Understanding Genetic Control of Biotic Stress Resistance in Sorghum for Applied Breeding

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ABSTRACT

Sorghum yield and production stability are constrained by various biotic stresses such as different insects and diseases. The biotic stresses not only reduce the yields but results in poor grain quality thus hampering its marketability and utilization leading to severe economic losses. Development of host plant resistance is one of the cheapest and sustainable methods for managing the insect pests and diseases. Improvement in stress resistance will increase ecological fitness, reduce pesticide use, and facilitate creation of a sustainable production system with increased efficiency, profitability and to enhance grain quality/end-use traits. An integrated synergistic system involving plant breeding and genomics research using advanced molecular tools could increase the efficiency and precision of crop improvement. This chapter deals with recent developments with regard to sorghum adaptation to different production systems, major biotic stresses affecting sorghum production, understanding genetic control of biotic stress resistance, screening techniques developed, QTLs identified for various stresses and the progress made in cultivar development using this knowledge.

Keywords: grain sorghum, insect resistance, disease resistance, drought tolerance, sorghum grain yield, sorghum grain quality

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9.1 Introduction

Sorghum improvement deals with development of new crop cultivars, which are superior to existing cultivars for traits of interest including high yield, better quality, resistance to pests and diseases and specific usability traits (Reddy et al. 2011; Kumar et al. 2013). Availability of genetic variability for these traits, knowledge about their heritability and genetic control, and availability of effective screening methodologies/phenotyping tools are fundamental for success of any crop improvement program. In sorghum, a large collection of germplasm is available at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (~40,000 accessions) and other places with characterization information available for various morphological, agronomic and adaptive traits. Inheritance of major traits is well studied and phenotyping techniques are developed for efficient selection/screening for major traits of interest. There is continuous exchange of material and information across different research groups. As a result, a large number of sorghum cultivars were developed and commercialized across the world for traits of interest. For example, during the period of 1976 to 2010, a total of 242 sorghum cultivars were released in 44 countries using the ICRISAT-bred sorghum material by private and public sector organizations (Kumar et al. 2011a). The list is quite exhaustive if we consider cultivars developed by other centers in all sorghum growing countries. Focused sorghum improvement programs backed by germplasm sources, information on heritability and gene action for traits of interest, screening techniques, established selection procedures, massive adaptive trials in partners’ locations and above all, collaborative research, contributed for the large scale development and commercialization of improved cultivars in some of the agro-ecosystems. This chapter deals with recent developments with regard to sorghum adaptation to different production systems, major biotic stresses affecting sorghum production, understanding genetic control of biotic stress resistance, screening techniques developed and progress made in cultivar development using this knowledge.

9.2 Adaptation

Sorghum is produced in the rainy (hot) season in most parts of the world for various uses: food, feed, fodder, industrial starch, etc., in the semi-arid tropics of the world whereas in India it is grown in both rainy and post-rainy (cold) seasons (Kumar et al. 2011a). A limited sorghum area (mostly forages) is there in India under the summer season but is small compared to the global area of 40 m ha. Transplanted (known locally as muskware) sorghum is cultivated in areas around the Lake Chad in Nigeria, Chad and Cameroon, but again the area is small. Some of the important biotic stresses
affecting sorghum production across the major adaptations are drought, shoot fly, stem borer and foliar diseases, while some stresses specific to the adaptations are grain mold in the rainy season and charcoal rot in the post-rainy season. Understanding the adaptation and associated stress complex is critical in developing management methods for these stresses.

9.2.1 Rainy Season Sorghum

This is the most important adaptation globally spanning from May/June to August/September with more than 30 m ha sorghum area across various continents falling under this category. A variety of sorghums belonging to different races (direct or hybrid), different cultivar types (mostly hybrids and varieties) and different grain color (red, brown, white, etc.) types are grown for a variety of end-uses in more than 90 sorghum growing countries (House 1985). For an applied plant breeder, the target materials and criterion for selection depends upon the prevailing seed systems and the utilization pattern of the crop and the consumer preference, besides the adaptation traits. For example, medium tall dual-purpose sorghum hybrids with bold white grain are preferred in India for both food and fodder use whereas grain types with red pericarp are preferred for food and brewing purposes in East Africa while tall, long duration guinea white grain sorghums are preferred in West Africa for food. However, both in India and Africa, the white grain types are more acceptable for food purposes. Similarly, medium tall/short red grain sorghum hybrids are preferred in the USA, South America and Australia for mechanical harvesting for use as animal feed. In sorghum, plant height, pigmentation, time to flowering, crop duration, panicle exertion, panicle size, glume coverage, grain number, grain size and color and grain threshability are major selection criteria in addition to the grain yield. In dual purpose types, apart from grain yield, stover yield and quality are also important selection criteria. The important biotic constraints in rainy season sorghum include shoot fly, stem borer, midge, grain mold, striga (primarily in Africa) and among abiotic constraints, drought predominates (Sharma 1985; Thakur et al. 2006).

9.2.2 Post-rainy Season Sorghum

Post-rainy season sorghum is a unique adaptation specific to India with sorghum grown on 4.5 m ha area during September/October to January/February with residual and receding moisture in black soils. The post-rainy sorghum grain is preferred for food use owing to its bold globular lustrous nature. However, as per sensory evaluation test involving staple sorghum consumers, no differences were observed between the flat breads (unleavened) made from rainy (but matured under rain-free condition)
and post-rainy seasons sorghums (ST Borikar pers. comm.). Stover from post-rainy crop is the most important animal feed particularly in the dry periods. In addition to the agronomic traits mentioned under rainy season adaptation, photoperiod sensitivity, temperature insensitivity and grain luster, size and shape are the major selection criteria. Varieties are the cultivar choice but there is a good scope for hybrid development using the white grained rainy season adapted lines as female parents and land race restorers as pollinators. While terminal drought is the major production constraint, shoot fly, aphids and charcoal rot play havoc with post-rainy season production (Sharma et al. 2003; Haussmann et al. 2011).

9.3 Resistance Breeding

Sorghum is affected by various biotic factors, indicated as above leading to severe reduction in productivity and production in different production systems. Development of host plant resistance is the cheapest and sustainable method for managing pests and diseases. Where ever feasible, combining genetic and management methods are more effective in overcoming these constraints.

