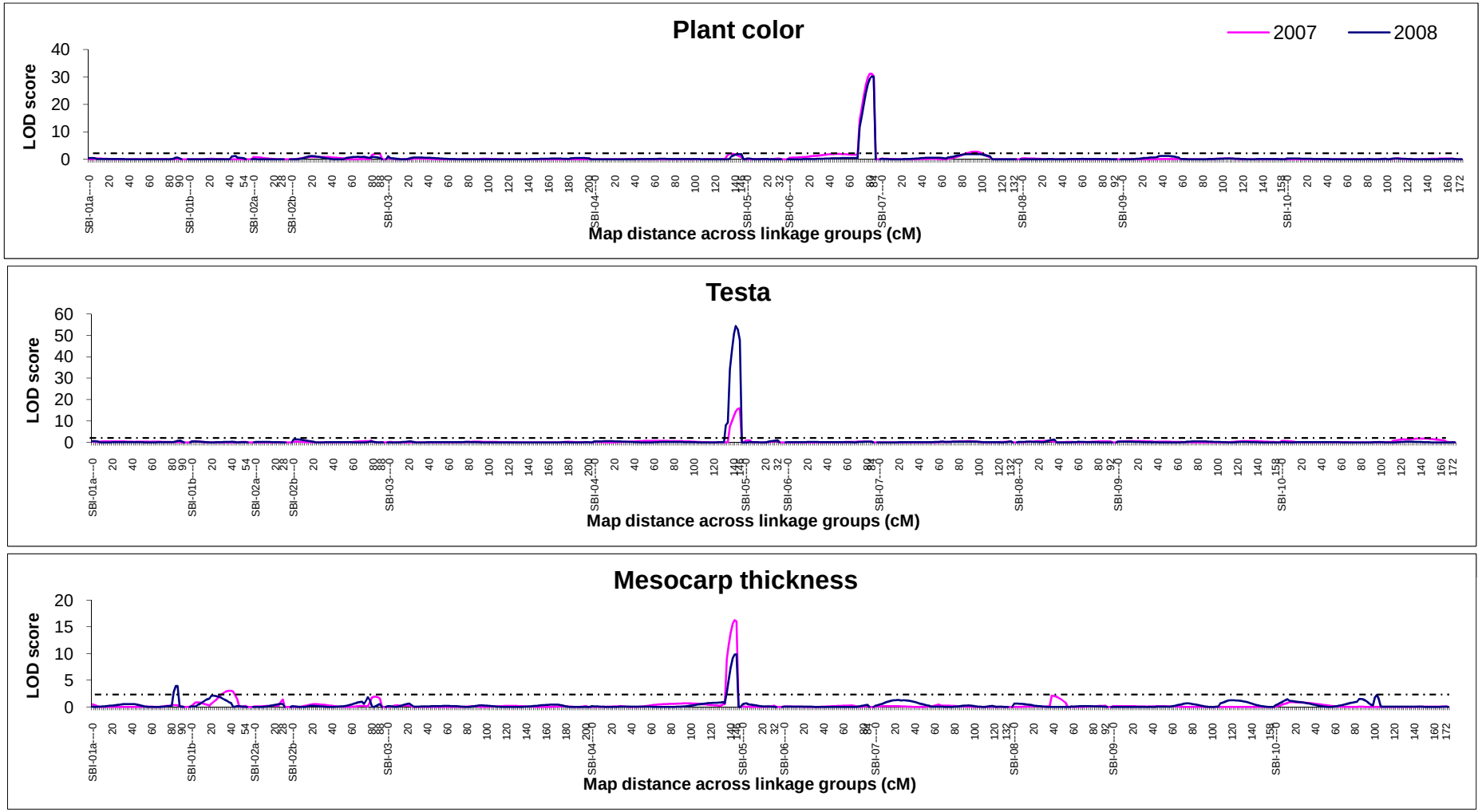


Figure 20. QTL LOD peaks detected for plant color, testa and mesocarp thickness using 2007 and 2008 rainy season data sets

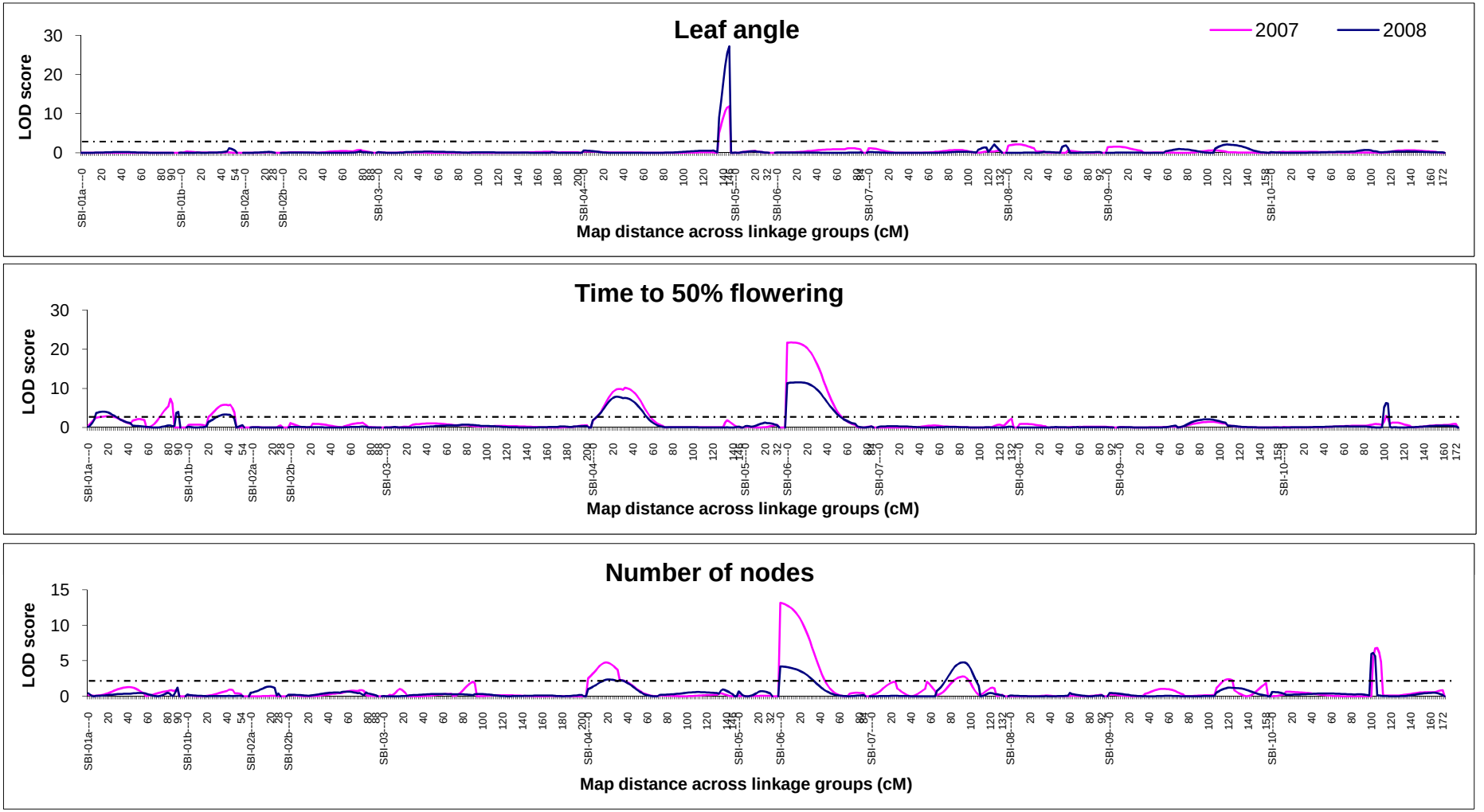


Figure 21. QTL LOD peaks detected for leaf angle,time to 50% flowering and number of nodes using 2007 and 2008 rainy season data sets

