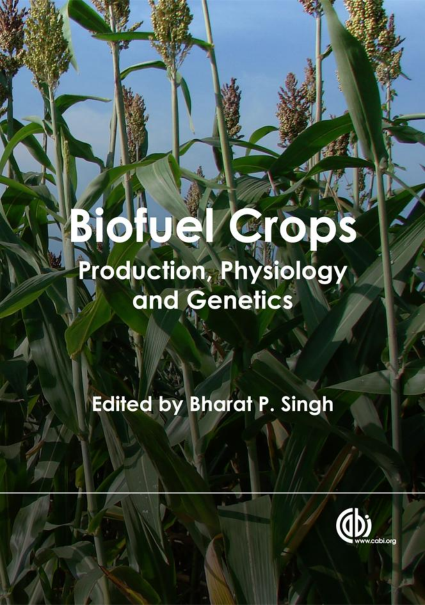

Biofuel Crops

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9 Sweet Sorghum: Genetics, Breeding and Commercialization

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9.1 Introduction

Sweet sorghum, similar to grain sorghum except for its juice-rich sweet stalk, has been grown in the USA (for syrup) and Africa (for fodder) for many centuries and is considered to be a potential bioethanol feedstock, and is expected to meet food, feed, fodder, fuel and fibre demands. Some sweet sorghum lines attain juice yields of about 78% of total plant biomass, containing from 15 to 23% soluble fermentable sugar (by comparison, sugarcane has 14–16%) (Reddy *et al.*, 2008). The sugar is composed mainly of sucrose (70–80%), fructose and glucose. Most of the sugars are distributed in the stalk, with about 2% in the leaves and inflorescences (Vietor and Miller, 1990), making the crop particularly amenable to direct fermentable sugar extraction.

9.2 Sweet Sorghum Characteristics and Utilization

The term sweet sorghum is used to distinguish varieties of sorghum with high concentration of soluble sugars in the plant stalk sap or juice compared to grain sorghum, which has relatively less sugar and juice in the stalks. Sweet sorghum is a C_4 species with wide, flat leaves and a round or elliptical head full of grain at the stage of maturity. It has, like grain sorghum, traditionally been under cultivation for nearly 3000 years. It can be grown successfully in the semi-arid tropics, where other crops fail to thrive, and is highly suitable for cultivation in tougher dryland growing areas. It can produce very high yields with irrigation. During very dry periods, sweet sorghum can go into dormancy, with growth resuming when sufficient moisture levels return (Gnansounou *et al.*, 2005). It can be grown easily on all continents, in tropical, subtropical, temperate, semi-arid regions as well as in poor quality soils. It is known as the sugarcane of the desert and also 'the camel among crops' for its drought-hardy characteristics (Sanderson *et al.*, 1992). It has higher drought tolerance and water use efficiency (WUE) compared to maize, and yields, like those of *Miscanthus*, range from 18 to 36 dry t ha^{-1} biomass $year^{-1}$ on low-quality soils with minimal inputs of fertilizer and water. In Indiana (USA), studies showed that sweet sorghum cultivars produce 25 to 40 t dry mass ha^{-1} with an input of from 0 to 60 kg ha^{-1} of nitrogen fertilizers. The high WUE and low N requirements of sorghum also

Biofuel Crops: Production, Physiology and Genetics (ed. B.P. Singh) provide significant advantages to the growers, because sorghum fits into a normal rotation scheme with maize and soybean, yet has lower production costs and employs similar production equipment. Its ratooning ability enables multiple harvests per season, a feature that could expand the geographical range of sorghum cultivation. For example, in Nebraska (USA), cold-tolerant sweet sorghum planted in April yielded 22 t dry biomass ha^{-1}, and a ratoon crop harvested from the same material in mid-October gave an additional 12 t ha^{-1} (Ali *et al*., 2008). The grain stalk juice and bagasse (the fibrous residue that remains after juice extraction) can be used to produce food, fodder, ethanol and power. Owing to these favourable attributes, it is often referred to as a 'smart' crop (William D. Dar, Director General, ICRISAT) (Fig. 9.1).