9.3.1 Genetic Basis for Host Plant Resistance to Insect Pests

Nearly 150 insect species have been reported as pests on sorghum (Sharma et al. 1993), of which sorghum shoot fly (*Atherigona soccata*), stem borers (*Chilo partellus*, and *Busseola fusca*), aphid (*Melanaphis sacchari*), sorghum midge (*Stenodiplosis sorghicola*), and mirid head bugs (*Calocoris angustatus* and *Eurystylusoldi*) are the major pests worldwide. They cause an estimated loss of US$1,089 million in the semi-arid tropics (ICRISAT 1992). Early planting, use of pest-resistant cultivars, inter/mixed cropping, and need-based application are the major components of pest control in sorghum (Sharma 1985). Host-plant resistance is one of the most effective and economic means of pest management in sorghum. It is compatible with other methods of pest control and there is no cost involvement for the farmers (Sharma 1993). Screening for resistance to insects under natural infestation is unreliable, and takes a long time to identify lines with stable resistance to the target pests. Therefore, several field, cage, and screen house techniques have been standardized for evaluating sorghum germplasm, breeding lines, mapping populations and transgenic plants for resistance to different insect pests (Sharma et al. 1992b, 2003).
9.3.2 Sorghum Shoot Fly

Sorghum shoot fly, *A. soccata*, is a major pest of sorghum in Asia, Africa and Mediterranean Europe. The larva cuts the growing point, resulting in wilting and drying of the central leaf, known as a deadheart. The damaged plants produce side tillers, which may also be attacked. The shoot fly population begins to increase in July, peaks in August–September and declines thereafter. The interlard-fishmeal technique is used for increasing shoot fly abundance under field conditions which involves planting four rows of a susceptible cultivar (such as CSH 1, or Swarna) 20 days before the sowing of test material. Moistened fishmeal is spread uniformly 1 week after seedling emergence or kept in plastic bags in the interlards to attract shoot flies from the surrounding areas. Four infester rows should be planted for every 20 rows of the test material. One generation of the shoot fly is completed on interlards, and the emerging flies infest the test material (Taneja et al. 1985a; Sharma et al. 1992b). Data on number of eggs and the plants with eggs, plants with deadhearts should be recorded when there are maximum differences between the susceptible (>80% deadhearts in Swarna) and resistant (<40% deadhearts in IS 18551) checks, or record data twice at 14 and 21 days after seedling emergence. Also record the number of tillers, and tillers with panicles at maturity as a measure of genotype’s recovery resistance. Grain yield under protected and unprotected conditions can also be used as a measure of resistance to sorghum shoot fly.

Cultivated germplasm has low to moderate levels of resistance (Sharma et al. 2003), while wild relatives of sorghum have very high levels of resistance to this insect (Kamala et al. 2008). Resistance to shoot fly is due to: (1) non-preference for oviposition; (2) antibiosis; and (3) recovery resistance (tillers produced following deadheart formation in the main plant, which survive to produce a productive panicle). Presence of trichomes (microscopic hairs on the lower surface of leaves) has been found to contribute to oviposition nonpreference (Sharma and Nwanze 1997). Deadhearts, oviposition, leaf glossiness, trichomes on the abaxial surface of the leaf, and several chemicals on the leaf surface and flavonoids are important marker traits to select for resistance for shoot fly (Dhillon et al. 2005; Anandan et al. 2009; Chamarthi et al. 2011a,b). Resistance to shoot fly is both constitutive and inducible (Chamarthi et al. 2012b), is inherited quantitatively, and predominantly controlled by additive gene action. The sources of resistance to shoot fly are genetically diverse (Dhillon et al. 2005; Chamarthi et al. 2012a). To develop shoot fly-resistant sorghum hybrids, both parents should be bred for resistance (Dhillon et al. 2006; Sharma et al. 2007).

While breeding for shoot fly resistance, resistant sources in desirable agronomic background (ICSV 702, ICSV 705, ICSV 708, PS 21318, PS
30715-1 and PS 35805) as well as other sources (IS 18551, IS 2146, IS 1054, IS 2312) were used in crossing programs. Following trait-based pedigree breeding approach, a large number of shoot fly-resistant seed parents for both rainy (ICSA-/B-409 to ICSA-/B-436) and post-rainy season adaptation (ICSA-/B-437 to ICSA-/B-463) were developed (Reddy et al. 2004). More recently, new sources of resistance IS 923, IS 1057, IS 1071, IS 1082, IS 1096, IS 2394, IS 4663, IS 5072, IS 18369, IS 4664, IS 5470 and IS 5636 are in use for development of shoot fly resistant hybrid parents. High yielding, shoot fly resistant hybrid parents were developed and heterotic hybrids were produced using these parents. The need for having shoot fly resistance in both female and male parents for producing shoot fly resistant high yielding hybrids has been demonstrated (Kumar et al. 2008). In sorghum, large a number of Quantitative Trait Loci (QTLs) governing component traits of shoot fly resistance have been identified (Fig. 9-1) by phenotyping and genotyping of biparental mapping populations and developed dense-linkage maps (Satish et al. 2009; Aruna et al. 2011).

At ICRISAT, four validated QTLs (identified from donor IS 18551) imparting shoot fly resistance (QTL A on sorghum chromosome SBI-10, QTL E on sorghum chromosome SBI-07, QTL G on sorghum chromosome SBI-10, and QTL J on sorghum chromosome SBI-05) governing different component traits such as leaf trichome density (QTL G), reduced oviposition and deadhearts incidence (QTL A and QTL E) and leaf glossiness (QTL G and QTL J) were introgressed into two elite genetic backgrounds of BTx623 and 296B through MABC (Ramu et al. 2010). Using these validated introgression lines as donors, the QTLs are currently being transferred to a number of elite cultivars and hybrid parents.

