Chapter 3
Phenotyping in Sorghum [*Sorghum bicolor* (L.) Moench]

Are Ashok Kumar, Hari C. Sharma, Rajan Sharma, Michael Blummel, P. Sanjana Reddy, and Belum V.S. Reddy

Abstract Sorghum is one of the most important cereal crops grown in the semi-arid tropics (SAT) of Asia, Africa and Americas for its food, feed, fodder and fuel value. Sorghum production is constrained by several biotic and abiotic stresses. Genetic enhancement of sorghum for grain and stover yield, nutritional quality and plant defense traits (abiotic and biotic) which stabilize the crop performance requires thorough knowledge on crop genetic and crop breeding principles. Rapid progress in biotechnology provided powerful and cost-effective molecular/genomic tools to develop desired products in sorghum. However, development of robust and efficient phenotyping methods for traits of interest is critical to make use of these new tools. There is no publication with efficient phenotyping protocols for sorghum research compiled at one place for use by sorghum workers. This book chapter is an attempt to fill that gap and we hope various phenotyping methods discussed hereunder will be useful to sorghum researchers in developing improved products by using them in combination with appropriate breeding/genomic tools.

Keywords Sorghum • Yield and quality • Biotic and abiotic stresses • Breeding • Phenotyping • Genotyping • Genomics

A.A. Kumar (✉) • H.C. Sharma • R. Sharma • B.V.S. Reddy
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, Andhra Pradesh, India
e-mail: a.ashokkumar@cgiar.org

M. Blummel
International Livestock Research Institute (ILRI), ICRISAT, Patancheru 502324, Andhra Pradesh, India

P.S. Reddy
Directorate of Sorghum Research, Rajendranagar 500030, Andhra Pradesh, India

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3.1 Introduction

Sorghum is an often cross-pollinating (6 % cross-pollination on an average) diploid (2n = 2x = 20) belonging to Gramineae family with a genome (730 Mb), about 25 % the size of maize or sugarcane. It is a C_4 plant with higher photosynthetic efficiency and higher abiotic stress tolerance (Nagy et al. 1995; Reddy et al. 2009) Its small genome makes sorghum an attractive model for functional genomics of C_4 grasses. Drought tolerance makes sorghum especially important in dry regions such as northeast Africa (its center of diversity), India and the southern plains of the United States (Paterson et al. 2009). Genetic variation in the partitioning of carbon into sugar stores versus cell wall mass, and in perenniality and associated features such as tillering and stalk reserve retention, make sorghum an attractive system for the study of traits important in perennial cellulosic biomass crops (Paterson et al. 1995). Its high level of inbreeding makes it an attractive association genetics system. Sorghum is one among the climate resilient crops that can better adapt to climate change conditions (Cooper et al. 2009; Reddy et al. 2011). This chapter deals with the biology and classification of sorghum, major sorghum improvement methods, traits of global importance in sorghum improvement research and various phenotyping methods used for improving sorghum for these traits. We hope it serves as a practical tool for the sorghum workers across the world.

3.1.1 Global Importance

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop globally and is the dietary staple of more than 500 million people in over 30 countries, primarily in the developing world. It is grown on 40 m ha in more than 90 countries in Africa, Asia, Oceania, and the Americas. Among those, USA, Nigeria, India, Mexico, Sudan, China, and Argentina are the major sorghum producers globally. Sorghum accounts for 6 % of the global coarse cereals production in the world and is particularly well suited to hot and dry agro-ecologies in the world. Global sorghum productivity is low (1.4 t ha^{-1}) with wide variation in different parts of the world (Reddy et al. 2011).

Sorghum grain is mostly used directly for food (55 %), and is consumed in the form of porridges (thick or thin) and flat breads. However, sorghum is also an important feed grain (33 %), especially in Australia and the Americas. Stover (crop residue after grain harvest) is an important feed source to livestock in mixed crop-livestock systems prevalent in semi-arid tropics. Of late, sweet sorghum with sugar-rich juicy stalks is emerging as an important biofuel crop (Reddy et al. 2008). Sorghum grain is a rich source of micronutrients, particularly Fe and Zn (Kumar et al. 2011a) and is also a rich and cheap source of starch. Thus, sorghum is a unique crop with multiple uses as food, feed, fodder, fuel and fiber. It is generally grown in rainy season (spring) but in India and in some parts of Africa it is grown in both rainy and postrainy seasons (Reddy et al. 2009).

Table 3.1 Five basic and ten hybrid races

Basic races	Intermediate/hybrid races
1. Race *bicolor* (B)	6. Race *guinea-bicolor* (GB)
2. Race *guinea* (G)	7. Race *caudatum-bicolor* (CB)
3. Race *caudatum* (C)	8. Race *kafir-bicolor* (KB)
4. Race *kafir* (K)	9. Race *durra-bicolor* (DB)
5. Race *durra*(D)	10. Race *guinea-caudatum* (GC)
	11. Race *guinea-kafir* (GK)
	12. Race *guinea-durra*(GD)
	13. Race *kafir-caudatum* (KC)
	14. Race *durra-caudatum* (DC)
	15. Race *kafir-durra* (KD)

3.1.2 Taxonomy and Classification

Sorghum was first described by Linnaeus in 1753 under the name Holcus. In 1974, Moench distinguished the genus *Sorghum* from genus *Holcus* (Celarier 1959; Clayton 1961). Subsequently, several authors have discussed the systematics, origin and evolution of sorghum since Linnaeus (de Wet and Huckabay 1967; de Wet and Harlan 1971; Doggett 1988; Dahlberg 2000). Sorghum is classified under the family *Poaceae*, tribe *Andropogoneae*, subtribe *Sorghinae*, genus *Sorghum Moench* (Clayton and Renvoize 1986). Some authors further divided the genera into five subgenera: *sorghum*, *chaetosorghum, heterosorghum, parasorghum* and *stiposorghum* (Garber 1950; Celarier 1959). Variation within these five subgenera except the subgenera *sorghum* has been described (Celarier 1959). *Sorghum bicolor* sub spp. *bicolor* contains all of the cultivated sorghums. Doggett (1988; Dubey 1994) described them as annual plants, with stout culms up to 5 m tall, often branched, and frequently tillering.

Harlan and de Wet (1972) have developed a simplified classification of cultivated sorghum which proved to be of real practical utility for sorghum researchers. They classified *Sorghum bicolor* (L.) Moench, subspp. *bicolor* into five basic and ten hybrid races as depicted below (Table 3.1).

The 15 races of cultivated sorghum can be identified by mature spikelets alone, although head type is sometimes helpful. The classification is based on five fundamental spikelet types (Harlan and de Wet 1972). However, some of the commercial grain sorghum types are utilized in improvement programs, the characteristics of which are given in Table 3.2.

The Biodiversity International [formerly International Plant Genetic Resources Institute (IPGRI)] Advisory Committee on Sorghum and Millets Germplasm has accepted and recommended this (Harlan and de Wet 1972) classification to be used in describing sorghum germplasm (IBPGR/ICRISAT 1980). Large genetic diversity reported in sorghum and sorghum gene bank at ICRISAT holds ~38,000 global collections of sorghum germplasm which represents 80 % of the variability in sorghum (Kumar et al. 2011a).

Table 3.2 Characteristics of commercial grain sorghum types

Grain sorghum type	Brief morphological description	Geographical location
Durra	Hairy rachis, flattened kernels and dry stalks	Mediterranean, Near East, Middle East
Shallu	Partly pubescent involute glumes, cone-shaped lax panicles, corneous kernels, dry and non-sweet stalks	India, tropical Africa
Guineense	Involute and nearly glabrous glumes and compact panicles	Central and Western Africa
Kafir	Awnless, compact cylindrical panicles and juicy non-sweet stalks	South Africa
Kaoliang	Stiff stalks, thick hard rind, stiff spreading and few panicle branches, and dry and no-sweet stalks	Eastern Asia
Milo	Yellow midrib, transverse wrinkle of the glumes, compact, awned panicles, large round kernels	East Africa
Feterita	Large kernels, brown testa, and dry and non-sweet stalks	Sudan
Hegari	Rounded kernels, brown testa mid-compact ellipsoid and branched panicles, and white kernels with a bluish-white appearance	Sudan

3.2 Floral Biology and Crop Improvement Methods

Sorghum is a short day plant, and blooming is hastened by short days and long nights. However, varieties differ in their photoperiod sensitivity (Quinby and Karper 1947). In traditional varieties, reproductive stage is initiated when day lengths return to 12 h. Floral initiation takes place 30–40 days after germination. Usually, the floral initial is 15–30 cm above the ground when the plants are about 50–75 cm tall (House 1980). Floral initiation marks the end of the vegetative phase. The time required for transformation from the vegetative primordial to reproductive primordial is largely influenced by the genotype and the environment. The grand growth period in sorghum follows the formation of a floral bud and consists largely of cell enlargement. Hybrids take less time to reach panicle initiation, more days to expand the panicle and a longer grain filling period than their corresponding parents (Maiti 1996).