Sweet sorghum candidate traits in relation to utilizable options are listed in Table 9.1. These important characteristics, along with its suitability for seed propagation, mechanized crop production and comparable ethanol production capacity compared to sugarcane and sugarbeet makes sweet sorghum a viable alternative source for ethanol production (Table 9.2).

Fig. 9.1. Improved sweet sorghum cultivar 'ICSV 25274'.

9.2.1 Food–fuel trade-off

It is often stated that sweet sorghum cultivars do not produce grain yield or the grain yield is very less compared to that of grain sorghum. Studies at ICRISAT showed that sweet sorghum hybrids had higher stem sugar yield (11%) and higher grain yield (5%) compared to grain sorghum types, while sweet sorghum varieties had 54% higher sugar yield and 9% lower grain yield compared to non-sweet stalk varieties in the rainy season. On the other hand, both sweet sorghum hybrids and varieties had higher stalk sugar yields (50% and 89%) and lower grain yields (25% and 2%) in the post-rainy season. Thus, there is little trade-off between grain and stalk sugar yields in the sweet sorghum hybrids in the rainy season while the trade-off is less in varieties in the post-rainy season (Srinivasa Rao *et al*., 2009, 2010; Kumar *et al*., 2010).

This is further supported by other published work (Zhao *et al*., 2009) showing that there is significant soluble sugars content in the stems (79–94%) during the post-anthesis period, with the hybrids exhibiting significantly high soluble sugars content over varieties with the same maturity period, and effects of year, harvest time and genotype on calculated ethanol yield (CEY) are highly significant. The experimental data on the relationship between stalk sugar traits and grain yield showed that the regression coefficient of stalk sugar yield on grain yield is not significant, thereby indicating that the grain yield is not affected when selection is done for stalk sugar yield. Hence a selection programme can aim to improve both the traits simultaneously. When sweet sorghum complements sugarcane production, this does not require additional land area, but simply the insertion of a cycle of sweet sorghum between two cycles of sugarcane. The impact on food security is less, even if sweet sorghum replaces a cycle of legumes (groundnut, cowpea).

Table 9.1. Candidate traits of sweet sorghum as biofuel feedstock (Reddy *et al.*, 2005, 2008; Srinivasa Rao *et al.*, 2009, 2010).

As crop	As ethanol source	As bagasse	As raw material for industrial products
Short duration (3–4 months) C_4 dryland crop Good tolerance of biotic and abiotic constraints Meets fodder and food needs Non-invasive species Low soil N_2O and CO_2 emission Seed propagated	Amenable to ecofriendly processing Less sulfur in ethanol High octane rating Automobile friendly (up to 25% of ethanol–petrol mixture without engine modification)	High biological value Rich in micronutrients Use as feed, for power co-generation or biocompost Good for silage making	Cost-effective source of pulp for paper making Dry ice, acetic acid, fuel oil and methane can be produced from the co-products of fermentation Butanol, lactic acid, acetic acid and beverages can be manufactured

Table 9.2. Comparison of sweet sorghum with other bioethanol feedstocks (Reddy *et al.*, 2008; Almodares and Hadi, 2009; Srinivasa Rao *et al.*, 2009; Girase, 2010; Wortmann *et al.*, 2010).

Characteristics	Sugarcane	Sugarbeet	Maize	Sweet sorghum
Crop duration	12–13 months	5–6 months	3–4 months	4 months
Growing season	One season	One season	All seasons	All seasons
Propagation	Setts (40,000 ha^{-1})	Seed (3.6 kg ha^{-1}; pellet)	Seed (25 kg ha^{-1})	Seed (8 kg ha^{-1})
Soil requirement	Grows well in drained soil	Grows well in sandy loam; also tolerates alkalinity		All types of drained soil
Water management	Requires water throughout the year (36,000 m^3 ha^{-1})	Requires water, 40–60% compared to sugarcane (18,500 m^3 ha^{-1})	Requires water (12,000 m^3 ha^{-1})	Less water requirement; can be grown as rain-fed crop (8000 m^3 ha^{-1})
Crop management	Requires good management 250 to 400N: 125P:125K	Requires moderate management 120N:60P:60K	Requires good management 130N:60P:60K	Easy management; low fertilizer 90N:40P
[a]Stalk/beet/grain yield (t ha^{-1})	60–85	85–100	5–10	45–65
Sugar content on weight basis	10–12%	15–18%		7–12%
Sugar yield (t ha^{-1})	5–12	11.25–18		3–7
Ethanol yield from juice (l ha^{-1})	4,350–7,000	7,100–10,500	2,150–4,300	2,475–3,500
Harvesting	Harvested mechanically	Harvested mechanically	Harvested mechanically	Very simple; predominantly manual and mechanical harvesting at pilot scale