9.3.3 Spotted Stem Borer

Spotted stem borer, C. partellus is a common pest in Asia and East and southern Africa. The first indication of stem borer infestation is the appearance of small-elongated windows (pin holes) in whorl leaves. The third-instar larvae migrate to the base of the plant, bore into the shoot, and damage the growing point resulting in production of a deadheart. Larvae continue to feed inside the stem throughout the crop growth. Extensive tunneling of the stem and peduncle leads to drying up of the panicle, production of a partially chaffy panicle or peduncle breakage. Stem borer infestation starts about 20 days after seedling emergence, and the deadhearts appear on 30 to 40 day old-crop. Screening for resistance to spotted stem borer can be carried out under natural and artificial infestation (Jotwani 1978; Taneja and Leuschner 1985b; Sharma et al. 1992). Several selection criteria have been evaluated to select for resistance to spotted stem borer (Singh et al. 2010).
Several lines with low to moderate levels of resistance have been identified in the cultivated germplasm (Sharma et al. 2003; Muturi et al. 2012a,b). However, wild relatives of sorghum have shown high levels of resistance to *C. partellus* (Kamala et al. 2012). Using the phenotypic tools...
and available genetic information, some improved lines were developed at ICRISAT using pedigree method that showed considerable resistance to spotted stem borer. They include ICSV 700, ICSV 708, ICSV 711, ICSV 714, ICSV 717, ICSV 89008, ICSV 89010, ICSV 93046 and ICSV 25056 to 25162 (Agarwal BL, Sharma HC, Taneja SL, Reddy BVS, and Stenhouse JH, unpubl.). Some cytoplasmic maintainer and male-sterile lines showing resistance towards stem borer, developed at ICRISAT are ICSB 464, ICSB 467, ICSB 468, ICSB 469, ICSB 472, ICSB 473, and ICSB 474 (Reddy et al. 2004).

Resistance to stem borer is conferred by several morphological and biochemical traits (Sharma and Nwanze 1997; Singh et al. 2011). The nature of resistance to stem borer is additive, and partially dominant over susceptibility (Pathak 1985; Pathak and Olela 1983; Rana et al. 1984; Sharma et al. 2007). Inheritance of resistance to foliar damage, deadheart, stem tunneling and number of exit holes has earlier been reported to be governed by additive gene action (Nour and Ali 1998). In a recent study, involving selected parents and hybrids, the general (GCA) and Specific Combining Ability (SCA) estimates suggested that leaf feeding score, number of nodes, overall resistance score, panicle initiation, recovery score and stalk length (dominance type of gene action) have been found to be associated with resistance to spotted stem borer, governed by additive type of gene action, their correlation and direct effects in the same direction, and explained 65.3% of the variation in deadhearts, and thus could be used as marker traits to select and breed for resistance to C. partellus in sorghum (Sharma et al. 2007).

Development of stem borer resistant transgenics is advancing slowly. A construct containing the Cry1Ac gene from Bacillus thuringiensis and a wound inducible promoter mpiC1 has been successfully introduced into a sorghum cultivar at ICRISAT (Girijashankar et al. 2005). The main function of this gene is to produce δ-endotoxin, a crystal protein which is toxic to the larvae of spotted stem borer.

Many studies have been carried out to understand the genetic makeup of spotted stem borer resistance in sorghum through QTL analyses. Vinayan et al. (2010) developed 266 Recombinant Inbred Line (RIL) population from a cross between ICSV 745 and PB 15520 wherein female parent ICSV 745 is susceptible and male parent PB 15520 shows resistance towards spotted stem borer. They used 90 polymorphic Simple Sequence Repeat (SSR) markers spanning 1,289.4 cM distance in all 10 sorghum chromosomes. Altogether 29 QTLs were found to be associated with different traits such as dead hearts, stem tunneling, leaf feeding, recovery resistance and overall resistance were detected across two environments. The putative QTL on SBI 07 was strongly associated with stem tunneling and is stable because of a non-cross over type of interaction, thus providing a targeted marker assisted selection. QTLs for other traits such as seedling basal pigmentation,
plant color, testa pigmentation, mesocarp thickness and leaf angle are also identified on SBI 06 and SBI 04 suggesting the presence of candidate genes on these chromosomes. *In silico* analysis of the regions/QTLs associated with stem borer resistance in sorghum on chromosome SBI 07, SBI 04 and SBI 02 showed homology with maize chromosome 1 genomic regions containing spotted stem borer resistance. Yueying Li et al. (2010) constructed a genetic linkage map with a mapping population developed from a cross between ICSV 745 (susceptible parent) and PB15881-3 (resistant parent) along with mapping of stem borer resistant QTLs in the RIL population.

### 9.3.4 Sorghum Midge

Sorghum midge, *S. sorghicola*, larvae feed on the developing ovary resulting in production of empty spikelets. The damaged panicles present a blasted appearance. Midge damaged spikelets have a pupal case attached to the glumes or have a small exit hole of the midge parasite on the upper glume. Techniques to screen for midge resistance have been described by various authors (Jotwani 1978; Page 1979; Sharma et al. 1988a,b, 1992b). Identification of hot-spot locations is useful to screen for resistance to sorghum midge. Hot-spot locations for sorghum midge are Dharwad, Bhavanisagar, and Pantnagar in India, Sotuba in Mali, FarakoBâ in Burkina Faso, Alupe in Kenya, and Kano in Nigeria. Midge infestations are also high at several locations in Australia, the USA and Latin America.

Phenotyping for midge resistance can be done by using infester row technique and no-choice headcage technique (Sharma et al. 1988b). Percentage chaffy spikelets is the most appropriate to evaluate sorghum lines for midge resistance. The midge infested panicles can also be evaluated at crop maturity visually on a 1 to 9 scale (1 = <10%, and 9 = >80% midge-damaged spikelets). Nonpreference and antibiosis are the major components of host plant resistance to sorghum midge. Several sources of resistance have been identified in the cultivated germplasm (Sharma et al. 1993, 2003). Resistance to midge is governed largely by additive gene action, although non-additive gene actions may also be involved (Sharma et al. 1994).