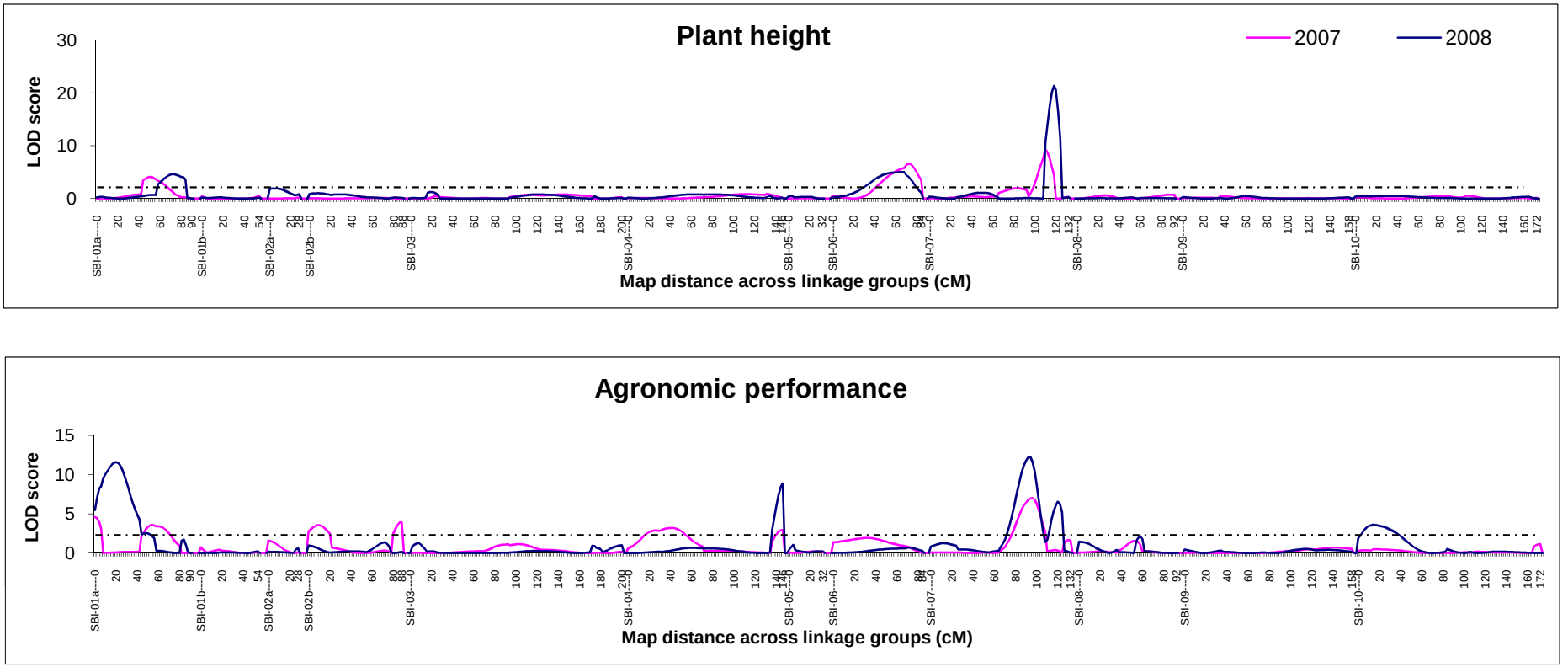


Figure 22. QTL LOD peaks detected for plant height and agronomic performance using 2007 and 2008 rainy season data sets

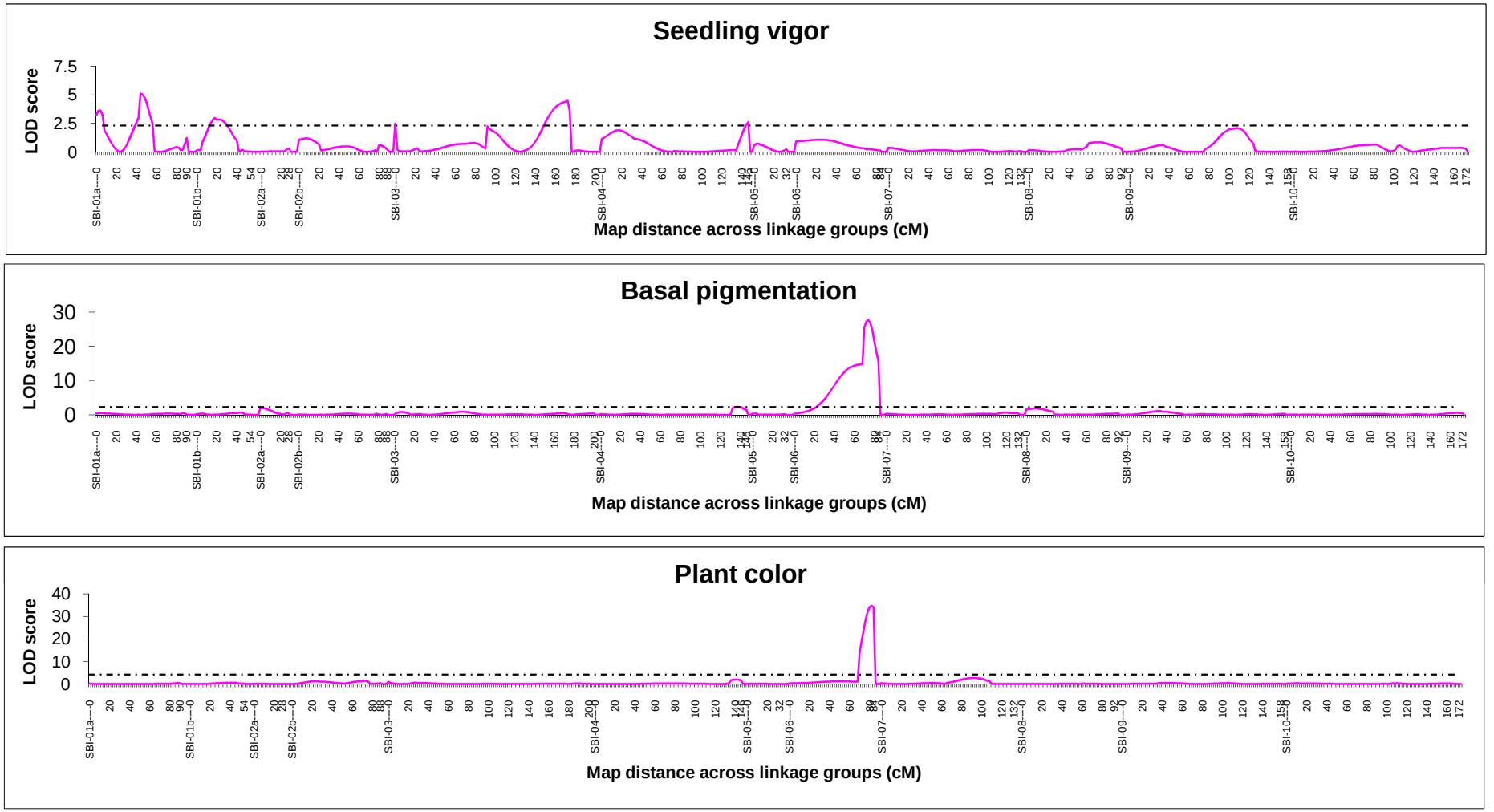


Figure 23. QTL LOD peaks for seedling vigor, basal pigmentation and plant color detected using across-season data set