[a] Stalk yield for sugarcane and sweet sorghum; beet yield for sugarbeet; and grain yield for maize.

9.3 Climate and Distribution

Sorghum (*Sorghum bicolor* (L.) Moench) is the fourth major cereal crop of the world in production and fifth in hectarage after wheat, rice, maize and barley. It is mostly grown in the semi-arid tropics (SAT) of the world wherein the production system is constrained by poor soils, low and erratic rainfall and low inputs resulting in low productivity. India is the largest sorghum grower in the world (7.7 Mha) followed by Sudan (5.6 Mha) and Nigeria (4.7 Mha). The USA is the largest producer followed by India and Nigeria (FAO, 2012). Sorghum is well adapted to the SAT and is one of the most efficient dryland crops to convert atmospheric CO_2 into sugar (Schaffert and Gourley, 1982). The crop can be grown in a wide range of climatic conditions as given below.

- Latitude: sweet sorghum can be grown between 40°N and 40°S latitude on either side of the Equator.
- Altitude: sorghum can be found at elevations between sea level and 1500 m. Most East African sorghum is grown between altitudes of 900 and 1500 m, and cold-tolerant varieties are grown between 1600 and 2500 m in Mexico.
- Temperature: sweet sorghum can be grown in the temperature range from 12 to 37°C and optimum temperature for growth and photosynthesis is from 32 to 34°C, day length from 10 to 14 h, optimum rainfall 550–800 mm and relative humidity 15–50%.
- Soils: alfisols (red) or vertisols (black clay loamy) with pH 6.5–7.5, organic matter >0.6%, depth >80 cm, bulk density <1.4 gcc, water holding capacity >50% field capacity, N >260 kg ha^{-1} (available), P >12 kg ha^{-1} (available), K >120 kg ha^{-1} (available) are optimal soil conditions for sorghum growth.
- Water: whilst sorghum will survive with a supply of less than 300 mm over the season of 100 days, it responds favourably with additional rainfall or irrigation water. Typically, sweet sorghum needs between 500 and 1000 mm of water (rain and/or irrigation) to achieve good yields, i.e. 50–100 t ha^{-1} total above ground biomass (fresh weight). Though sorghum is a dryland crop, sufficient moisture availability for plant growth is critically important for high yields. The great advantage of sorghum is that it can become dormant, especially in the vegetative phase, under adverse conditions and can resume growth after relatively severe drought. Early drought stops growth before panicle initiation and the plant remains vegetative; it will resume leaf production and flowering when conditions become favourable for growth again. Mid-season drought stops leaf development. Sorghum is susceptible to sustained flooding, but will survive temporary waterlogging much better than maize.
- Radiation: being a C_4 plant, sweet sorghum has high radiation use efficiency (RUE: about 1.3–1.7 g MJ^{-1}). It has been shown that taller sorghum types possess higher RUE because of better light penetration in the leaf canopy.
- Photoperiodism: most hybrids of sweet sorghum are relatively less photoperiod-sensitive. Traditional farmers, particularly in West Africa, use photoperiod-sensitive varieties. With photoperiod-sensitive types, flowering and grain maturity occurs almost during the same calendar days regardless of planting date, so that even with delayed sowing, plants mature before soil moisture is depleted at the end of the rainy season.

Based on the available literature, an attempt was made to depict diagrammatically the critical traits that have significant bearing on bioethanol productivity of the crop with regard to phenology (Fig. 9.2).