In a study by Tao et al. (2003) the quantitative trait loci associated with two of the mechanisms of midge resistance, antixenosis and antibiosis, were identified in a Recombinant Inbred (RI) population from the cross of sorghum lines ICSV 745 × 90562. Two genetic regions located on separate linkage groups were found to be associated with antixenosis and explained 12 and 15% variation, respectively, of the total variation in egg numbers/spikelet under no-choice cage technique. One region was significantly associated with antibiosis and explained 34.5% of the variation of the differences in egg and pupal counts in the RI population (Tao et al. 2003). Considerable progress has been made in the identification and utilization
of resistance to this insect. Several cultivars with high yield and resistance have been developed in different plant height and maturity backgrounds. ICSV 197, ICSV 745, ICSV 735, ICSV 758 and ICSV 88032 have high yield potential and at the same time high midge resistance (Agarwal et al. 1987, 1996, 2005; Sharma et al. 1994, 2005). A number of seed parents, ICSA-/B-488 to 544, with midge resistance have been developed for producing midge-resistant hybrids (Reddy et al. 2007).

9.3.5 Head Bugs

Head bugs, Calocoris angustatus and Eurystylus oldi, are serious pests of grain sorghum. The nymphs and adults suck the sap from the developing grain, resulting in tanning and shriveling of the grain. Head bug damage leads to both qualitative and quantitative losses in grain yield (Sharma and Lopez 1990). Head bug damage spoils the grain quality, and renders the food unfit for human consumption. Head bugs damage also increases the severity of grain molds. Techniques to screen for resistance to head bugs have been discussed by various authors (Sharma and Lopez 1992; Sharma et al. 1992a,b, 2003). As in case of sorghum midge, the phenotyping for head bug resistance can be effectively carried out using infester row technique and no-choice headcage technique. Head bug damage can be evaluated by tagging five panicles at random in each test genotype at the half-anthesis stage. Sample the panicles for head bugs at 20 days after flowering or infestation in a polyethylene or muslin cloth bag containing a cotton swab soaked in 2 ml of ethyl acetate or benzene. Count the total number of adults and nymphs. Evaluate head bug damage at maturity on a 1 to 9 scale (1 = all grains fully developed with a few feeding punctures, and 9 = most of the grains highly shriveled and almost invisible outside the glumes). Resistance to head bug was found to be dominant over susceptibility and dominance gene action is more important for the three resistant traits: grain damage rating, floaters percentage, germination percentage. Inheritance of resistance to C. angustatus is governed by additive gene action (Sharma et al. 1998), and to E. oldi by one dominant gene in two F2 populations, while resistance in the remaining two F2 populations is controlled by two dominant genes and in part by genes at two or more loci (Showemimo 2004). Three significant and seven putative QTLs conditioning head bug resistance was reported earlier (Deu et al. 2001, 2005). The significant QTLs, which explained an important part of the phenotypic variation, were placed on the genetic map. One of the QTLs that accounted for 13% phenotypic variance was mapped on Linkage Group 2 (Deu et al. 2001, 2005). Efforts have been made to develop cultivars and hybrid parents resistant to head bugs, and the seed ICSA-/B- 545 to 565 developed at ICRISAT showed considerable resistance to head bug infestation (Reddy et al. 2007).
9.3.6 Greenbug and Sugarcane Aphid

Greenbug, *Schizaphis graminum* (Rondani), has been a serious insect pest of small grains in the United States for many decades and a key insect pest of sorghum. This aphid sucks juice from and injects toxins into sorghum plant tissues and consequently causes damage to the plants (Teetes and Pendleton 2000). Greenbugs have a wide host range, occurring across all continents. As a result of the variable options of adaptation, various biotypes of greenbugs have evolved, and biotypes C, E, I, and K are virulent on sorghum in nature (Harvey et al. 1997). In the US, host plant resistance has been proven to be an effective means of controlling this pest, and resistant sorghum hybrids and cultivars have been used to avoid/reduce damage by greenbug (Huang 2011). More importantly, new sources of resistance to the key pests must be found continuously and incorporated into high-performance breeding lines for cultivar/hybrid development. Continuous improvement in crop defense against greenbugs is indeed dependent on the availability of the diverse genetic resources and judicious use of effective sources of resistance.

In order to conduct high-throughput evaluation, we have developed mass-screening techniques for evaluating sorghum germplasm for resistance to greenbugs, which involves screening at the seedling stage (Huang 2007). Recently, we have completed the evaluation of a large collection (approx. 42,000 accessions) of sorghum germplasm from various locations of the world for their response to greenbug feeding in a greenhouse of the USDA-ARS Plant Science Research Laboratory, Stillwater, Oklahoma. As a result, 21 germplasm accessions were identified to possess resistance to the greenbug (Huang 2011). In addition, genetic diversity of these resistance accessions was assessed using DNA markers Amplified Fragment Length Polymorphism (AFLP) to be precise. A high level of genetic variation was observed among these genetic sources and there is a broader genetic base in the germplasm collection than those resistance sources used in the current sorghum breeding programs (Huang 2011; Wu et al. 2006). These newly identified sorghum accessions resistant to greenbug offer additional sources to the sorghum breeding programs.

Information on the genetics of resistance is very useful to breeders for choosing breeding materials and deciding on breeding strategy and methodology to be adopted for their breeding programs. Diverse genes for resistance are needed to cope with the development of new biotype populations and to attain regional deployment of resistance genes. As for the genetics of resistance, classic genetic analysis using phenotypic data demonstrated that the inheritance of sorghum resistance to greenbug biotypes was relatively simple. Weibel et al. (1972) reported that inheritance of biotype C resistance was controlled by a single incompletely dominant gene. In other cases, authors reported one to five resistance genes from
different sources complementing each other while conditioning resistance (Olonju Dixon et al. 1990). Another study indicated that resistance to greenbug biotype I is incompletely dominant and controlled by two genes which may rely on complementary gene action (Tuinstra et al. 2001). Using the Composite Interval Mapping (CIM) and Multiple Interval Mapping (MIM), the authors have detected at least two QTLs on sorghum chromosome nine (SBI-09) responsible for the host plant resistance the greenbug (Wu and Huang 2008; Punnuri et al. 2013). One of the QTLs is a major one accounting for 55–80% of phenotypic variance while the second one accounts for 1–6% of variance for the resistance to green bug feeding (Wu and Huang 2008). There is good scope for Marker-Assisted Selection (MAS) for developing cultivars with greenbug resistance using these QTLs.