3.2.1 Mode of Reproduction and Artificial Hybridization

Sorghum is an often cross-pollinating crop and natural cross pollination varies from 0.6 to 30 % depending on the genotype, panicle type, wind direction and velocity (House 1980). Inflorescence is a raceme, consisting of one to several spikelets. The spikelets usually occur in pairs, one being sessile and the second borne on a short pedicel, except the terminal sessile spikelet, which is accompanied by two pedicelled

spikelets. In sorghum anthesis starts with the exertion of complete panicle from the boot leaf. Flowers begin to open 2 days after complete emergence of the panicle. The sorghum head begins to flower at its tip and anthesis proceeds successively downward. Anthesis takes place first in the sessile spikelets. It takes about 6 days for completion of anthesis in the panicle with maximum flowering at 3 or 4 days after anthesis begins. Anthesis takes place during the morning hours, and frequently occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. Maximum flowering is observed between 0600 and 0900 h. Because all heads in a field do not flower at the same time, pollen is usually available for a period of 10–15 days. At the time of flowering (anthesis), the glumes open and all the three anthers fall free, while the two stigmas protrude, each on a stiff style. The anthers dehisce when they are dry and pollen is blown into the air. Pollen in the anthers remains viable several hours after pollen shedding. Flowers remain open for 30–90 min. Dehiscence of the anthers for pollen diffusion takes place through the apical pore. The pollen drifts to the stigma, where it germinates; the pollen tube, with two nuclei, grows down the style, to fertilize the egg and form a 2n nucleus (Aruna and Audilakshmi 2008). Stigmas get exposed before the anthers dehisce subjecting to cross pollination. Pollination for crossing purposes should start soon after normal pollen shedding is completed during morning hours.

Sorghum is amenable for crossing and selfing quite easily. For selfing, after panicle exertion, bagging should be done by snipping off the flowered florets at the tip. Crossing is done by emasculation of selected panicles and dusting of pollen from identified plants. Hand emasculation is the most commonly practiced in sorghum. Because of this ease in crossing, hybridization is most commonly followed in sorghum for trait improvement.

3.2.2 Crop Improvement Methods

The crop improvement methods depend on the pollination control mechanisms and cultivar options. Considering that sorghum is predominantly a self-pollinated crop, breeding methods that are being followed in sorghum are those that are designed for self-pollinated crops. The hybrids are superior to pure lines. The discovery of cytoplasmic-nuclear male sterility helped to produce hybrids seed on mass scale using three-line system (A, B and R) for commercial cultivation of hybrids. Also, sorghum can be handled as cross pollinated crop for breeding purposes; the recurrent population methods can be deployed using genetic male sterility genes.

3.2.2.1 Pure Line Selection

Pure line selection is practiced in two situations (a) when there is a need to develop a variety from a land race population, and (b) while developing a variety from a segregating population. For e.g. in sorghum, for postrainy season adaptation, the

local landraces from Maharashtra were collected and single plant selections were made for a couple of generations and the performance for grain and stover yields of the lines were compared. The line showing better performance than the check variety for yield traits is released for commercial cultivation (Audilakshmi and Aruna 2008). In case of segregating populations, the individual plants are heterozygous in the beginning as they are the products of crossing between two homozygotes and attain homozygosity in successive generations upon self-pollination. Individual plant selections have to be carried out for at least 5–6 generations to achieve the desired level of homozygosity of a pure line. Higher number of plants (3,000–10,000) of segregating population is evaluated and selection is practiced to obtain desired plants.

3.2.2.2 Mass Selection

Mass selection differs from pure line selection, wherein a number of desirable plants (instead of only one), are selected and compositing is done on the harvested seed to produce the next generation (Allard 1960). This method has a few drawbacks, such as, it is not known whether the plants being grouped are homogenous and some of them if heterogeneous would segregate further in following generations, and repeated selection would be required (Sharma 1988). Mass selection is generally practiced to purify a variety. A large number of single plants are selected from impure variety population, each line progeny tested and similar type progenies bulked to form the pure seed lot. The success of the method depends upon high heritability, that is, the presence of additive gene action and minimal influence of genotype × environment interaction on the expression of the selected trait.

3.2.2.3 Hybridization-Based Methods

The term hybridization refers to crossing of two genetically different individuals as it combines the traits of two varieties and provides an opportunity to select plants with desirable features of both parents through recombination in the segregating progenies. As the natural variability for most traits is limited or already exploited, there is a need to create new variability by making artificial hybrids to make any further dent in developing improved varieties through selection in the segregating populations. As most of the traits of interest in sorghum are quantitatively inherited, sorghum breeders generally use pedigree method of selection in segregating populations. In pedigree method, the records of the ancestry or pedigree of each progeny is maintained and it is easy to trace back the parentage and selection. With the pedigree system, the F_2 generation represents the first opportunity for selection. Selection for superiority is based on the vigor and other agronomic features of progenies (families). In F_2, selection is limited to individuals. In F_3 and subsequent

generations, until a reasonable level of genetic homozygosity is reached, selection is practiced both within and between families. Of the >700 sorghum female parents (A-/B-pairs) developed by ICRISAT for various traits of global importance, more than 600 parents are used in crossing to develop them using pedigree method (Reddy et al. 2007).

Bulk population breeding is an economic method of obtaining homozygous lines in self-fertilized crops. However it is not widely used in sorghum. Back cross method is widely used in sorghum improvement particularly for resistance genes, transferring male sterility to the identified maintainer lines by test crossing. Similarly it is the most sought after method for transferring QTLs for shoot fly resistance and stay-green trait (Kumar et al. 2011a).

The choice of parents for hybridization programs is critical for its success and requires careful and critical evaluation of potential parents for various attributes such as yielding ability, disease resistance, adaptation, quality of the produce and morphological features relevant to crop management practices. Since new strains are intended to have superior yield potential than the existing varieties, one of the parents is invariably the adapted variety of the area. The other parent is primarily chosen for complimenting the specific weakness of the variety, which needs to be replaced. The general combining ability of a parent is likely to be reflected adequately in the parental performance of the trait. Besides selection of the parents on the yield performance and general and specific combining abilities in the partial diallel crosses or line × tester crosses, it is desirable to analyze the potential parents for important traits such as panicle length, number of primary/secondary branches, grain per primary branch, and grain size (Audilakshmi and Aruna 2008).

A single genetic male sterility recessive gene in homozygous condition confers male sterility. Population improvement methods can also be deployed in sorghum by making use of this system which provides long-term breeding strategy to derive diverse and broad genetic-based superior varieties/hybrid parents (Reddy and Ashok Kumar 2008). More than 50 sorghum hybrid parents (A-/B-pairs) at ICRISAT were developed using population improvement methods.

3.2.3 Marker Technologies and Genetic Transformation

Traditional methods of plant breeding have made significant contributions to sorghum improvement as indicated by the progress in productivity in different parts of the world (global average productivity 1.4 t ha^{-1}in 2007 compared to 1.1 t in 1970). However, the traditional methods have been slow in improving complex traits like grain yield, grain quality, drought tolerance, resistance to grain mold, shoot fly, midge, and Striga. For efficient genetic management of such traits, biotechnology offers new and potentially powerful tools to plant breeders. Of the several biotechnological tools, DNA marker technology and genetic transformation have wide application in sorghum improvement programs across the globe.

3.2.3.1 DNA Marker Technology in Sorghum

DNA markers have the potential to enhance the operation of a plant breeding program through a number of ways ranging from finger printing of elite genetic stocks, assessment of genetic diversity, addressing genome evolution, phylogeny relevant to germplasm management, increasing the efficiency of selection for difficult traits through their tight linkages with DNA markers, to make environment-neutral selection for map based cloning (Ejeta et al. 2000; Subudhi and Nguyen 2000). The long-term utility of marker-assisted selection in sorghum improvement is likely to be jointly determined by the identification and mapping of phenotypes with a direct impact on productivity and quality but which are difficult to study and manipulate by classical means (Paterson 1994).