9.4 Taxonomy, Botanical Description and Reproductive Biology

9.4.1 Taxonomy and distribution

The genus *Sorghum* is a variable genus with 24 species divided into five subgeneric sections based on taxonomic differences: Eu-sorghum, Chaetosorghum, Heterosorghum, Para-sorghum and Stiposorghum (de Wet, 1978; Price *et al*., 2006). *S. bicolor, S. arundinaceum, S. drummondii,*

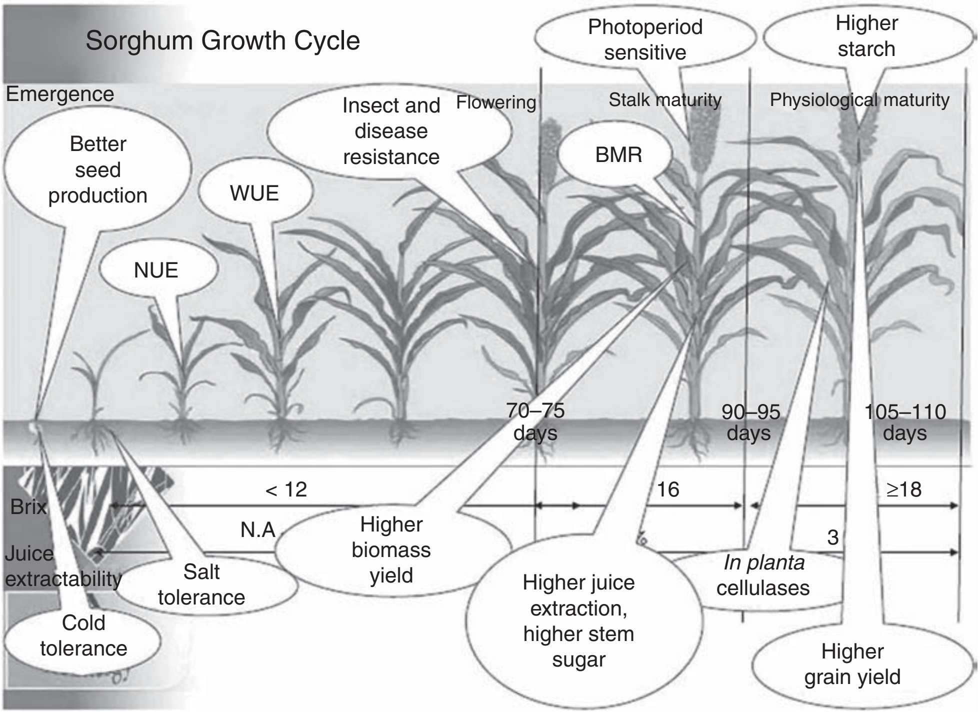

Fig. 9.2. Critical traits to be focused on for genetic enhancement in order to maximize bioethanol productivity in sweet sorghum (Gibson, 2010).

S. halepense, S. propinquum and *S. almum* form the section Eu-sorghum. The first three species form the primary gene pool while the latter three species form the secondary gene pool of sorghum. All species other than the Eu-sorghum section form the tertiary gene pool (Dahlberg, 2000). The species *S. bicolor* (2n = 20) is further divided into three subspecies *bicolor, arundinaceum* and *drummondii*. Subspecies *bicolor* includes all cultivated races and they are further divided into basic and intermediate races. The five basic races include bicolor, guinea, caudatum, kafir and durra and the ten intermediate races are those between any two of those types, classified primarily based on grain shape, glumes and panicle.

9.4.2 Reproductive biology

Breeding procedures that are used with a particular crop species are determined by its mode of reproduction. Understanding the details of phenology, i.e. floral biology, pollination, fertilization and seed development (Fig. 9.2) in a crop make it possible to develop orderly and efficient breeding procedures.

Panicle initiation

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). Tropical sweet sorghum varieties initiate the reproductive stage when day lengths return to 12 h. Floral initiation takes place 30 to 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 growth due to meristematic activity. The time required for transformation from the vegetative apex to reproductive apex is largely influenced by genetic characteristics and the environment (photoperiod and temperature). The grand period of growth in sorghum follows the formation of a floral bud and consists largely of cell enlargement. Hybrids take less time to reach panicle initiation and are relatively less influenced by photoperiod and temperature (Srinivasa Rao *et al.*, 2009).