Furthermore, plant genomics has proven to be the great new tools for both efficient identification of resistance gene(s) and a better understanding of the host resistance mechanisms. For example, cDNA microarray technology has revealed the transcriptional changes in sorghum seedlings based on the results from a parallel system, greenbug resistant and susceptible genotypes, leading to the detection of the 157 differentially-expressed transcripts that responded to infestation by greenbug biotype I (Park et al. 2006). These experiments showed comprehensive gene activities resulted from upregulating or activating existing defense pathways in sorghum seedlings in response to greenbug feeding. For further analysis, the genes that showed differential expression were cloned and sequenced. The resultant cDNA sequences were then annotated by comparison to the GenBank databases using the BLASTX search program. Sequence similarity search allowed putative functions to be assigned to 16 cDNA clones/genes that are directly or indirectly involved in host defense against greenbug attack. The detailed studies also suggested the defense responses against greenbug in sorghum are coordinately modulated by versatile molecular regulators such as salicylic acid, jasmonic acid, abscisic acid and phytohormones (Park et al. 2006).

Sugarcane aphid, *M. sacchari* is a serious pest of sorghum in Asia and Africa. It feeds on the under surface of leaves and secretes honeydew. Aphid infestation is high during severe moisture deficit conditions. The infestation starts from lower leaves and proceeds upwards. Under severe infestation, the plants become pale yellow, with soot molds, wither and dry up. Infestation becomes severe by panicle initiation stage. Screening for resistance to aphids can be carried out under natural infestation in the field or infesting the test material under greenhouse conditions using uniform number of insects per plant at the flag leaf stage (Sharma HC unpubl.). Sources of resistance have been identified in the cultivated germplasm (Sharma and Dhillon 2005). Genetic control of aphid resistance is not fully
understood, but it is quantitatively inherited and the variability for the trait is low (House 1980). A set of 30 lines comprising of germplasm and improved maintainer and restorer lines was screened for resistance to sugarcane aphid, *M. sacchari* at ICRISAT center, Patancheru; DSR, Sholapur; MAU, Parbhani, and MPKV, Rahuri. Under natural infestation, the aphid damage scores ranged from 2.33 to 9.0, and the genotypes ICSB 321, ICSB 323, ICSR 165, 61523, 61588, RAS 25 and DSV 5 exhibited moderate levels of resistance (damage rating of <4.0 compared to 9.0 in the susceptible check, Swarna) (Sharma HC unpubl.).

### 9.4 Genetic Basis for Host Plant Resistance to Diseases

A number of diseases are of major concern in sorghum producing areas across the world. Most important among these are the grain molds, downy mildew (*Peronosclerospora sorghi*) and charcoal rot (*Macrophomina phaseolina*). Anthracnose (*Colletotrichum graminicola*), downy mildew, and maize dwarf mosaic virus are the more important diseases in the Americas. Breeding for resistance is the best method of disease control especially for crops such as sorghum being grown by resource poor farmers (House 1985).

#### 9.4.1 Grain Mold

Grain mold is an important biotic constraint to the sorghum production in Asia and parts of Africa, especially when the rains coincide with grain development on the panicle. The improved white grain medium duration genotypes are more prone to grain mold attack compared to the late maturing types as their grain development coincides with heavy rainfall. A complex of pathogenic and saprophytic fungi causes grain mold, and the major fungi associated with early infection events are *Fusarium* spp., *Curvularia lunata*, *Alternaria alternata* and *Phoma sorghina* (Thakur et al. 2003, 2006). Several species of *Fusarium* associated with the grain mold complex have been shown to produce mycotoxins, such as fumonisins and trichothecenes that are harmful to human and animal health (Thakur et al. 2006; Sharma et al. 2011). Phenotyping for grain mold reaction is done under field conditions during the rainy season (June–September). No artificial inoculation is required since sufficient natural inocula of mold fungi are present during the rainy season over sorghum fields in India and other countries for natural field epiphytotic conditions (Thakur et al. 2007; Bandyopadhyay et al. 1988). The test lines are sown in the first half of June so that grain maturing stages coincides with periods of frequent rainfall in August–September. To enhance mold development, high humidity (>90% RH) is provided through sprinkler irrigation of test plots twice a day for 30 minutes each between 10 and 12 noon, and between 4 and 6 PM.
on rain-free days from flowering to physiological maturity (when most grains in the middle of the panicle develop a black layer at the hilum). The visual Panicle Grain Mold Rating (PGMR) is taken at the prescribed physiological maturity (Thakur et al. 2006) using a progressive 1 to 9 scale, where 1 = no mold infection, 2 = 1–5%, 3 = 6–10%, 4 = 11–20%, 5 = 21–30%, 6 = 31–40%, 7 = 41–50%, 8 = 51–75% and 9 = 76–100% molded grains on a panicle to categorize the test entries into resistant (1–3 score), moderately resistant (3.1–5.0 score), susceptible (5.1–7.0 score) and highly susceptible (>7.0 score). The resistant and susceptible checks are invariably included for comparison. More recently, a greenhouse screening method has been developed at ICRISAT Patancheru that facilitates screening sorghum lines against an individual mold pathogen under controlled conditions (Thakur et al. 2007).

Resistance to grain mold is a polygenic trait and both additive and non-additive gene action in conditioning resistance has been reported. The mechanisms important in breeding white, grain mold resistant sorghums are: hard corneous endosperm, thin pericarp, and thick wax layer on pericarp, fast grain filling rate, large glume coverage, pigmented glumes and open panicles. Antifungal proteins may also play an important role in imparting grain mold resistance in sorghum (Menkir et al. 1996; Audilakshmi et al. 1999; Reddy et al. 2000). In a recent study, grain mold incidence, kernel milling hardness, grain density, plant height, panicle peduncle length, foliar-disease incidence and plant color were measured on 125 F5 genotypes derived from a cross of Sureño and RTx430. Quantitative trait loci were detected by means of 130 mapped markers (44 microsatellites, 85 AFLPs, one morphological-trait locus) distributed among 10 LGs covering 970 cM. One to five QTLs affected each trait, with the exception of grain density for which no QTLs were detected. Grain mold incidence was affected by five QTLs each accounting for between 10 and 23% of the phenotypic variance (Klein et al. 2001).