Construction of linkage map is the most fundamental step required for a detailed genetic study and to follow marker-assisted breeding approach in any crop (Tanksley et al. 1989). The use of DNA markers in marker-assisted breeding is based on the tight linkages found between these markers and genes of interest. Such linkage infers the presence of a desirable gene by assaying for the DNA marker. For example, while transferring disease resistance gene to susceptible cultivars traditionally, progenies are screened for the presence of disease resistance genes by inoculation with the pathogen. With DNA-marker technology screening the plants with several different pathogens simultaneously is possible without the need to inoculate the pathogens (Lu 1994). However, expression of such resistance genes under variable field environments needs to be tested. Sorghum genome mapping based on DNA markers began in early 1990s and since then several maps of sorghum have been constructed (Subudhi and Nguyen 2000). Several qualitative traits and QTLs of agronomic importance have been mapped with the help of different classes of DNA markers. Some of them include QTLs for yield components like kernels weight panicle^{-1}, threshing (%), dehulling yield (%) (Rami et al. 1998; Deu et al. 2000; Hart et al. 2002), panicle length (Pereira et al. 1995; Rami et al. 1998; Deu et al. 2000), tiller number (Paterson et al. 1995; Hart et al. 2002), flowering or maturity (Crasta et al. 1999), number of seed branches panicle^{-1} (Pereira et al. 1995), 100/1,000 seed weight (Pereira et al. 1995; Rami et al. 1998; Deu et al. 2000), number of seeds panicle^{-1} (Rami et al. 1998; Paterson et al. 1998; Deu et al. 2000) and seed size (Paterson et al. 1998). Apart from grain yield components, fodder quality traits like stay-green (Tuinstra et al. 1996; Tuinstra et al. 1997; Crasta et al. 1999; Xu et al. 2000; Subudhi et al. 2000a, b; Tao et al. 2000; Haussmann et al. 2002) and juicy midrib (Xu et al. 2000) have been investigated and mapped. Depending on their relative effects and position, many QTLs could be used as targets for marker-assisted selection and provide opportunity for accelerating breeding programs (Subudhi and Nguyen 2000).

The QTL studies (Tuinstra et al. 1996, 1997; Crasta et al. 1999; Xu et al. 2000; Ejeta et al. 2000; Kebede et al. 2001) identified several genomic regions of sorghum associated with pre- and post-flowering drought tolerance. The molecular genetic analysis of QTLs influencing stay-green trait, an important post-flowering drought resistance (Xu et al. 2000; Tao et al. 2000; Haussmann et al. 2002) resulted in the identification of up to four QTLs. Subudhi and Nguyen (2000) confirmed all the

four QTLs (Stg-1, -2, -3, -4) that were identified earlier by Xu et al. (2000) by evaluating Recombinant Inbred Line (RIL) populations derived from B 35 and BTx 700 in two locations for 2 years. By generating a dense linkage map using RFLP markers, Ejeta et al. (2000) mapped the locus for one of the better characterized mechanisms of resistance to Striga.

For disease resistance in sorghum, Rami et al. (1998) for the first time detected three QTLs explaining 33.8 % of phenotypic variations in grain mold incidence. Later, Rooney and Klein (2000) identified five QTLs on linkage groups D, E, F, G and I with each QTL accounting for 10–24 % of the phenotypic variation for grain mold. Rodriguez-Herrera et al. (1999) found that eight grain mold resistant RILs from Sureno × TX 430 had consistently higher levels of anti-fungal proteins than those in susceptible lines. Klein et al. (2001) also identified five QTLs for grain mold each accounting between 10 and 23 % of phenotypic variation whose expression varied with location and the year tested.

For insect resistance, Sajjanar (2002) identified eight QTLs for shoot fly resistance components. One major QTL for glossiness was detected on linkage group J with phenotypic expression ranging from 34.3 to 46.5 % in the three screening environments with highest expression in postrainy season. The largest consistent effect for glossiness due to this QTL on linkage group "J" co-mapped with genomic regions associated with dead hearts (%) under high shoot fly pressure. This QTL may be a useful target for MAS for shoot fly resistance in sorghum.

At ICRISAT–Patancheru, India, QTL mapped for shoot fly resistance using RILs populations derived from BTx 623 × IS 18551 and 296B × IS 18551. A linkage map with reasonable genome coverage has been constructed and six QTLs have been identified in at least two screening environments. The phenotypic variance explained by each of these QTL ranged from 62.9 % for glossiness to 4.5 % for seedling vigor (Ramesh et al. 2005). Satish et al. (2009) identified 29 QTLs for five component traits of shoot fly resistance using the RIL populations of the cross 296 B × IS 18551. Interestingly, some more additional QTL regions where resistance alleles were contributed by the susceptible parent (296B) are also identified. All these can be used in MAS for shoot fly resistance improvement in sorghum.

3.2.3.2 Genetic Transformation Technology

Recent advances in transgenic technology have enabled the transfer of agronomically desirable traits into crop species from diverse sources across reproductive barriers. Entire process of crop improvement through transgenic technology can be divided into (a) production of transgenic plants, (b) transgenic breeding program, (d) release of products. Sorghum is recalcitrant to tissue culture and thereby to genetic transformation compared to other cereals (Seetharama et al. 2003). Model genotypes that can be readily transformed with far greater efficiency and reproducibility are not available in sorghum, and thus the genotypes of interest are directly used (Visarada 2008). In order to overcome the difficulties encountered in *in vitro* protocols, *in planta* methods and direct transformation of developing tissues with

Table 3.3 Transformation of sorghum with agronomically important traits at research level

Trait	Transgenes	Method	Organization	References
Resistance to stem borer	*Bt cry1Ac*	Bombardment	ICRISAT, India	(Girijashankar et al. 2005)
Resistance to stem borer	*Bt cry1Aa* & *cry1B*	Bombardment	DSR (ICAR), India	(Visarada et al. 2004)
Resistance to stalk rot	*Rice chitinase*	*Agrobacterium* and bombardment	Kansas Univ., USA	(Zhu et al. 1998; Krishnaveni et al. 2001)
Drought resistance	*HVA1*	Bombardment	Michigan Univ., USA	(Devi et al. 2004)
Drought resistance	*mtlD*, *p5CSf129A* and *codA*	*Agrobacterium* and bombardment	CRIDA (ICAR), India	(Maheswari et al. 2006)
Anthracnose tolerance	*Chitinase* (*harchit*) *and chitosanase* (*harcho*)	*Particle bombardment*	KIRDI, Kenya	(Moses et al. 2011)

gene guns are employed, though the transformation efficiency is far lower than the methods described above. After production, the transgenic plants are evaluated for the levels of expression of transgene trait and the stable inheritance of the transgene in subsequent generations. Development of transgenic sorghum plants for agronomically important traits at research level is presented in Table 3.3.

3.3 Crop Improvement Objectives and Phenotyping for Major Traits of Interest

Sorghum improvement deals with production of new crop cultivars which are superior to existing cultivars for traits of interest. Availability of genetic variability for these traits, knowledge about their heritability and inheritance, availability of effective phenotyping methodologies are fundamental for success of any crop improvement program. In fact, the efficiency of phenotyping and its robustness decides the success of the crop improvement program in terms of producing a tangible product or technology. In sorghum, a large collection of germplasm is available at ICRISAT (~38,000 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 developed for efficient selection/screening for major traits of interest. There is continuous exchange of material and information across research groups. As a result, a large number of sorghum cultivars were developed and commercialized across the world for traits of interest. For e.g. during the period 1976–2010, a total of 242 sorghum cultivars were released in 44 countries using the ICRISAT-bred sorghum material by the private and public

3.3.1.2 Postrainy Season

It is a unique adaptation to India (approximately 4.5 m ha) where the crop is grown from September/October to January/February with residual and receding moisture in black soils. The postrainy sorghum grain is preferred for food use in India owing to its bold globular lustrous nature. However, no differences were observed between the flat breads made from rainy (but matured under rain-free condition) and postrainy sorghums (ST Borikar, personal communication). The stover from postrainy crop is the most important animal feed particularly in the dry periods. In addition to the traits mentioned under rainy season adaptation, photoperiod sensitivity, temperature insensitivity and grain luster are the major selection criterion. Varieties are the cultivar choice but there is 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 postrainy season production (Kumar et al. 2011a).