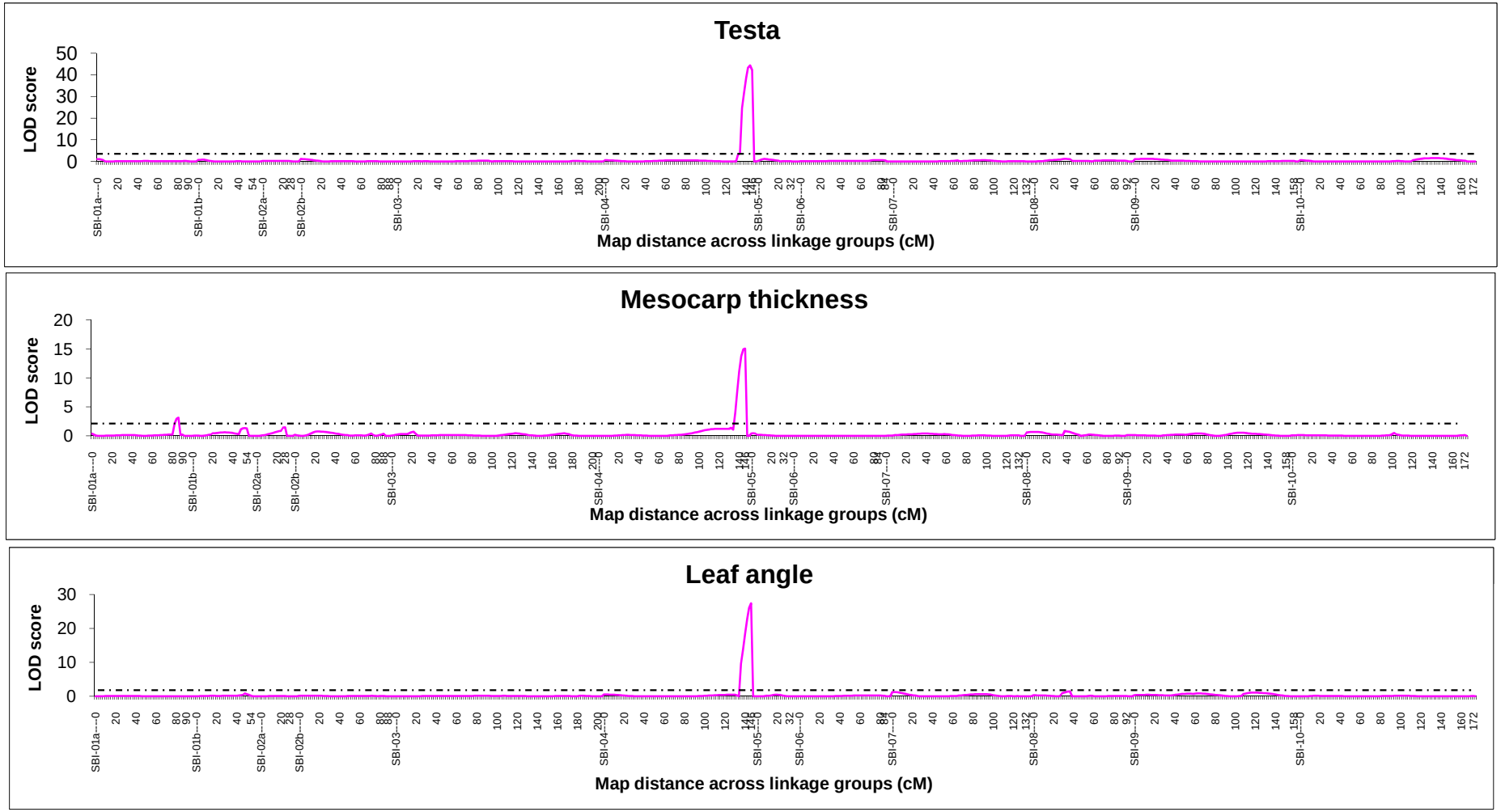


Figure 24. QTL LOD peaks detected for testa (presence vs absence), mesocarp thickness and leaf angle using across-season data set

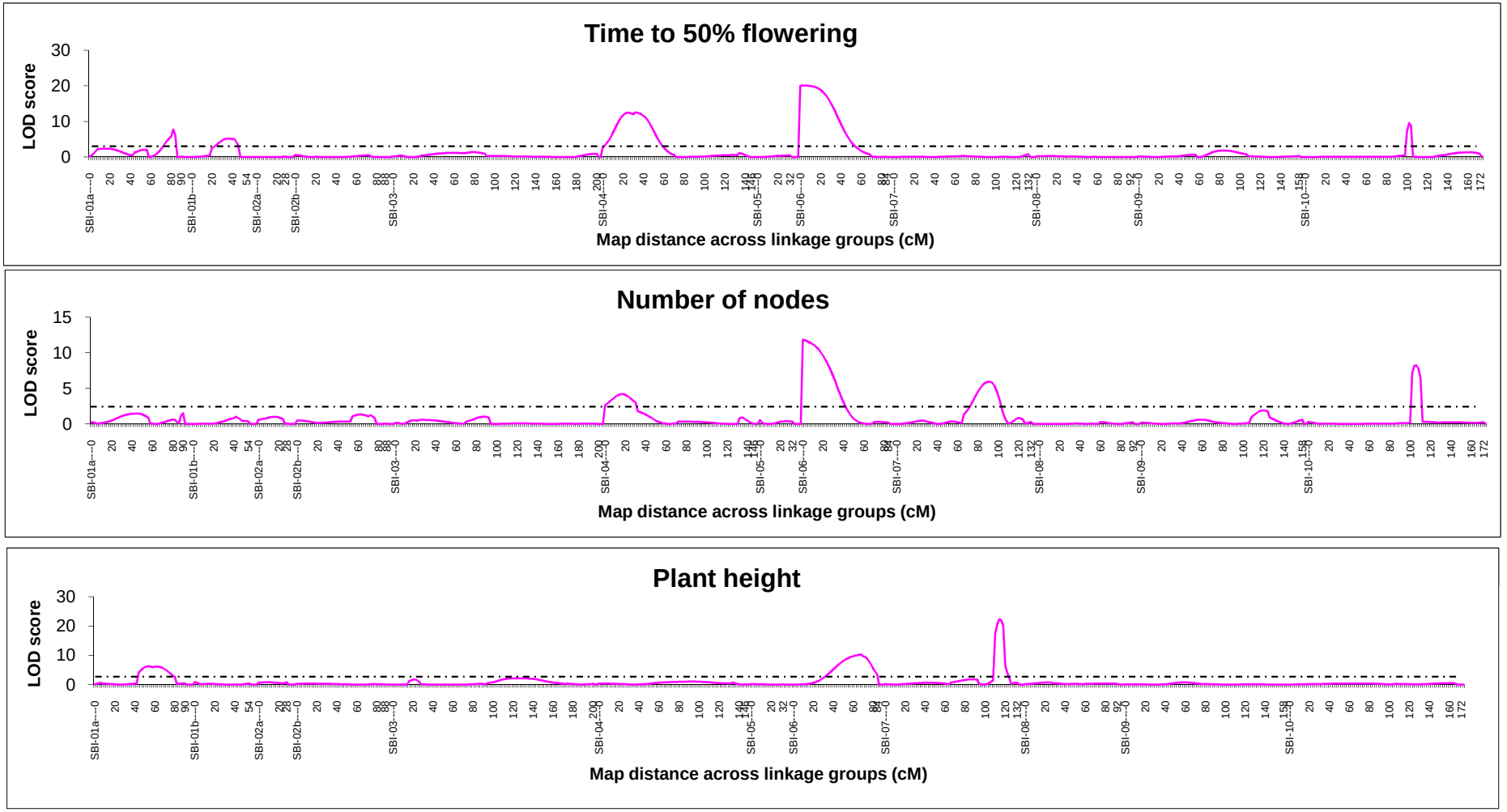


Figure 25. QTL LOD peaks for time to 50% flowering, number of nodes and plant height detected using across-season data set