From the applied breeding point of view, it was reported that to develop grain mold resistant hybrids, at least one parent should possess grain mold resistance (Kumar et al. 2011b). Several resistant accessions (IS 2815, IS 21599, IS 10288, IS 3436, IS 10646, IS 10475 and IS 23585) have been used in breeding to develop restorer lines, varieties and hybrid parents. White/chalky white-grained mold resistant accessions such as IS 20956, -21512, -21645 IS 2379 and -17941 have been selected from the sorghum minicore collection (Sharma et al. 2010). In a trait-specific breeding program, a number of grain mold resistant lines with maintainer reaction have been converted into male-sterile lines. Fifty-eight seed parents with A1 cytoplasm with white grain, red grain and brown grain have been developed. Also, the grain mold resistant accession IS 9470 with A1 (milo), A2, A3 and A4 (maldandi), and IS 15119 with A3 and A4 (maldandi) cytoplasms have been converted
into male-sterile lines and these have been characterized. More recently, some test hybrids developed using mold resistant advanced hybrid parents (A- and R-lines) have shown promising results for mold resistance and grain yield at ICRISAT (Kumar et al. 2011a; Thakur et al. 2007).

9.4.2 Anthracnose and Leaf Blight

Sorghum anthracnose caused by *Colletotrichum sublineolum* Hann. Kabátet Bub. (syn. *C. graminicola* (Ces.) G.W. Wils.), is one of the most important foliar disease of sorghum (Marley et al. 2001; Valério et al. 2005). Estimated grain losses caused by anthracnose are about 50% on susceptible cultivars (Thakur et al. 2007). Leaf blight caused by *Exserohilum turcicum* (Pass) Leonard and Suggs is another widely distributed and the most damaging foliar disease of sorghum, causing significant grain losses due to the reduction of the photosynthetic leaf area (Bergquist et al. 2000).

Screening techniques for phenotyping both the diseases are the same. Both greenhouse and field screening for these diseases have been standardized (Thakur et al. 2007). For field screening, the test lines are evaluated along with the susceptible check H 112 in the anthracnose/leaf blight screening nurseries. Anthracnose screening is carried out during the rainy season and the leaf blight nursery is planted in the late rainy season. The inoculum of both the pathogens (*C. sublineolum* and *E. turcicum*) is multiplied by inoculating autoclaved sorghum grains with an actively growing pure culture of a local isolate and incubating at 28±1°C for 10 days under a 12-hour photoperiod. The test entries in the screening nursery are whorl-inoculated with infested sorghum grains (colonized by *C. sublineolum* or *E. turcicum*) @ 3 to 4 grains/plant at 30 days after seedling emergence. High humidity is maintained with overhead sprinklers twice a day on rain-free days until the soft dough stage. Disease severity is recorded on 10 uniformly flowering plants at the soft-dough stage using a progressive 1–9 scale, where 1 = no disease and 9 = 76–100% leaf area covered with lesions (Thakur et al. 2007). Based on the disease score, the test lines are categorized as resistant (1.0–3.0 score), moderately resistant (3.1–5.0 score), susceptible (5.0–7.0 score) and highly susceptible (>7.0 score).

Anthracnose resistance is governed by a single recessive gene (House 1980). Coleman and Stokes (1954) reported that resistance to anthracnose in sorghum line is encoded by two closely linked dominant genes, each conferring resistance to different phases of the disease. Jones (1979) and Tenkouano (1993) both reported that resistance to anthracnose in SC326-6 was controlled by a single genetic locus with multiple allelic forms, while Boora et al. (1998) reported that a single recessive gene conferred resistance in SC326-6. Segregation studies by Mehta et al. (2005) using 235 lines in 1999 and 146 lines in 2000 detected a 3:1 ratio of resistant to susceptible
phenotypes in the F\textsubscript{2} generation suggesting that a single dominant gene, \textit{Cg1}, in sorghum line SC748-5 confers resistance to anthracnose.

Perumal et al. (2009) worked on identification of molecular markers that co-segregate with \textit{Cg1}, a dominant gene for anthracnose resistance originally identified in cultivar SC748-5. To identify molecular markers linked with the \textit{Cg1} locus, F\textsubscript{2} plants derived from a cross to susceptible cultivar BTx623 were analyzed with 98 AFLP primer combinations. Four AFLP markers that co-segregate with disease resistance were identified, of which Xtxa6227 mapped within 1.8 cM of the anthracnose resistance locus and all four AFLP markers have been previously mapped to the end of sorghum linkage group LG-05. Sequence scanning of Bacterial Artificial Chromosome (BAC) clones spanning this chromosome led to the discovery that Xtxp549, a polymorphic SSR marker, mapped within 3.6 cM of the anthracnose resistance locus. To examine the efficacy of Xtxa6227 and Xtxp549 for marker-assisted selection, 13 breeding lines derived from crosses with sorghum line SC748-5 were genotyped. In 12 of the 13 lines, the Xtxa6227 and Xtxp549 polymorphism associated with the \textit{Cg1} locus was still present, suggesting that Xtxp549 and Xtxa6227 could be useful for marker-assisted selection and for pyramiding of \textit{Cg1} with other genes conferring resistance to \textit{C. sublineolum} in sorghum (Perumal et al. 2009). QTL analysis of resistance to three foliar diseases, viz. target leaf spot, zonate leaf spot and drechstera leaf blight was undertaken in sorghum using 168 F\textsubscript{2} recombinant inbred lines derived from a cross between “296B” (resistant) and “IS18551” (susceptible) parents. The genomic region flanked by plant color locus (\textit{Plcor}) and SSR marker Xtxp95 on chromosome SBI-06 harbored a disease-response QTL for all the three diseases caused by different fungal pathogens. It is hypothesized that this region on sorghum chromosome SBI-06 could harbor a cluster of disease-response loci to different pathogens as observed in the syntenic regions on rice chromosome 4 and maize chromosome 2 (Mohan et al. 2009).

In a recent study, using 14,739 SNP markers, Upadhyaya et al. (2013) mapped eight loci that are linked to anthracnose resistance in sorghum through association analysis of 242 diverse sorghum mini core accessions evaluated for anthracnose resistance for two years in the field under artificial inoculation. Based on consistency of association strength across the testing environments and markers, loci 1, 2, 3, 4, and 5 showed strong linkage to the resistance phenotype. Four of the eight loci (2, 3, 5, and 6) were on chromosome 1, two (loci 1 and 8) on chromosome 6 and loci 4 and 7 were located on chromosomes 8 and 10, respectively. Except locus 8, disease resistance related genes were found in all loci based on their physical distance from linked Single Nucleotide Polymorphism (SNP) markers.