Panicle emergence

During the period of rapid cell elongation, floral initials develop into an inflorescence. About 6–10 days before flowering, the boot will form as a bulge in the sheath of the flag leaf. This will occur, in a variety that flowers in 60–65 days, about 55 days from germination. Sorghum usually flowers in 55 to more than 70 days in warm climates, but flowering may range from 30 to more than 100 days. These observations are valid for tropical sweet sorghums, while temperate sorghums that mature in 5 months take 20–30 days longer for panicle emergence.

Panicle structure

The inflorescence is a raceme, which consists of one or several spikelets. It may be short, compact, loose or open, composed of a central axis that bears whorls of primary branches on every node. The racemes vary in length according to the number of nodes and the length of the internodes. Each primary branch bears secondary branches, which in turn bear 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 pediceled spikelets. On the pediceled spikelet, the pedicels vary in length from 0.5 to 3.0 mm, and usually are very similar to the internodes. The first and second glumes of every spikelet enclose two florets; the lower one is sterile and is represented by a lemma, and the upper fertile floret has a lemma and palea. Two lodicules are placed on either side of the ovary at its base. The androecium consists of one whorl of three stamens. The anthers are attached at the base of the ovule by a very fine filament and are versatile and yellowish. The gynoecium is centrally placed and consists of two pistils with one ovule from which two feathery stigmas protrude. Many of these floral characters, such as anther colour, stigma colour, stigma length, length of pedicel, etc. are important traits for cultivar identification and classification.

Sessile spikelets

The sessile spikelet contains a perfect flower. It varies in shape from lanceolate to almost rotund and ovate and is sometimes depressed in the middle. The colour is green at flowering, which changes to different colours such as straw, cream, yellow, red, brown, purple, or almost black at grain maturity. The intensity and extent of colouring on the glumes is variable. Glumes vary from quite hairy to almost hairless. The seed may be enclosed by the glume or may protrude from it, being just visible to almost completely exposed.

Pedicelled spikelets

These are much narrower than the sessile spikelets, usually lanceolate in shape. They can be smaller, the same size, or longer than the sessile spikelets. They possess only anthers but occasionally have a rudimentary ovary and empty glumes.

Anthesis and pollination

Anthesis starts after panicle emergence from the boot leaf. Flowers begin to open 2 days after full emergence of the panicle. Floret opening or anthesis is achieved by swelling of the lodicules, and is followed by the exertion of anthers on long filaments and of stigmas from between the lemma and palea. The sorghum head begins to flower at its tip and flowers successively downward over a 4 or 5 day period. Flowering takes place first in the sessile spikelets from top to bottom of the inflorescence. It takes about 6 days for completion of anthesis in the panicle with maximum flowering at 3 or 4 days after anthesis begins. Flowering proceeds downwards to the base in a horizontal plane on the panicle. When flowering of the sessile spikelets is halfway down the panicle, pedicellate spikelets start to open at the top of the panicle and proceed downwards. The flowering phase of pedicellate spikelets overtakes the flowering phase of sessile spikelets before they reach the base of the inflorescence (Maiti, 1996). Anthesis takes place during the morning hours, and frequently occurs just before or just after sunrise, but may be delayed on cloudy damp mornings. It normally starts around midnight and proceeds until 10am depending on the cultivar, location and weather. Maximum flowering is observed between 6 and 8:30am. 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, 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 air. Pollen remains viable several hours after 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. Glumes close shortly after pollination, though the empty anthers and stigmas still protrude (except in the long glumed types). The florets of some of the very long-glumed types do not open for fertilization, a phenomenon known as cleistogamy.

Cytoplasmic male sterility has been found in sorghum (A1–A4 systems) and has made possible the development of a hybrid seed industry. A good male-sterile plant will not develop anthers, but in some instances dark-coloured shrivelled anthers with no viable pollen will appear. Partially fertile heads are also observed, and although the anthers frequently have viable pollen, the quantity is less than in normal plants.