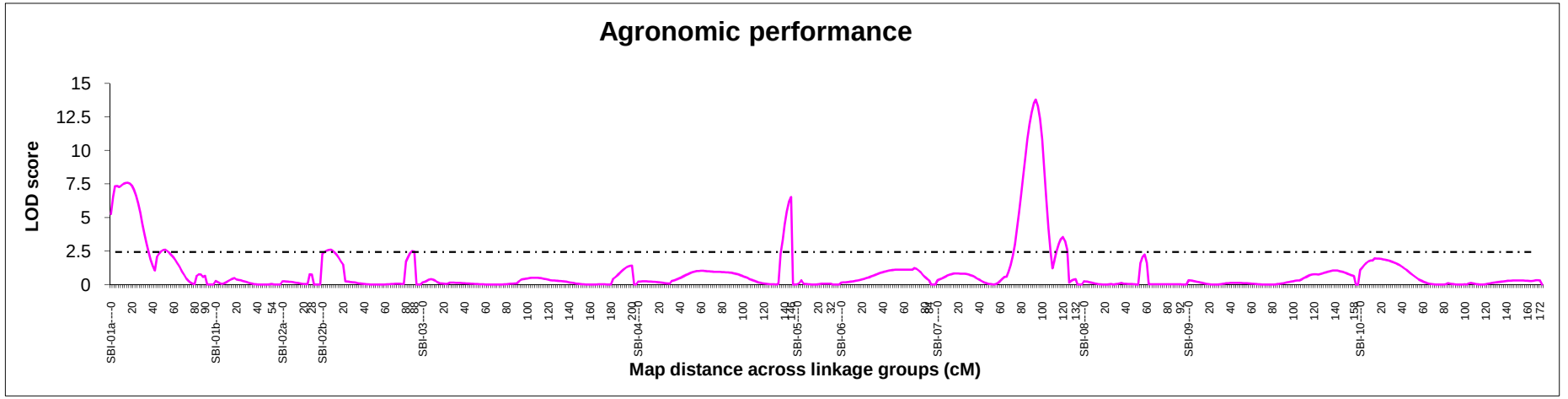


Figure 26. QTL LOD peaks detected for agronomic performance using across-season data set