3.3.2 Yield and Yield Attributes

Grain yield is the most important trait in sorghum breeding as in other crops; however stover yield is equally important in sorghum particularly in countries like India. Breeding for grain yield improvement is carried out by selecting genotypes directly for grain yield and for component traits. For higher yield, genotypes with a plant height of around 1.5 m are desirable which are amenable for mechanical harvesting with medium maturity duration (100–120 days). Longer duration types give higher yields but the length of growing period (LGP) in most sorghum growing areas does not allow for breeding long duration types, with the exception of West Africa. If we reduce the crop duration, it is likely that the yield goes down. Therefore the breeder has to first fix the plant height and maturity duration for a given environment. However, in the context of climate change, longer duration types need to be maintained in the breeding program considering the fact that when temperatures increases by 2 °C, the longer duration types behave as medium duration types and produce higher yields than other types (Cooper et al. 2009). Another important consideration is photoperiod sensitivity. It is the ability of a genotype to mature at a given period in the calendar year irrespective of its planting date. It is feasible to identify the photoperiod-sensitive genotypes by planting them in different dates (at 15 or 30 days interval) and recording the days for 50 % blooming in the genotypes. The genotypes that take less time for flowering when planted late can be considered photoperiod-sensitive. In sorghum improvement in West Africa and postrainy sorghum in India, photoperiod sensitivity is a key trait. Among the component traits, long panicles, bold grains, number of grains per panicle, 100-seed weight contribute for grain yield and most of these traits have high heritability enabling the plant breeder to improve for these traits through simple selection. The gap between flag leaf sheath and panicle base should be minimum to have good grain filling and the

glume coverage on grains is to be less for higher threshability. Grain size can be visually judged and grain color can be selected as per the consumer /market preference in the given adaptation (Reddy et al. 2009; House 1980).

3.3.2.1 Grain and Stover Yield

In areas where sorghum stover is important as animal feed, breeding dual-purpose types is the best choice. Heterosis for grain and stover yield is high in sorghum and therefore hybrids development should be targeted. A heterosis of 30–40 % for grain yield is reported compared to the best varieties (Kumar et al. 2011a). Hybrid parents' development is critical for exploiting heterosis and therefore genetic and cytoplasmic diversification of hybrid parents is a major breeding objective. Population improvement is also being followed for improving the grain and stover yields.

Quality of grain and stover is as important as grain yield. This is more so in the postrainy season sorghum where consumers prefer bold, lustrous white grain types, which is generally available only in landrace varieties (Reddy et al. 2009). The grain luster is visually scored on a scale 1–3 where 1 = lustrous and 3 = dull among the white grained types. The genetic base of these landraces is narrow and therefore it is more challenging to improve for postrainy season adaptation. Similarly heterosis is low when both parents are derived from landraces. A more practical method for developing postrainy season hybrids is by using rainy season adapted lines (mostly *caudatum* types) as females and landrace varieties as pollinators. While improving the stover yield, one has to keep in the mind the stover digestibility, protein content in addition to the stover yields. The stover yields have to be recorded on oven dried samples after harvesting the grains and for stover quality, indirect selection using NIRS is the most practical method.

3.3.2.2 Height and Maturity

Plant height is a major consideration in sorghum improvement and in fact it is one the criteria for classifying sorghums as grain sorghums, dual-purpose sorghums, fodder sorghums, sweet sorghums and forage sorghums. In sorghum, four loci are known to be involved in the control of plant height. These genes are assigned the symbols Dw1, Dw2, Dw3, and Dw4. Tallness is partially dominant to dwarfness. The zero dwarf type (dominant [DW-] at all loci) may reach a height of 4 m. The change from four to three dominant genes may result in a height change of 50 cm or more. If genes at one or more of the loci are recessive, the difference in height resulting from the recessive condition at an additional locus may have a smaller effect in reducing plant height. The difference between a 3-dwarf (recessive genes [dw dw] at three loci) and a 4-dwarf type may be only 10 or 15 cm (House 1980). Breeders have to keep in mind these facts while selecting genotypes with appropriate height. The plant height is always recorded from base of the plant to tip of the panicle. Plant height and days to flowering data gives an idea about the genotype in terms of suitability for various uses.

Quinby (1967) identified factors at four loci that influence maturity, Ma1, Ma2, Ma3, and Ma4. Generally tropical types are dominant (Ma-) at all four of these loci, and a recessive condition (mama) at any one of them will result in more temperate zone adaptation which takes more time for maturity. Most sorghum improvement programs target medium maturity types (crop duration less than 120 days) as they yield high, however the targeted maturity is to be decided based on the length of growing period (LGP) of the target area. In general, sorghum takes 35–40 days from flowering to maturity. The grain is to be harvested at physiological maturity stage. The hilum turns dark at physiological maturity and this is an important criterion for harvesting (House 1980).

3.3.3 Resistance Breeding

Sorghum is affected by various biotic and abiotic factors leading to severe reduction in productivity and production. A combination of genetic and management methods are more effective in overcoming these constraints.

3.3.3.1 Phenotyping for Host Plant Resistance to Insect Pests

Nearly 150 insect species have been reported as pests on sorghum (Sharma 1993), of which sorghum shoot fly (*Atherigona soccata*), stem borers (*Chilo partellus,* and *Busseolafusca*), aphid (*Melanaphis sacchari*), sorghum midge (*Stenodiplosis sorghicola*), and mirid head bugs (*Calocorisangustatus* and *Eurystylusoldi*) are the major pests worldwide. They cause an estimated loss of $1,089 million in the semi-arid tropics (International Crops Research Institute for 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. 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. 1992a, 2003).

Sorghum Shoot Fly, *Atherigona soccata*. Sorghum shoot fly, *A. soccata* is a key pest of sorghum in Asia, Africa, and the Mediterranean Europe. The larva cuts the growing point, resulting in wilting and drying of the central leaf, known as a dead-heart. 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. Infestations are high when sorghum plantings are staggered due to erratic rainfall.

Interlard-Fishmeal Technique (*Multi-choice Field-Screening*). Adequate shoot fly density for resistance screening can be achieved by manipulating the sowing date, using infester rows, and spreading fishmeal (which attracts the shoot flies) in the field (Sharma et al. 1992a). Shoot fly population can be monitored through fishmeal-baited traps to determine the periods of peak abundance of the shoot fly (Taneja and Leuschner 1985a). This information can be used for planting the test material so that the susceptible stage of the crop coincides with the optimum shoot fly pressure. Late-sown crops are subjected to high shoot fly infestation. At ICRISAT-Patancheru, sowing test material in mid-July in the rainy season, and during October in the postrainy season is effective to screen for resistance to shoot fly. The interlard-fishmeal technique, which is useful for increasing shoot fly abundance under field conditions, involves planting four rows of a susceptible cultivar (such as CSH 1, or 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 and Leuschner 1985a; Sharma et al. 1992a).

No Choice Cage-Screening Technique. To confirm resistance to shoot fly observed under field conditions, and to study the resistance mechanisms, the cage-screening technique developed by Soto (Soto 1972) has been modified to simulate field conditions. The cage-screening technique can be used for multiple- or no-choice tests. For a multiple-choice test, the test genotypes are sown in the field in 3.4×2 m beds, with a row spacing of 15 cm. Ten days after seedling emergence, the plants are covered with a 3.4×2×1 m screened cage, and the shoot flies are introduced into the cage. The shoot flies are collected from fishmeal-baited traps in the field (Sharma et al. 1992a). Eggs and deadhearts are recorded after 1 week. For a no-choice test, only one genotype is sown in 1×1 m beds. Six beds can be covered with a 2×3×0.5 m cage having six compartments. Twenty shoot flies are released into each compartment, and observations are recorded as described above.

Damage Evaluation for Resistance Screening. 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. Resistance can also be measured in terms of leaf glossiness (1=highly glossy, and 5=nonglossy) and trichome density on the undersurface of leaves (Sharma and Nwanze 1997). These traits are associated with resistance to shoot fly.

Spotted Stem Borer, Chilo partellus. Spotted stem borer, *Chilo partellus* is common in Asia and east and southern Africa. The first indication of stem borer infestation is the appearance of small-elongated windows (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 the production of a deadheart. Normally, two leaves dry up as a result of stem borer damage. Larvae continue to feed inside the stem throughout the crop growth. Extensive tunneling of the stem and peduncle leads to drying up of the panicle, production of a partially chaffy panicle or peduncle breakage. Stem borer infestation starts about 20 days after seedling emergence, and the deadhearts appear on 30–40 day old-crop. In northern India, moth catch in light traps begins to increase during the last week of July and peaks during August to September, while in southern India, the peak in moth catches has been recorded during January to February. 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. 1992a).