Several sorghum lines have been identified as moderately to highly resistant to both anthracnose and leaf blight. Some of the lines with stable
anthracnose resistance are: IS 3547, IS 6958, IS 6928, IS 8283, IS 9146, IS 9249, IS 18758, M 35610, A 2267-2, SPV 386 and ICSV 247. Four accessions IS 473, IS 23521, IS 23644 and IS 23684 have been found to have stable resistance to both leaf blight and anthracnose (Sharma et al. 2012). At ICRISAT Patancheru, in a trait-specific breeding program, some of these lines with white-grain have been used to develop resistant lines and hybrid parents. Some anthracnose tolerant hybrid seed parents, such as ICSA/B 260 to ICSA/B 295 are available at ICRISAT (Reddy et al. 2007). Similarly, some leaf blight tolerant hybrid seed parents, such as ICSA/B 296 to ICSA/B 328 were developed during 1989 to 1998 and are available at ICRISAT, Patancheru (Reddy et al. 2007; Thakur et al. 2007).

9.4.3 Stalk Rot

The Charcoal/stalk rot of sorghum is caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. It is a major disease in dry regions of Asia, Africa, the Americas and Australia. The disease is relatively more severe and destructive on high yielding sorghum cultivars when grain filling coincides with low soil moisture in hot dry weather (Mughogho et al. 1984). Disease affected stalks become soft at the base and often lodges even due to moderate wind or by bending the plants. Thus pre-mature lodging is the most apparent symptom of charcoal rot. When an infected stalk is split open, the pith is found disintegrated across several nodes. The cortical tissues are disintegrated and vascular bundles get separated from one another. Numerous minute, dark, charcoal-colored sclerotia of the pathogen are formed on these vascular tubes. The disease reduces grain yield and stover quality. Loss in grain yield is mainly due to lodging of the crop, and loss in stover quality (and yield) is due to rotting and decaying of the stalk.

Phenotyping for charcoal rot involves artificial inoculation of the test lines with a toothpick infested with inoculum of *M. phaseolina*. The tooth picks are inoculated with actively growing pure culture of the virulent local isolate of *M. phaseolina* and incubated at 25±1°C for 10 days. The test lines are grown in the field in the post-rainy season and are artificially inoculated by inserting the toothpick infested with inoculum of *M. phaseolina* into the second internode of the stalk at 10 days after 50% flowering. Irrigation is withheld in the experimental plots at 50% flowering to ensure adequate soil moisture stress to facilitate disease development. The inoculated plants in the test lines are scored for charcoal rot severity at the physiological maturity (25–35 days after inoculation) using a 1 to 5 scale, where: 1 = one internode invaded, but rot does not pass through any nodal area; 2 = two internodes; 3 = three internodes; 4 = more than three internodes; and 5 = most internodes extensively invaded, shredding of stalk and death of plant.
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Data are also recorded for percent soft rot, and length of infection. Charcoal rot rating of test lines is compared with that of the known resistant and susceptible checks to identify resistant lines.

There is limited variability for resistance to charcoal rot disease and apparently more than one gene is involved in controlling the resistance (House 1985). Due to complex quantitative inheritance of resistance to the disease, very little progress has been made in breeding for charcoal rot resistance (Mukury 1992; Rosenow 1992). Selection of stiff-stalk and non-senescent (stay-green) types with high productivity is considered important in breeding for charcoal rot resistance (Maunder 1993). Recently, five QTL conditioning stalk rot resistance have been identified and one of the QTLs explained close to 32% phenotypic variance for this trait (Reddy et al. 2008).

Sorghum genotypes that show the stay-green trait (e.g., E36-1 and B35) are generally tolerant to charcoal rot (Reddy et al. 2009). Some other lines, such as SLB 7, SLB 8, SLR 17 and SLR 35 are also reported to be tolerant to charcoal rot. Drought tolerant, lodging resistant and non-senescent sorghum genotypes are supposed to have good tolerance to charcoal rot (Kumar et al. 2011a). However, finding such genotypes with high grain yield under a desirable agronomic background are often not easy. Involving the stay-green trait sources in crosses with other high yielding lines, several improved hybrid parents have been developed. Among the hybrid seed parents, ICSA/B 307, -351, -371, -373, -375, -405, -589, -675, -678 and 702, and among male parents/varieties ICSV 21001 through 21025 are quite promising for the stay-green trait (Reddy et al. 2007). Based on number of nodes infected, infection length and percent soft, two hybrids (ICSA 675 x SPV 1411 and ICSA 675 x ICSV 700) have been found tolerant to charcoal rot (Sharma et al. unpubl.).

9.4.4 Head Smut

Head smut of sorghum caused by \[\text{Sporisorium reilianum}\ (Kuhn)\ \text{Langdon\ and\ Fullerton}\], is an economically important disease of sorghum worldwide. \textit{S. reilianum} causes various symptoms affecting the inflorescence and occasionally the foliage in sorghum (Frederiksen and Odvody 2000). The causal agent also attacks maize, and other related species such as Johnsongrass, Sudangrass, \textit{Euchlaena mexicana}, and \textit{Teosinte} spp. Pathogenicity of various sources of \textit{S. reilianum} varies depending on the hosts; thus, different races of \textit{S. reilianum} are recognized based on their host specificity. Frederiksen et al. (1975) and Frowd (1980) reported four races of \textit{S. reilianum} in the United States. Herrera and Vallejo (1988) reported three races in Mexico. Dodman and Obst (1985) reported race 3 of \textit{S. reilianum} in
Southeast Queensland, Australia. Recently, Zhang et al. (2011) documented that there are four races found in the sorghum fields in China.