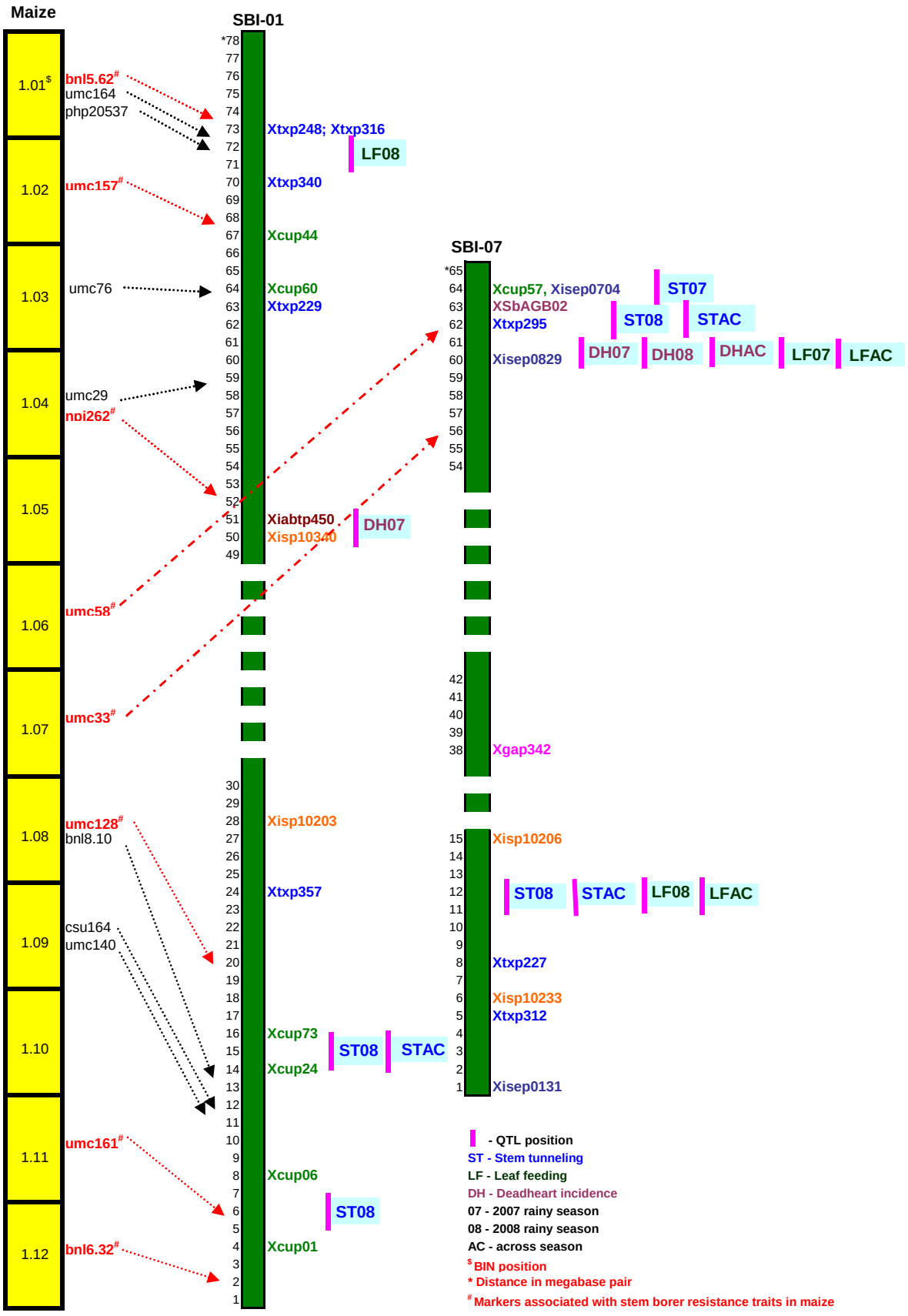


Figure 27. Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,

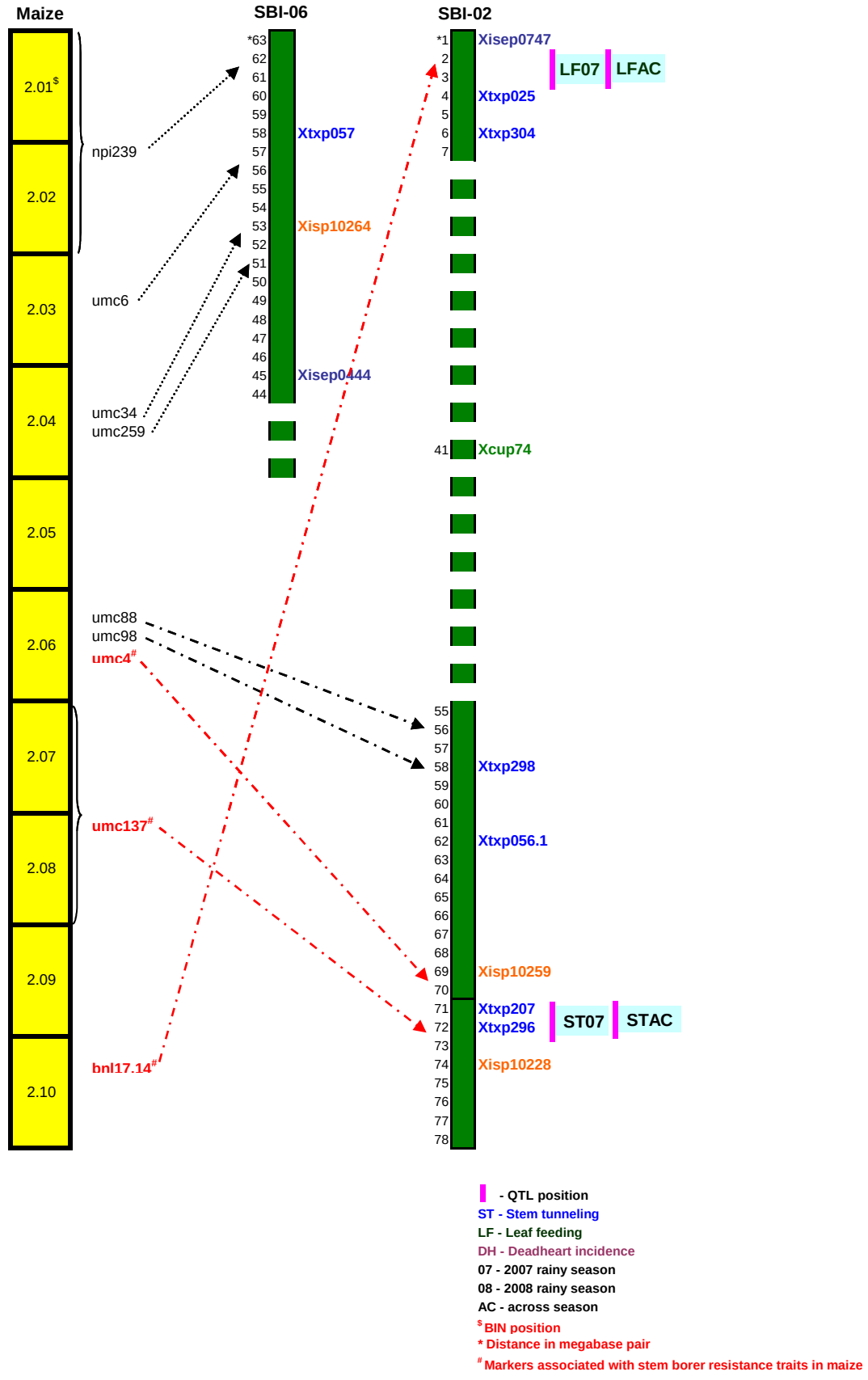


Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,

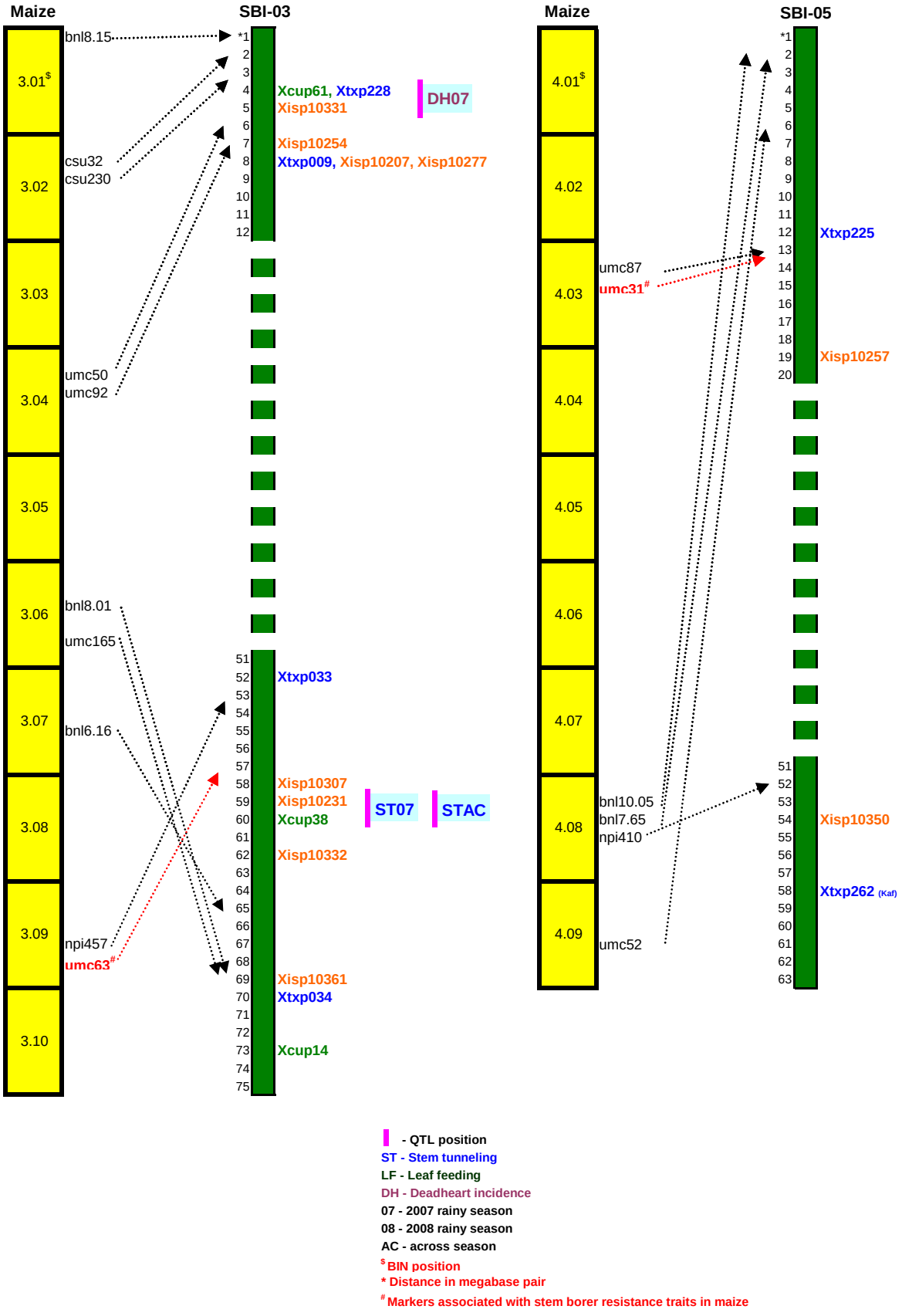
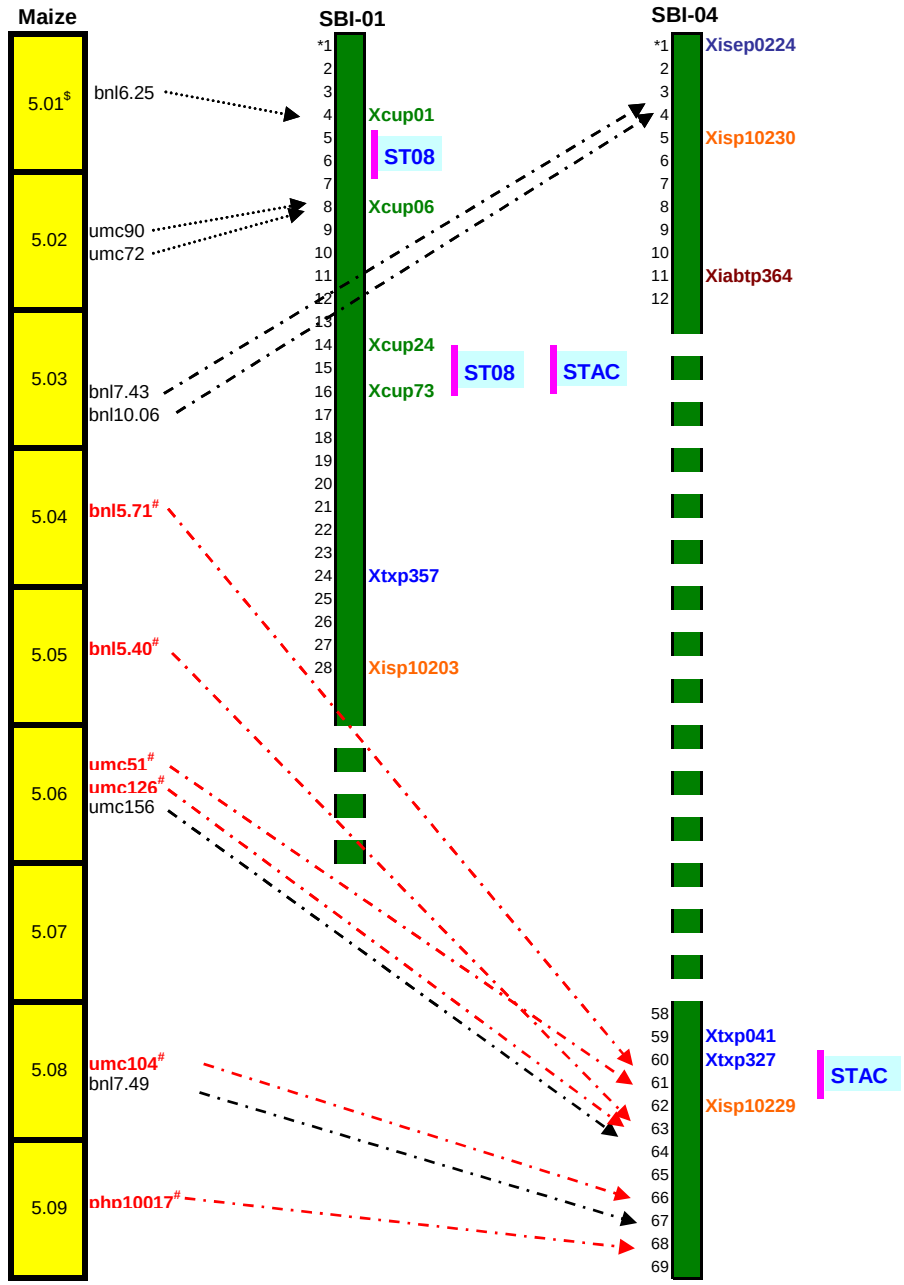


Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,



- QTL position
ST - Stem tunneling
LF - Leaf feeding
DH - Deadheart incidence
07 - 2007 rainy season
08 - 2008 rainy season
AC - across season
$ BIN position
* Distance in megabase pair
# Markers associated with stem borer resistance traits in maize

Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,

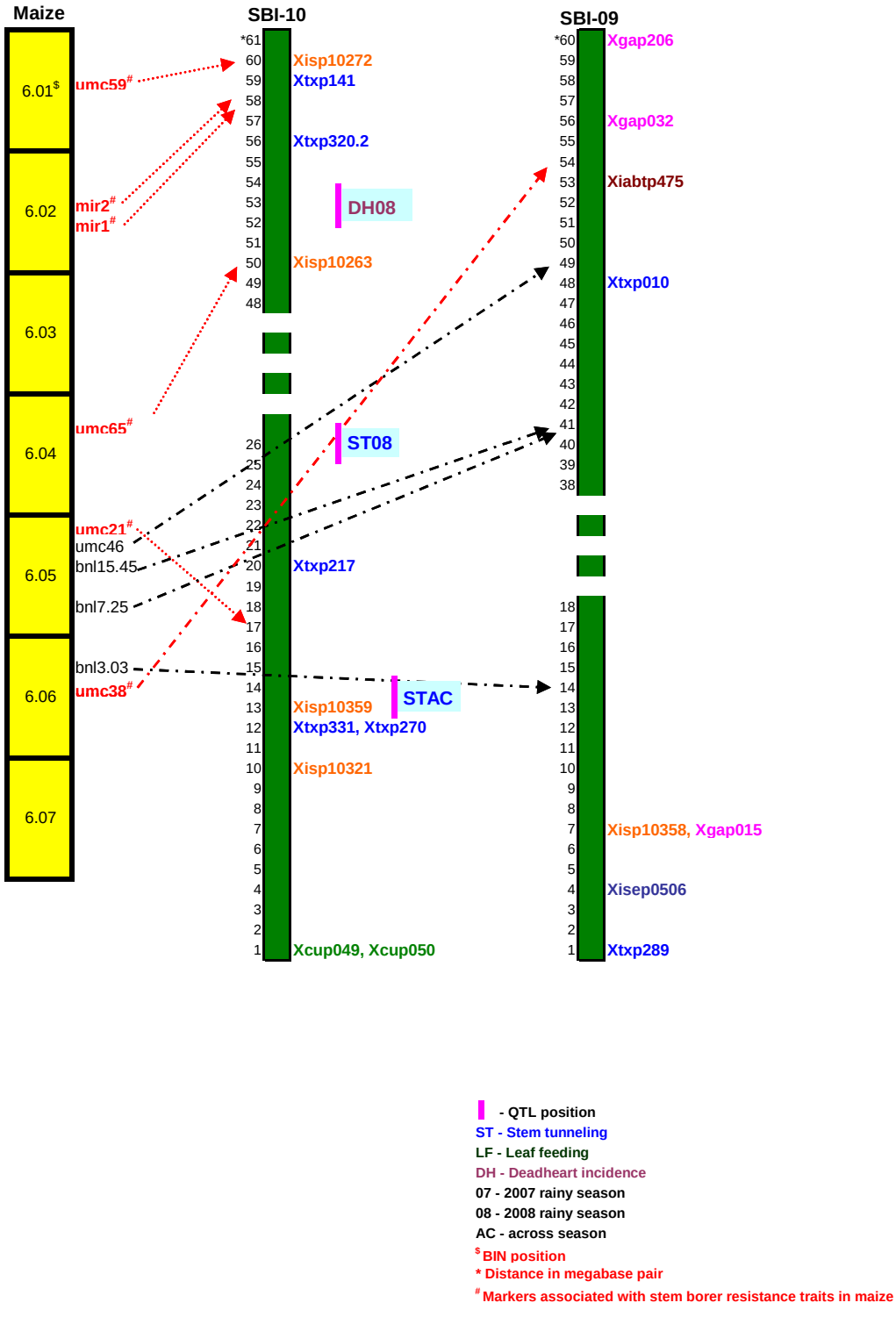


Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,

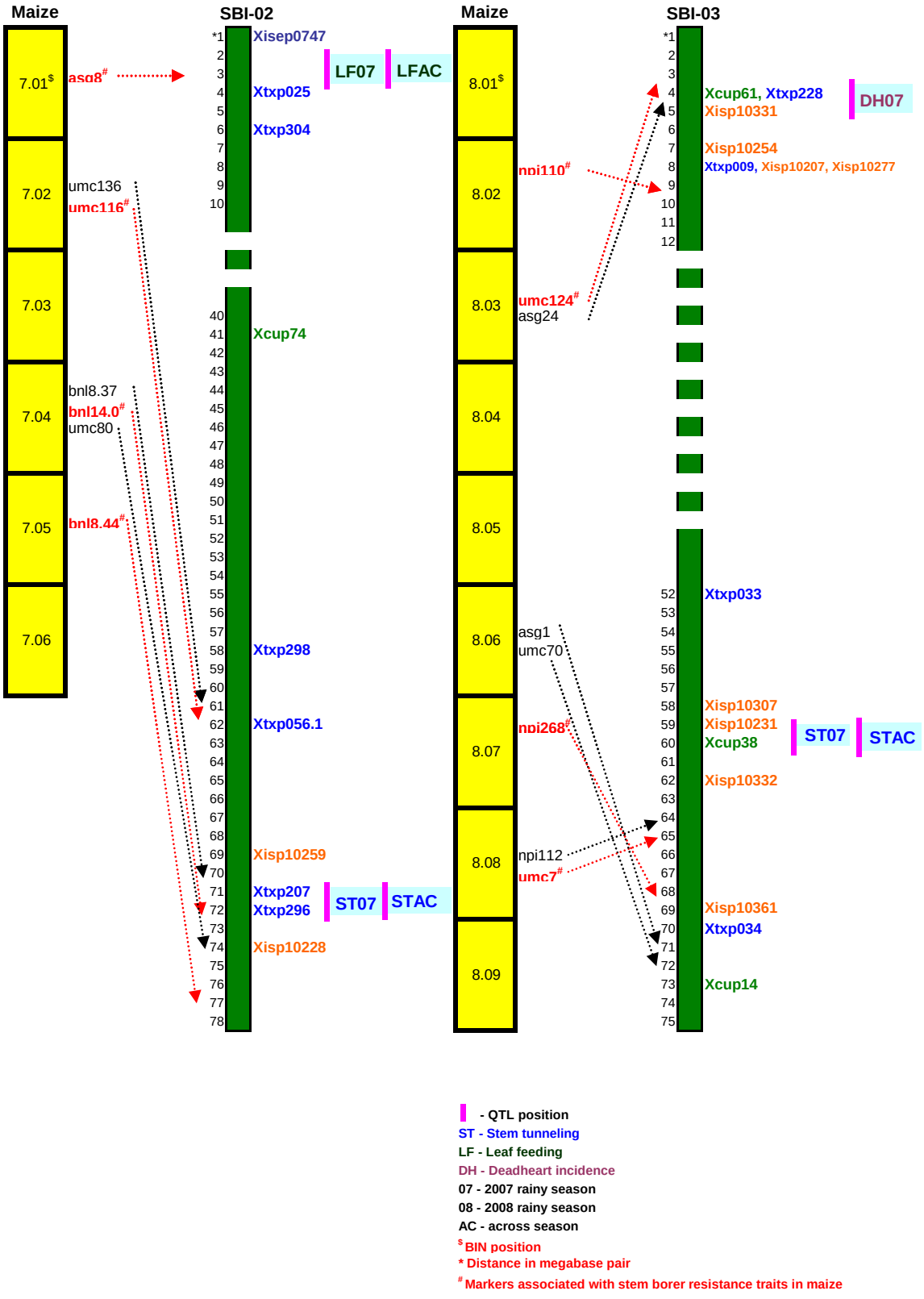


Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

contd.,

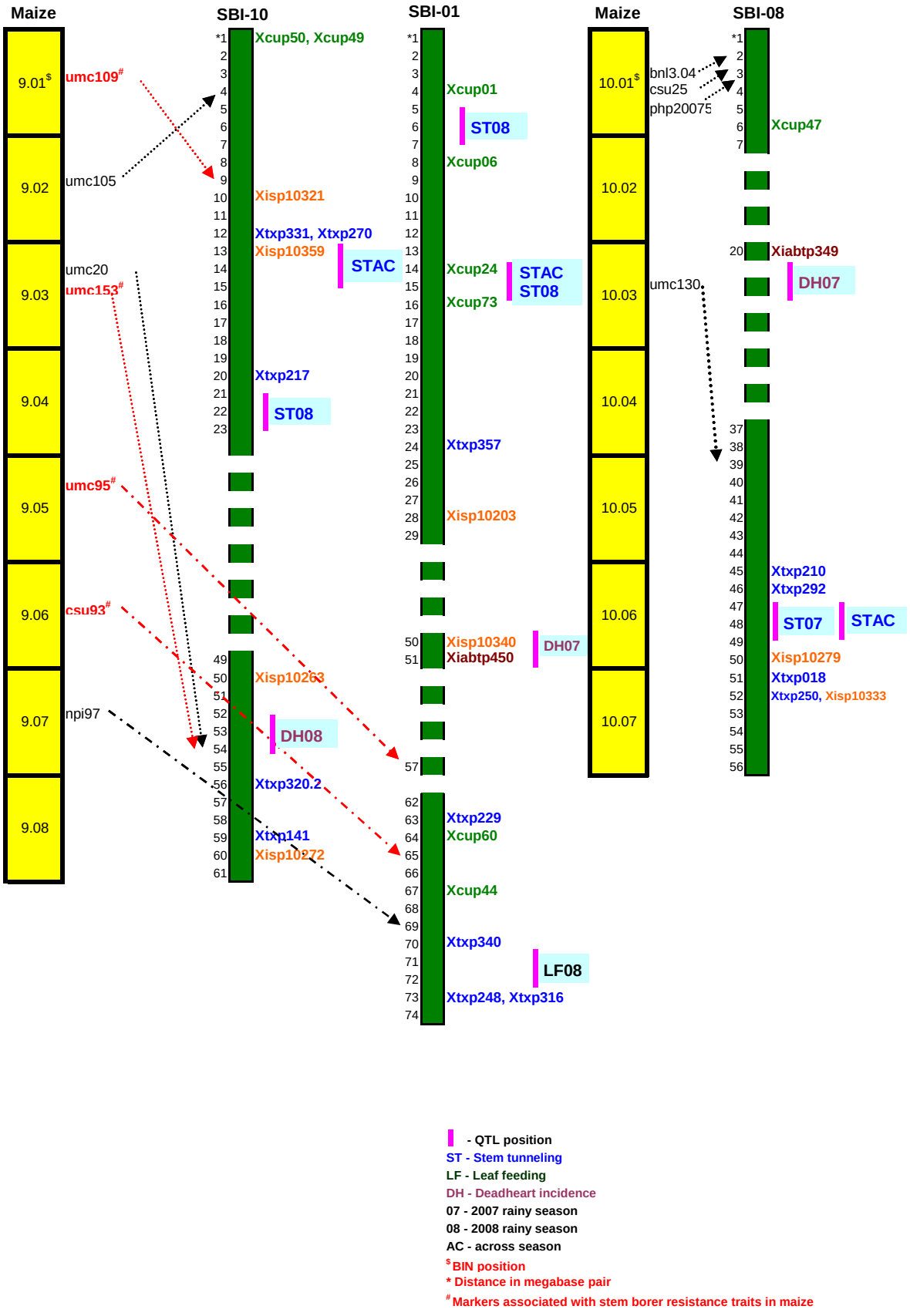


Figure 27 (Contd.,). Comparative map of maize and sorghum genomic regions associated with stem borer resistance component traits