Use of Hot-Spots. Screening for stem borer resistance can be carried out at hot-spot locations, where the pest populations are known to occur naturally and regularly at levels that often result in severe damage. Hot-spot locations for *C. partellus* are Hisar in Haryana and Warangal in Andhra Pradesh, India; Agfoi and Baidoa in Somalia; Panmure and Mezarbani in Zimbabwe; Kiboko in Kenya; and Golden Valley in Zambia.

Sowing Date. To screen for resistance under natural infestation, especially at the hot-spot locations, adjust the sowing date of the crop such that the crop is at a susceptible stage when the stem borer abundance is at its peak. Determine the periods of maximum borer abundance through pheromone traps, light traps, or by monitoring borer infestation in the crop planted at regular intervals. In northern India, *C. partellus* is most abundant in August to September, and the crop sown between the 1st and 3rd week of July suffers maximum stem borer damage. At ICRISAT-Patancheru, maximum number of moths in the light traps have been recorded during September, followed by smaller peaks during November and February (Sharma et al. 1992a).

Mass Rearing and Artificial Infestation. Artificial infestation with laboratory-reared insects has been successfully used for screening test material for resistance to *C. partellus* (Taneja and Leuschner 1985b; Dang et al. 1970; Reddy and Davies 1979). For field infestation, the Bazooka applicator, developed at the International Maize and Wheat Improvement Center (Trigo) 1977), has been modified to suit the requirements for infesting sorghum. Infest 15–20-day-old plants in the field with 5–7 larvae per plant. Deadheart formation decreases progressively as the infestation is delayed. Shoot fly infestation interferes with screening for resistance to stem borer. Spray fenvalerate or endosulfan to suppress shoot fly infestation one week before artificial infestation with stem borer. Screening for resistance to stem borer, *C. partellus* can also be carried out using diet incorporation assay (Kumar et al. 2005).

Damage Evaluation. Stem borer attack in sorghum causes leaf damage, deadheart formation, stem and peduncle tunneling, and production of chaffy panicles. Record the extent of leaf feeding 2 weeks after artificial infestation, and 4–5 weeks after crop emergence under natural infestation. Record the total number of plants, the number of plants showing the leaf-feeding symptoms, and the leaf-feeding score on a 1–9 scale (1 = <10 % leaf area damaged, and 9 > 80 % leaf area damaged). Data on deadhearts is recorded 3 weeks after artificial infestation, and 4–6 weeks after crop emergence under natural

infestation. Record the total number of plants, plants showing borer deadhearts, and the visual score (1–9 scale) (1=<10 % plants with deadhearts, and 9=>80 % plants with deadhearts). At crop maturity, record observations on the number of partial and completely chaffy panicles, the number of broken panicles. Recovery resistance could be recorded in terms of number of plants with tillers and the number of tillers with productive panicles on a 1–9 scale (1=>80 % plants with 2–3 uniform and productive tillers, and 9=<20 % plants with one or no productive tillers). Data on stem tunneling may be recorded by measuring plant height and the peduncle length in five plants at random in each plot. Measure the stem and peduncle tunneling separately and express it as a percentage of stem/peduncle length. The use and importance of various criteria to select for stem borer resistance have been discussed by Singh et al. (2010).

Sugarcane Aphid, Melanaphis sacchari. 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. The infested leaves begin to die, first turning yellow-brown at the edges. 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. 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. Crops planted between 20 September and 15 October are heavily infested by the aphids.

Screening Under Greenhouse or Net-House Conditions. The plants can be infested artificially by stapling a 10 cm aphid infested leaf cutting to the 5th leaf of each plant under screen house or under nylon net in the field at the flag leaf stage. The nylon net excludes the natural enemies and results in fast build-up of the aphid population. The test lines can also be tested for aphid resistance by using clip cages. Fifth leaf at the boot leaf stage can be infested with ten mature aphids inside a 5 cm diameter leaf cage. The cages are placed in the mid-portion of each leaf. Rate of multiplication of the aphids inside the clip cages can be recorded after 10 days (Sharma HC, unpublished).

Damage Evaluation. Aphid damage can be evaluated at the hard–dough stage on a 1-9 scale (1=plants with a few aphid colonies with no apparent feeding symptoms, 9=5-6 leaves with severe aphid damage, and completely covered with aphid colonies). Under no-choice screen house and clip cage methods, data can also be recorded on numbers of aphids, and this also provides information on antibiosis mechanisms of resistance to the aphids.

Sorghum Midge, Stenodiplosis sorghicola. 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. The major difficulty in identifying source material with stable resistance against sorghum midge is the variation in the flowering of sorghum cultivars and day-to-day variation in midge populations. Because of these problems, genotypes rated as resistant under natural infestation often turn out to be susceptible in the

following seasons or at other locations. Techniques to screen for midge resistance have been described by Jotwani (1978), Page (1979), and Sharma et al. (1988a, 1992a).

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

Sowing Date. To screen test the material for resistance to sorghum midge under natural conditions, it is necessary to determine the appropriate time for sowing at different locations. Determine the periods of maximum midge density through fortnightly sowings of a susceptible cultivar. Adjust sowing dates so that the flowering of the test material coincides with greatest insect density. At ICRISAT-Patancheru, maximum midge damage has been observed in the crop planted during the 3rd week of July. The peak in midge density occurs during October, and a second but smaller peak has been observed during March in the postrainy season, for which planting is carried out during mid-December (Sharma et al. 1992a).

Infester Row Technique. Midge abundance can be increased through infester rows and spreading sorghum panicles containing diapausing midge larvae in the infester rows (Sharma et al. 1988a). Four infester rows of a susceptible cultivar such as CSH 1 should be planted 20 days before the test material after every 20 rows of the test material. Alternatively, early-flowering (40–45 days) lines (IS 802, IS 13249, and IS 24439) can be sown along with the test material. Midge-infested chaffy panicles containing diapausing midge larvae, collected during the previous season should be moistened 10–15 days to stimulate the termination of larval diapause and spread in the infester rows at initiation of flowering in the infester rows. Midge population multiplies for 1–2 generations on the infester rows before infesting the test material, and increases midge damage by three to five times. High relative humidity is important for adult emergence, oviposition, and subsequent damage. Use overhead sprinkler irrigation to increase relative humidity in midge-screening trials during the postrainy season or periods of low relative humidity. Group the test material according to maturity (early, medium, and late) and height (dwarf, medium, and tall) for proper comparisons. The test material can also be planted twice at 15-day intervals to minimize the chances of escape from midge damage.

No Choice Head Cage Technique. Caging midge flies with sorghum panicles inside a head cage to screen for midge resistance under uniform insect pressure (Sharma et al. 1988b). Collect 20 adult female midges in a plastic bottle (a 200 ml aspirator) between 0800 and 1100 from flowering sorghum panicles and release 40 midges into each cage, and repeat the operation the next day. Infest 5–10 panicles in each genotype, depending upon the stage of material and the resources available. Midge damage decreases as the time of collection and release advances from 0830 to 1230 h. Examine the cages 5–7 days after infestation and remove any other insects such as head bugs, panicle-feeding caterpillars, and predatory spiders from inside the cage. Remove the cages 15 days after infestation and evaluate the midge

damage. The head cage technique is quite simple, easy to operate, and can be used on a fairly large scale to confirm the field resistance of selected genotypes.

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

Percentage chaffy spikelets is the most appropriate criterion by which to evaluate sorghum lines for midge resistance. Record midge damage in 250 spikelets collected from five panicles at random at 15 days after flowering or at maturity. In samples collected at the milk stage, squeeze the chaffy spikelets between the thumb and first finger or with forceps, and record the numbers of spikelets producing a red ooze (this indicates midge damage). Express the data as a percentage of chaffy or midge-damaged spikelets. The midge infested panicles can also be evaluated at crop maturity visually on a 1–9 scale (1=<10 %, and 9=>80 % midge-damaged spikelets). The test material can be maintained under infested and non-infested conditions by using a cloth bags or sprayed with insecticides at flowering to control the sorghum midge. Harvest all panicles from the middle row(s) at the time of maturity and record grain yield. Express the loss in grain yield in the infested plots or panicles as a percentage of the grain yield in non-infested plots or panicles. Glume size and tightness, which are associated with resistance to sorghum midge, can also be evaluated on a 1–5 scale (1 = glume short, shining and tight, and 5 = glumes long, nonglossy, and soft upon touch) (Sharma and Nwanze 1997).