Several good sources of resistance to head smut have been identified and characterized (Peterson et al. 2009; Zhang et al. 2011). Some resistance sources have been utilized in various sorghum breeding programs (Rooney et al. 2002; Zhang et al. 2011). Inheritance of resistance to head smut is variable, depending upon the source of resistance, the race of pathogen (i.e., race-specific reaction), and the environment where host-pathogen reaction is evaluated (Cao et al. 1988; Magill et al. 1996). A race-specific form of resistance to head smut has been reported (Magill et al. 1996). Molecular tags for head smut resistance would be very useful because the disease is not expressed until heading, and because there is typically a very high rate of escapes. Mapping population and DNA markers (AFLP) were developed to identify genomic region(s) harboring the genetic factors associated with resistance to head smut, but none was detected yet. Nevertheless, continuous efforts using SNP markers to map the genetic loci conditioning resistance to head smut are in progress (Magill et al. 2002), and molecular makers linked to head smut resistance should be available in the near future.

9.5 Genetic Basis for Striga Resistance

The witch weed \(\text{(Striga spp.)}\), Striga a serious parasitic angiosperm of cereal crops, is the most limiting biotic factor in the production of sorghum in sub-Saharan Africa (Ejeta 2007a). The weed survives by extracting water and nutrients from the host plant and produces phytotoxins which are harmful to the host crop. It causes a characteristic “witch” appearance of the host crop manifested by stunting and withering. The yield losses range from 20–80% and even total crop failure in severe infestation. Up to 5 and 95% yield losses have been recorded for resistant and susceptible sorghum hybrids, respectively (Obilana 1980). Striga seeds remain dormant and viable in the soil for up to 20 years. With every planting, some of the dormant seeds, stimulated by crop exudates, germinate and infest the host crop while reproducing and increasing the Striga seeds in the soil thus escalating the problem. Several host resistance mechanisms have also been suggested in the literature including low germination stimulant production, low production of the haustorial initiation factor, avoidance mechanisms, presence of physical barriers, Hypersensitive Response (HR) and antibiosis (Ejeta 2000). Low germination stimulant production is the only mechanism that has been studied and exploited for breeding purposes (Hess et al. 1992; Ejeta 2000). Haustoria formation and attachment occur on the hosts and non-host roots in a similar manner, but parasitic penetration in the non-host is arrested only at the epidermis of the root with clear necrosis. An in \textit{vitro} culture is an important tool in identification of Striga resistance genes and
characterization of their mechanisms of expression. With the development of the agar gel assays (Hess et al. 1992), important sources of resistance were identified and, reliable genetic information generated (Ejeta et al. 1992). An extended agar gel assay was developed for screening for resistance to striga (Mohamed et al. 2010).

Significant variation was found in the tested genetic materials for various Striga resistance traits due to significant genotype × environment interactions and high parasite variability, multi-locational testing of breeding materials is essential. Both additive and dominant gene action are involved in Striga resistance and grain yield under conditions of Striga infestation. Due to the significant contribution of dominance effects, evaluations of testcrosses under Striga infestation are essential in later stages of a hybrid breeding program (Haussmann et al. 1999 ftp://ftp.gwdg.de/pub/tropentag/proceedings/1999/referate/STD_C3.pdf verified on April 7, 2013). Putative QTLs conditioning Striga resistance have been reported by Ejeta (Ejeta 2005). Haussmann and group reported that across sites, composite interval mapping detected 11 QTLs and nine QTLs in sets 1 and 2 of RI population RIP-1, explaining 77 and 80% of the phenotypic variance for area under the Striga number progress curve (ASNPC), respectively. The most significant RIP-1 QTL corresponded to the major-gene locus lgs (low stimulation of Striga seed germination) in LG I. In RIP-2, 11 QTLs and nine QTLs explained 79 and 82% of the phenotypic variance for ASNPC in sets 1 and 2, respectively. Five QTLs were common to both sets of each RIP, with the resistance alleles deriving from IS9830 or N13. Since their effects were validated across environments, years and independent RIP samples, these QTLs are excellent candidates for MAS (Haussmann et al. 2004).

The best characterized resistance phenotype against Striga is low germination stimulant production. Cultivar differences in sorghum to stimulate Striga germination are well correlated to field resistance (Hess et al. 1992). Low Striga germination stimulant production in sorghum is controlled by recessive alleles at a single locus (Vogler et al. 1996). A bioassay for this character has been exploited in developing Striga resistant sorghum cultivars (Hess et al. 1992). The nature of induction of these genes is now known, although the relationship between the activity of these genes and the formation of germination stimulants has not yet been clearly established (Bouwmeester et al. 2003).

Beyond low germination stimulant production by host plants, several other resistant phenotypes are being discovered and to some degree exploited in breeding programs. A laboratory method was used to screen wild and cultivated sorghums for the ability to cause haustorial initiation of germinated S. asiatica (L.) Kuntze, and wild accessions of sorghum were found that showed reduced haustorial formation (Rich et al. 2004). Exudation of phytotoxins by the host that kill unattached parasites has
been reported in sunflowers resistant to *O. cumana* (Serghini et al. 2001). Pyramiding genes for multiple mechanisms of Striga resistance has been used, pyramided and Striga resistance traits of LGS, LHF, HR, and IR Stacking genes for Striga resistance was done into both improved modern cultivars with high yield as well as African landraces that possess unique adaptation and fit in specific niches of local environments (Ejeta 2007a). These genes have been transferred to sorghum cultivars and deployed in various countries in Africa (Ejeta 2007b).

### 9.6 Conclusion

Developing host plant resistance to biotic stresses has been a challenging job for sorghum workers because of the complexities involved in variation in pest genotypes, complexity in genetic control and difficulty in effective phenotyping for these stresses. In spite of this, sorghum improvement has come a long way from using simple classical methods to using advanced molecular tools for biotic stress resistance improvement. Efforts are underway to use new genomic tools for sorghum improvement facilitated by the availability of aligned genome sequence. The integrated genetic maps (Mace and Jordan 2011; Ramu et al. 2010) will be quite handy for the development of more efficient breeding systems in sorghum to better exploit heterosis and breed for host plant resistance to various stresses. Currently the genotyping tools are increasingly available and more affordable and therefore trait phenotyping should be given high priority. The progress in developing host plant resistance to biotic stresses in the future depends upon the quality of the phenotyping data we generate and most appropriate genomic tools we use for establishing trait-marker associationships and their deployment in breeding programs.

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