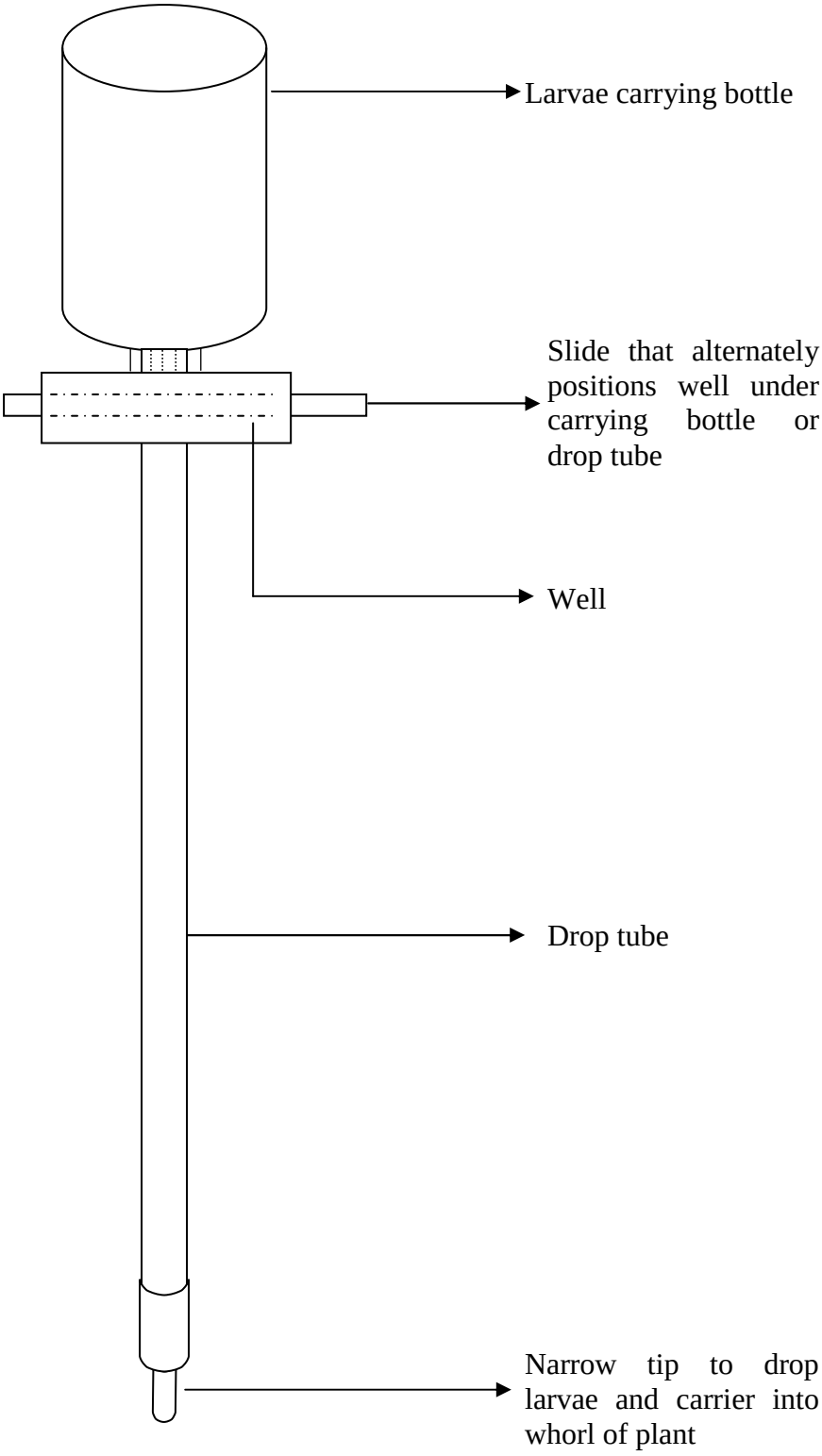


Plate 1. Bazooka applicator for dispensing spotted stem borer neonate larvae

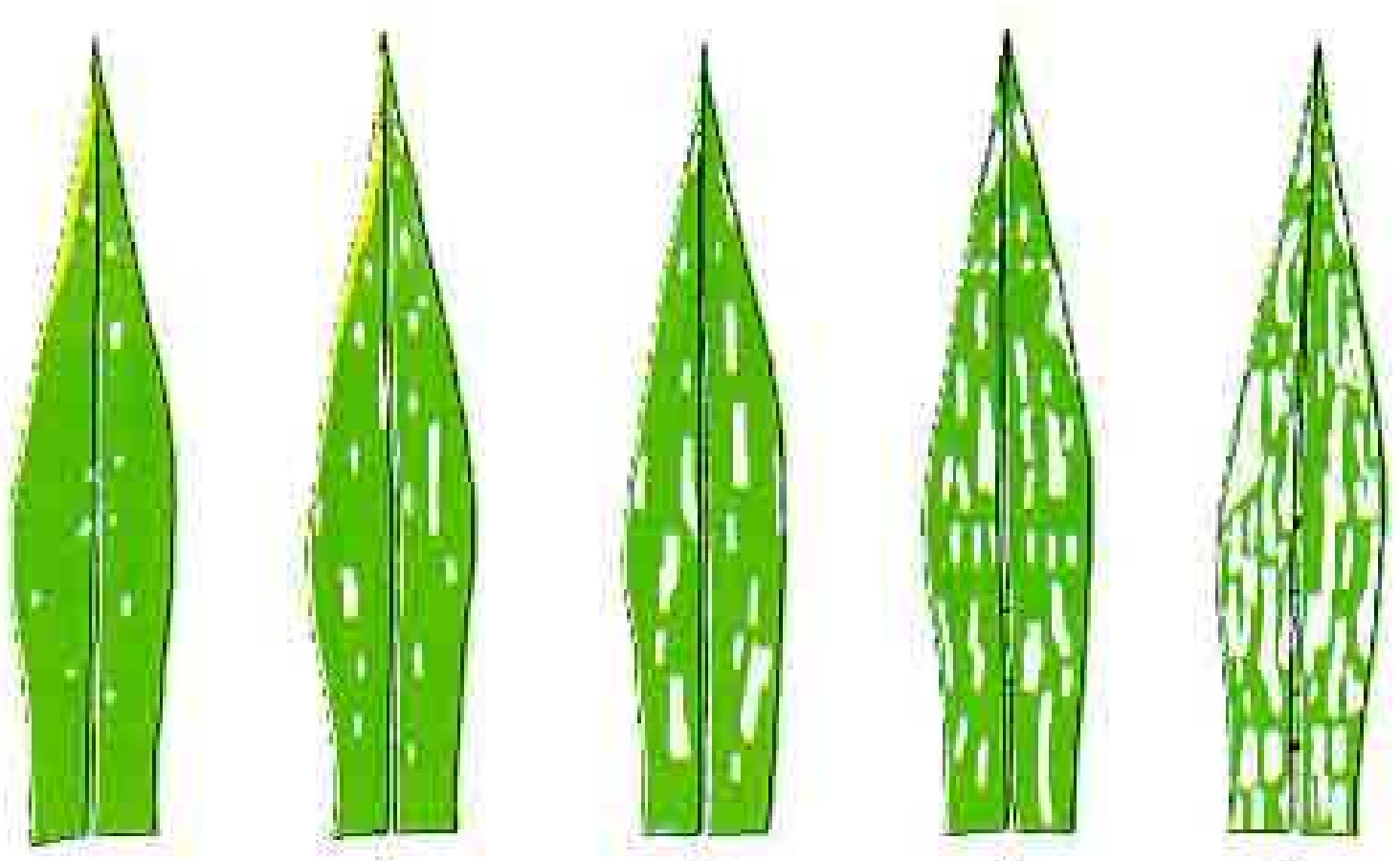


Plate 2. Pictorial representation of leaf feeding damage (score 1 to 9) by spotted stem borer larvae

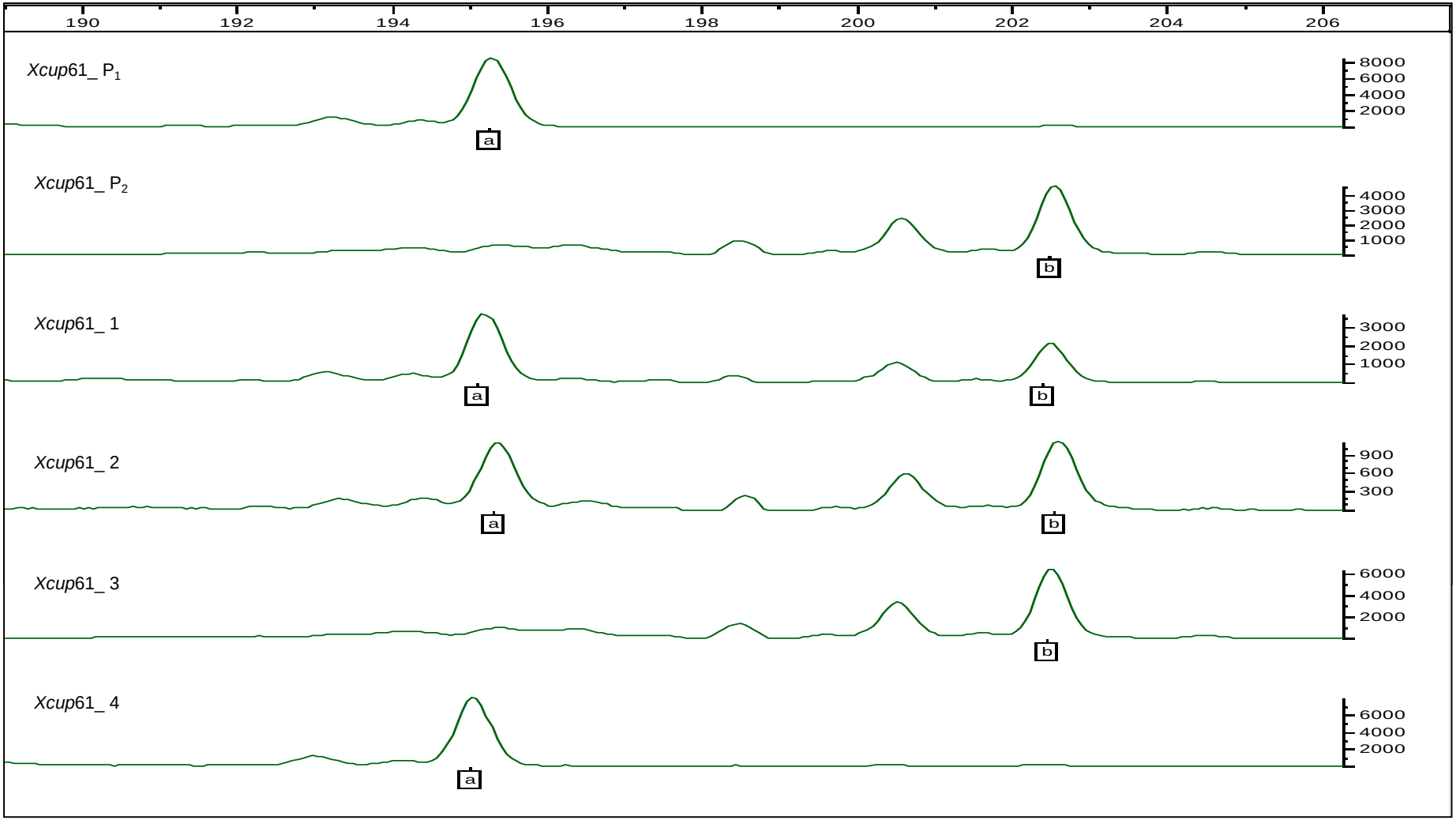


Plate 3. Image of PCR product of SSR marker screened using ABI-3130