Head Bug, Calocoris angustatus. Head bugs, *C. angustatus* is a serious pests of grain sorghum in India, while *E.oldi* is important in West Africa. 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. Such grain also shows poor seed germination. Head bug damage also increases the severity of grain molds. Techniques to screen for resistance to head bugs have been discussed by Sharma and Lopez (1992) and Sharma et al. (1992a, b, 2003).

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

Sowing Date. Adjust sowing dates such that flowering of the test material coincides with maximum head bug density. Determine the periods of maximum head bug abundance through fortnightly sowings. Maximum bug numbers at ICRISAT-Patancheru have been recorded during September and a second but smaller peak has

been recorded during March. Crops sown during the 2nd week of July suffer the maximum head bug damage.

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

No Choice Head Cage Technique. To overcome the problem of variation in flowering among the test cultivars, and fluctuations in insect abundance, the head cage technique developed for midge resistance screening has been found to be useful to screen for resistance to head bugs as well (Sharma et al. 1992b). Collect ten head bug pairs in a 200-ml plastic bottle aspirator and release them inside the cage. Examine the infested panicles after 1 week and remove panicle-feeding caterpillars or predatory spiders if any. Remove the muslin cloth bag along with the bugs 20 days after infestation, kill the bugs with ethyl acetate or benzene (2 ml bag^{-1}), or keep the bags in deep-freeze for 30 min. Count the total number of bugs in each cage. Evaluate the panicles for head bug damage at maturity as described under damage evaluation.

Damage Evaluation for Resistance Screening. Sorghum head bugs suck the sap from developing ovary and result in shriveling and tanning of the grains. Head bug damage can be evaluated by tagging five panicles at random in each genotype at the half-anthesis stage. Sample the panicles for head bugs at 20 days after flowering or infestation in a polyethylene or muslin cloth bag containing a cotton swab soaked in 2 ml of ethyl acetate or benzene. Count the total number of adults and nymphs. Evaluate head bug damage at maturity on a 1–9 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).

Harvest all panicles from the middle row(s) of each plot or genotype at maturity and record panicle and grain weight. Plots or panicles of lines being tested can also be maintained under infested and un-infested conditions by using cloth bags to exclude the head bugs or chemical control. Express the loss in grain yield of infested plots or panicles as a percentage of the grain yield in non-infested plots or panicles. Grain weight and percentage floaters in sodium nitrate solution can also be used as selection criteria (Sharma and Lopez 1992). Take a sample of 1,000 grains at random from each replication or panicle. Equilibrate the moisture content (24 h at 37 °C), and record the grain weight. Prepare a sodium nitrate solution of a specific density of 1.31 in a beaker. Place the 1,000 grain sample in the beaker containing sodium nitrate solution, and count the number of grains floating on the surface, and express them as a percentage of the total number of grains. Glume covering of the grain and grain

hardness that are associated with resistance to head bugs can also be used as an indirect criterion to select for resistance to head bugs (Sharma et al. 1992b).

Grain Mold. Grain mold is a major production constraint in Asia and parts of Africa. The white grain medium duration genotypes are more prone to grain mold attack 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). Damage resulting from early infection includes reduced kernel development, discoloration of grains, colonization and degradation of endosperm, and decreased grain density, germination and seedling vigor (Thakur et al. 2006). Several species of *Fusarium* associated with 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 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 for natural field epiphytotic conditions (Bandyopadhyay et al. 1988; Thakur et al. 2007). The test lines are sown in the first half of June so that grain maturing stages coincided 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 min 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 1–9 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) reaction types. 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 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. To develop grain mold resistant hybrids, at least one parent should possess grain mold resistance (Kumar et al. 2011b). Hard grain and colored glumes contribute to grain mold resistance in white grain types and red grain types possess better grain mold resistance than white grain types. 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 mini-core 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 A_1 cytoplasm with white grain, red

grain and brown grain have been developed. Also, the grain mold resistant accession IS 9470 with A_1 (*milo*), A_2, A_3, and A_4 (maldandi), and IS 15119 with A_3 and A_4 (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).

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). Severe infection and disease development occur during prolonged periods of cloudy, warm, humid and wet weather. Sorghum plants are more vulnerable to infection from flowering through the grain development phase. The pathogen causes seedling blight, leaf blight, stalk rot, head blight and grain molding, and thus limits both forage and grain production. Among these, foliar anthracnose is the most pronounced and devastating on forage and grain sorghum, especially on sweet sorghum cultivars.

Leaf blight caused by *Exserohilum turcicum* (Pass) Leonard and Suggs, is another widely distributed, and most damaging foliar disease of sorghum, causing significant grain losses due to the reduction of the photosynthetic leaf area (Bergquist 2000). In case of early infection in susceptible cultivars, up to 50 % grain yield losses may occur. However, in case of late infection, disease development is slow and yield losses are minimal. The disease is considered more important on dual-purpose grain sorghum, but is especially severe on sweet sorghum (Hennessy et al. 1990; Thakur et al. 2007).

Screening techniques for phenotyping of both the diseases are same. Both greenhouse and field screening for these diseases have been standardized. For field screening, the test lines are evaluated along with susceptible check H 112 in the anthracnose/leaf blight screening nurseries. Anthracnose screening is carried out during rainy season and leaf blight nursery is planted in the late rainy season (2nd week of September) at ICRISAT, Patancheru, India. 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-h photoperiod. The accessions in the screening nursery are whorl-inoculated with infested sorghum grains (colonized by *C. sublineolum* or *E. turcicum*) @ 3–4 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). Greenhouse screening involves spray-inoculation of 21-day-old plants with the inoculum of *C. sublineolum* or *E. turcicum* (1×10^5 conidia ml^{-1}) using a handheld atomizer. Inoculated plants are incubated in a humidity chamber (25 °C,

RH >95 %) for 24 h, and then transferred to greenhouse benches under mist to maintain high humidity. Disease severity is recorded on a 1–9 scale as described above, 14 days after inoculation.

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. 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. Similarly some leaf blight tolerant hybrid seed parents, such as ICSA/B 296 to ICSA/B 328 were developed during 1989–1998 and are available at ICRISAT, Patancheru (Thakur et al. 2007).

Charcoal Rot. 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, 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 and Pande 1984). In India, the postrainy (*Rabi*) sorghums that are generally grown on residual soil moisture often get exposed to soil moisture stress during the grain filling stage if there are no rains. Dry weather conditions during this time may further increase the moisture loss from the soil. Under such a situation, plants are severely stressed due to increased senescence in root and stem cells that adversely affects the production and translocation of carbohydrates in the plant parts. These conditions predispose plants to infection by the charcoal rot fungus. Affected stalks become soft at the base and often lodge even due to moderate wind or by bending the plants. Thus pre-mature lodging is the most apparent symptom of charcoal rot. When 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 tooth pick 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 field in the post rainy season and are artificially inoculated by inserting 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 test lines are scored for charcoal rot severity at the physiological maturity (25–35 days after inoculation) using a 1–5 scale, where: 1 = one internode invaded, but rot does not pass through any nodal area; 2 = two internodes; 3 = three

internodes; 4=more than three internodes; and 5=most internodes extensively invaded, shredding of stalk and death of plant (Thakur et al. 2007). Data are also recorded for per cent 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.

Sorghum genotypes that show stay-green trait (e.g., E36-1 and B35) are generally tolerant to charcoal rot. 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. However, finding such genotypes with high grain yield under 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, -376, -405, -589, -675, -678 and 702, and among male parents/varieties ICSV 21001 through 21025 are quite promising for stay-green trait. Based on number of nodes infected, infection length and per cent soft, two hybrids (ICSA 675×SPV 1411 and ICSA 675×ICSV 700) have been found tolerant to charcoal rot.

Striga. The witch weed (*Striga* spp.), a serious parasitic angiosperm of cereal crops, is the most limiting biotic factor in the production of sorghum in sub-Saharan Africa (Ejeta 2007). 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 to 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 et al. 2000). Low germination stimulant production is the only mechanism that has been studied and exploited for breeding purposes (Hess et al. 1992; Ejeta et al. 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 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 by Mohamed et al. (2010) for screening for resistance to striga.

Abiotic Stresses. As sorghum is grown in a range of environments across tropical and temperate regions, it is subjected to various abiotic stresses in different growing countries. The inherent climatic variability and the projected changes in climate profoundly influence the sorghum production in these regions. Most important

abiotic stresses affecting sorghum include drought, heat, salinity, acid soils etc. Efforts are underway to address these issues in various sorghum improvement programs.

Drought Tolerance. Drought is the most important abiotic constraint and the crop may get exposed to drought during any stage of the growth. The response of sorghum plant varies with the growth stage at which the drought occurs and therefore one needs to breed for different droughts. Four growth stages in sorghum are considered vulnerable to drought: germination and seedling emergence, post-emergence or early seedling stage, midseason or pre-flowering, and terminal or post-flowering. Terminal drought is the most limiting factor for sorghum production worldwide. In sub-Saharan Africa, drought at both seedling establishment and terminal stages is very common. In India, the rainy season sorghum most often faces mid-season or end of season drought but end of season drought is a common phenomenon in postrainy sorghum. The variable moisture availability at both pre-flowering and post-flowering stages during the rainy season can have severe impact on grain and biomass yield. Similarly the terminal drought severely affects the grain and stover yields in postrainy season. The extent of grain yield losses due to drought stress depends on the stage of the crop and the timing, duration, and severity of drought stress (Reddy et al. 2009).

Sorghum responses to moisture stress at all four growth stages have been well characterized. Variation in these responses has been observed and found to be heritable. Since the phenotypic responses of genotypes differing in drought tolerance can be masked if drought occurs at more than one stage, screening techniques have been developed to identify drought-tolerant genotypes at each of the growth stages, separately. Of the several mechanisms to circumvent drought stress in sorghum, drought escape (related to shorter maturity durations), drought avoidance (maintenance of higher leaf water potential, LWP), and drought tolerance (related to greater osmotic adjustment, OA) are important and have been well characterized. However, LWP and OA did not correlate well enough with grain yield in field conditions to merit selection based on them; in addition, screening techniques developed based on LWP and OA were not cost effective in sorghum breeding. Empirical screening based on imposing drought at various growth stages and measuring plant morphological and yield responses is the most effective approach. Long mesocotyl in seedling establishment and recovery from mid-season stress after release by rains are important traits that can be easily deployed in lines. The stay-green trait has been well exploited to enhance post-flowering drought tolerance in sorghum.

At ICRISAT, growth-stage-specific breeding for drought tolerance, which involves alternate seasons of screening in specific drought and well-watered environments, has been used to breed sorghum that can yield well in both high-yield-potential environments as well as in drought-prone environments (Reddy et al. 2009). Since hybrids exhibits relatively better performance than open pollinated (OP) cultivars for grain yield under water-limited environments, hybrid cultivar development (including their parents) should be given strategic importance for enhancing sorghum production in water-scarce environments (Celarier 1959). The progress in enhancing drought tolerance in sorghum through conventional

approaches is limited by the quantitative inheritance of drought tolerance and yield coupled with the complexity of the timing, severity and duration of drought. Biotechnology appears to offer promising tools, such as marker-assisted selection, for genetic enhancement of drought tolerance in sorghum. Four stable and major QTLs were identified for the stay-green trait and are being introgressed through MAS into elite genetic backgrounds at ICRISAT, QDPI, Purdue University, and Texas A&M University (Nagy et al. 1995). However drought phenotyping assumes critical importance for using any of these methods.

High Temperature Tolerance. Sorghum grows well in a temperature range of 15–40 °C but temperatures below and above this may have a bearing on crop germination, establishment, flowering and seed setting. It was reported that sorghum flowers and set seed under high temperatures (up to 43 °C) provided soil moisture is available (House 1985). In many regions of the world, sorghum production encounters heat and drought stress concurrently but heat and drought tolerances are unique and independent traits (Jordan and Sullivan 1982). Despite the level of adaptation of sorghum in the semi-arid tropics, seedling establishment is still a major problem. Failure of seedling establishment due to heat stress is one of the key factors that limits yields and affect stability of production (Peacock 1982). Thomas and Miller (1979) reported that sorghum seedlings respond differently when exposed to varying temperatures, and genetic variation for thermal tolerance in sorghum has been shown to exist in certain lines that are capable of emerging at soil temperature of about 55 °C. Peacock et al. (1993) and Howarth (1989) have discussed the need for greater diversity in sorghum seedling tolerance to heat in superior genotypes, as this will improve the crop establishment in the semi-arid tropics. Genetic variability for heat tolerance among the genotypes at seedling stage was demonstrated by Wilson et al. (1982). Using screening techniques such as leaf disc method (Jordan and Sullivan 1982) and leaf firing ratings by ICRISAT breeders, genetic variability past the seedling stage was demonstrated and positive correlation found between grain yield and heat tolerance thus making breeding for heat tolerance a viable option. Genetic variability for heat tolerance in sorghum was also reported by other researchers (Sullivan and Blum 1970; Seetharama et al. 1982; Jordan and Sullivan 1982). Understanding the genetic control of heat tolerance in sorghum is a prerequisite for formulating an appropriate breeding program. Khizzah et al. (1993) studied four sorghum parental lines RTx430, BTx3197, RTx7000, and B35 and their F_1 and reciprocals, and F_2 progenies during their reproductive phase to assess the genetic basis of heat tolerance in sorghum. They reported that inbreds were more heat tolerant compared to their F_1 progenies. Also, cultivars which had good late season-field drought tolerance appeared to be heat tolerant, suggesting a possible relationship between drought and heat responses. They also reported cytoplasmic effects for heat tolerance. Using the F_2 frequency distribution of the crosses with B 35, Khizzah et al. (1993) made the following assumptions, (a) two loci were responsible for expression of heat tolerance, and (b) complete dominance at both gene pairs, but one gene when dominant is epistatic to the other. Reported low to high heritability of heat tolerance in sorghum suggests the feasibility of genetic enhancement. B35 and BTx3197 could be

used as sources for heat tolerance in sorghum improvement programs (Khizzah et al. 1993). The importance of additive gene effects over dominance effects for heat tolerance index was reported by Setimela et al. (2007). However, selection for heat tolerance has limited success as (a) laboratory techniques to screen for heat tolerance have not been effective in improving heat tolerance in field studies; (b) field screening for heat tolerance is difficult to manage and is often confounded with drought tolerance (Rooney 2004). Due to the confounding effects, though the heat and drought tolerance are independent traits, the selection for drought tolerance traditionally has been assumed to improve heat tolerance.

Salinity and Acid Soils. Of all the soil mineral stresses or chemical toxicities, acidity, and associated Al^{3+} toxicity and salinity are probably the most important constraints to sorghum productivity in tropical environments. Saline and sodic soils cause mineral stresses on approximately 0.9 billion hectares of land (Gourley et al. 1997). Salinity causes reduction in germination (Igartua et al. 1994), growth (Maiti et al. 1994), yields (Macharia et al. 1994) and modifies the physiological and biochemical processes of the plant (Dubey and Singh 1999) in sorghum. Salinity causes more serious damage in the seedling emergence stage than in any other stage in sorghum (Macharia et al. 1994). Though sorghum is known to be relatively more tolerant to soil salinity than maize (Igartua et al. 1994; Krishnamurthy et al. 2007), genetic enhancement of sorghum for salinity tolerance would further increase sorghum productivity in such soils. High soil acidity (80 % Al^{3+} saturation) significantly reduce early vigor and green leaf area at maturity, and induce the lines to flower early, besides reducing the head and grain weights quite substantially in sorghum (Reddy et al. 2000).

An evaluation of a number of germplasm lines, breeding lines and a few popular cultivars indicated existence of significant genetic variability in sorghum for grain yield and other agronomic traits under saline soil conditions (Ramesh et al. 2005). Similarly, Krishnamurthy et al. (2003) have identified some elite sorghum varieties and improved lines promising for agronomic traits and also exhibited better salinity tolerance under induced salinity (at 250 µM NaCl solution; EC: 23.4 dS m^{-1}) in a series of pot-culture experiments. The best way to screen large number of genotypes is planting them in fields with high salinity (with an average ECe of 10 dS m^{-1} or more) and select for desirable agronomic traits and salinity tolerance (Reddy et al. 2010b). In highly saline areas in West Asia and Caucasus, sorghum along with pearl millet is a good option for forage production. Sweet sorghums with high juice brix (%) are likely to be tolerant to salinity.

Haug (1984) considered the capacity of the cellular membrane for binding Al^{3+} as a possible mechanism of Al^{3+} tolerance, whereby Al^{3+} is prevented from entering and accumulating in the cell. *In situ* selection in a given problem soil in the field is a reasonable and reliable approach. While some argue that selection in a given problem soil in natural field conditions is less desirable as it does not allow one to address resistance to Al^{3+} toxicity *per se*, as most acid soils produce both Al^{3+} and *Mn* toxicities, and both elements interact differentially with other elements such as Ca, P and Mg, others regard such an approach as a more practical approach, since such a work may result in selection of genotypes resistant to the different toxicity problems and

their interactions in such soils (Blum 1988). The open-panicled *Guinea* race and the hybrid *Guinea bicolor* lines had a higher overall percentage of acid tolerant sorghum entries than those of other races and hybrids evaluated (Gourley 1988). Variation in soil acidity stress factors with location, soil depth, rainfall, temperature, effective cation exchange capacity (ECEC), natural content of essential elements, level of toxic ions, p-fixation capacity and amount and quality of organic matter (OM) (Gourley et al. 1997), has made breeding for soil acidity tolerance, a complex and slow process. Nevertheless, much progress has been made since EMBRAPA sorghum breeding program for tolerance to acid soils (Schaffert et al. 1975), International Sorghum and Millets (INTSORMIL) sorghum acid-soil breeding project and Inter-American Development Bank funded ICRISAT project on "Research Network for developing sorghum for acid soils in Latin America" were implemented in Brazil and Columbia in 1981. Many good sources of *Al* toxicity tolerance have been identified (Gourley et al. 1997). In ICRISAT—Latin American Partnership Sorghum Program, diverse sets of 378 pairs of A/B-grain sorghum lines, 784 grain sorghum restorers/varieties and 94 forage sorghum lines were screened during 1996–1998 at Cali, Quilichao, La Libertad, Carimagua and Matazul and selections were made based on early vigor (scale 1–5, where 1 = most vigorous, 5 = least vigorous), plant height (m) at maturity, stay green at maturity (scale 1–5, where 1 = most green, 5 = least green), grain yield (t ha^{-1}) and biomass (t ha^{-1}) in grain sorghum lines. Fresh forage weight, recovery score on 1–5 scale (1 = high recovery, 5 = less recovery) after the first cut, and tiller number were also used as an additional criteria for advancing the forage sorghum lines (Reddy et al. 2000).

3.3.4 Nutritional Quality Traits

Sorghum being one of the major food crops in the world and has predominant role in meeting the dietary energy and micronutrient requirements in the low income group populations, improving sorghum nutrition quality is of paramount importance.

3.3.4.1 Protein and β-Carotene Contents

Protein content is relatively more studied in sorghum where in high genetic variability reported. Gains in protein content were reported by various authors (Virupaksha and Sastry 1968; Ramesh and Hudda 1994; De Mesa-Stonestreet et al. 2010). The best method for phenotyping for protein content is through using Microkjeldahl method or Technicon autoanalyser (TAA) method (Johnson and Craney 1971; Jambunathan et al. 1983). A study on limited number of germplasm lines, hybrid parents in sorghum did not show appreciable variability for β-carotene content in sorghum (Reddy et al. 2005). Similar is the case with yellow endosperm lines where in the β-carotene did not exceed 1.1 ppm. For phenotyping for this trait, spectrophotometry can be followed but estimation using High-Performance Liquid Chromatography (HPLC) gives more accurate information.

3.3.4.2 Grain Fe and Zn Concentration

Large scale screening of sorghum core germplasm accessions, hybrid parents and commercial hybrids showed high genetic variability for grain Fe and Zn concentrations and most of this variation is heritable (Reddy et al. 2005; Kumar et al. 2012). Significant positive association exists between grain Fe and Zn concentrations (r^2 = 0.6–0.8) and it is possible to simultaneously improve both the traits (Kumar et al. 2009; Reddy et al. 2010b). Additive gene action plays significant role in conditioning the grain Zn concentration while both non-additive and additive gene actions condition the grain Fe concentration (Kumar et al. 2013). The Fe and Zn concentrations can be estimated using Inductively Coupled Plasma Spectrometry (Houk 1986). This is a precise but destructive and laborious method. Most rapid and low cost method for assessing grain Fe and Zn concentrations is by using X-ray fluorescence spectrometry (XRF) method which is non-destructive and can be used routinely to screen the breeding materials. There is high correspondence between the values obtained by both the methods indicating that XRF can be used for assessing grain Fe and Zn concentrations particularly for discarding the poor lines in the breeding material.

3.3.5 Fodder Quality Traits

Sorghum is an important source of fodder particularly in Asia and Africa. While it is mostly the stover used as animal feed in these areas, forage sorghums are quite popular in Americas, Europe and Australia.

3.3.5.1 Forage/Fodder Yield and Animal Feed Quality

Conventional laboratory analysis cannot cope with phenotyping the large set of sample entries from multidimensional sorghum improvement programs. Near Infrared Spectroscopy (NIRS) is a non-evasive technique that can be employed for phenotyping for relevant sorghum stover/forage/ fodder traits after calibration and validation with laboratory fodder traits obtained with conventional chemical and biological laboratory analysis. We summarize here the good-of-fitness NIRS equations used for the prediction of nitrogen, cell wall (NDF) cellulose (ADF), lignin (ADL), *in vitro* digestibility (IVOMD), *in vitro* metabolizable energy (ME) and *in vitro* fermentation kinetics using a modified exponent model that included a lag phase (Table 3.4).

Blummel et al. (Kumar et al. 2010) investigated 24 sorghum stovers with sheep for organic matter digestibility (OMD) and intake (OMI), and for digestible organic matter intake (DOMI) and for relations between above laboratory fodder quality traits and these *in vivo* measurements (Table 3.3). For each of the *in vivo* measurements, chemical (NDF, ADF, ADL) and *in vitro* (IVOMD, ME) traits were identified which accounted for at least 50 % of the variation in the respective

Table 3.4 Blind prediction of chemical and *in vitro* fodder quality nutritional traits of sorghum stover by near infrared spectroscopy

Trait	Range		Mean	SD	SEP	R^2
N	0.1	2.5	0.7	0.4	0.1	0.94
NDF	34.2	84.3	66.8	6.5	2.7	0.83
ADF	27.5	60.1	42.6	6.5	2.0	0.91
ADL	1.7	12.1	4.6	1.9	0.6	0.82
ME	4.7	12.0	7.2	1.0	0.3	0.91
IVOMD	30.0	77.8	49.0	6.6	1.9	0.90
A	33.3	75.3	53.6	7.0	3.5	0.76
C	0.02	0.07	0.04	0.01	0.004	0.57
Lag	10.2	3.6	−1.9	3.11	0.8	0.92
T_{50}	6.3	33.00	16.0	4.4	1.8	0.82

animal performance traits. Using multiple regression procedures and stringent cross-validation ("*blind-predictions*") procedures OMD, OMI and DOMI could be predicted with R^2 for comparing observed and predicted values of 0.36, 0.65 and 0.75, respectively.

3.3.5.2 Stalk Sugar Traits

Sweet sorghum is a multi-purpose crop that yields food, fodder and fuel. It is being used for syrup and ethanol production in USA (http://nssppa.org/Sweet_Sorghum_FAQs.html verified on 5th December 2012) EU (http://esse-community.eu/verified on 5th December), China, Philippines, Mali, India and other countries (Reddy et al. 2008, 2010b; Wortmann et al. 2010). Phenotyping for stalk sugar and related traits in sweet sorghum is done by recording observations on the fresh stalk yield (t ha^{-1}), stem girth (cm), soluble solids concentration (°Bx), juice yield (t ha^{-1}) and/or juice volume (l t^{-1}), juice extraction (%) and sugar yield is extrapolated using the equation sugar yield (t ha^{-1}) = Juice yield × Brix (%) × 0.75 (Blümmel et al. 2010).

Sorghum improvement has come a long way from using simple classical methods like mass selection to advanced level of selection using molecular markers for trait improvement. Efforts are underway to use new genomic tools for sorghum improvement facilitated by the availability of aligned genome sequence. While the genotyping tools are increasingly available and more affordable now, the phenotyping is not receiving equal attention. One should keep in mind that without good quality phenotyping data, the genotyping data is of no use, no matter how it was generated. Therefore the progress in sorghum improvement in the years to come depends upon the quality of the phenotyping data that we generate for traits of interest and most appropriate use of genomic tools available.

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