Seed

The seed is a fruit or caryopsis. The ovule begins to develop as a light green, almost cream-coloured sphere; after about 10 days it begins to take size and becomes darker green. Maturity of grain follows a similar pattern to flowering. The development of grains follows a sequence of stages comprising milky, soft dough, hard dough to the final physiological maturity, when a black layer is formed at the hilar region due to the formation of callus tissue. It takes about 30 days for the seeds to reach maximum dry weight (physiological maturity). The seeds contain about 30% moisture at physiological maturity; they dry to about 10–15% moisture during the following 20–25 days (House, 1980). The crop can be preferably harvested at physiological maturity to take advantage of stalk sugars and grain. The seeds harvested and dried at physiological maturity have good quality and fetch a higher market price (Audilakshmi *et al.*, 2005). Seed size varies from very small (less than 1 g/100 seeds) to large (5 to 6 g/100 seeds).

9.5 Genetics

9.5.1 Genomics

Sorghum is an important target of genome analysis and genomic tool building among the C_4 grasses because the sorghum genome is relatively small (730 Mbp) (Paterson *et al.*, 2009), the cultivated species is diploid ($2n = 20$) and the sorghum germplasm is diverse (Dje *et al.*, 2000; Menz *et al.*, 2004; Casa *et al.*, 2005). The sorghum genome is appreciably smaller and less complex than the maize genome, and as a member of the *Saccharinae* subtribe, it is the ideal model for genetic studies as its fellow members sugarcane and *Miscanthus* are both polyploids that do not succumb easily due to sterility issues. As a consequence, numerous sorghum genetic, physical and comparative maps are available (Tao *et al.*, 1998; Boivin *et al.*, 1999; Peng *et al.*, 1999; Klein *et al.*, 2000; Haussmann *et al.*, 2002; Menz *et al.*, 2002; Bowers *et al.*, 2003). High-density reference maps of one intraspecific *S. bicolor* (Xu *et al.*, 1994; Bhattramakki *et al.*, 2000; Klein *et al.*, 2000; Menz *et al.*, 2002) and one interspecific *S. bicolor* × *S. propinquum* (Chittenden *et al.*, 1994; Bowers *et al.*, 2003) cross provide about 2600 sequence-tagged sites (based on low-copy probes that have been sequenced), 2454 AFLP and about 1375 sequence-scanned (based on sequences of genetically anchored BAC clones) loci. The two maps share one common parent (*S. bicolor* 'BT × 623') and are essentially co-linear (Feltus *et al.*, 2006). More than 800 markers mapped in sorghum are derived from other grasses (serve as comparative anchors), and additional sorghum markers have been mapped directly in other grass species or can be plotted based on sequence similarity. Anchoring of the sorghum maps to those of rice (Paterson *et al.*, 1995, 2004), maize (Whitkus *et al.*, 1992; Bowers *et al.*, 2003), sugarcane (Dufour *et al.*, 1997; Ming *et al.*, 1998), millet (Jessup *et al.*, 2003), switchgrass (Missaoui *et al.*, 2005), bermuda grass (Bethel *et al.*, 2006) and others provides for the cross-utilization of results to simultaneously advance knowledge of many important crops. To have a genetic, physical and cytological perspective of the *Sorghum bicolor* genome, Kim *et al.* (2005) selected 40 BAC clones that had different linkage group markers, 21 from linkage group 2 and 19 from linkage group 8. Multi-BAC probe cocktails were constructed for each chromosome from the landed BACs, which were also pre-evaluated for fluorescence *in situ* hybridization (FISH) signal quality, relative position and collective chromosome coverage. Comparison to the corresponding linkage map revealed full concordance of locus order between cytological and genetic analyses. A sorghum EST project (Pratt *et al.*, 2005) and associated microarray analyses of sorghum gene expression have been carried out (Buchanan *et al.*, 2005; Salzman *et al.*, 2005), a comprehensive analysis of sorghum chromosome architecture has been completed (Kim *et al.*, 2005), and an 8x draft of the sorghum genome sequences was released by the US Department of Energy's Joint Genome Institute and Center for Integrative Genomics in 2007 (http://www.phytozome.net; Paterson *et al.*, 2009). Globally, a total of 167,890 sorghum accessions are reported held in different germplasm collection centres representing about 86% of the total 194,250 accessions that have been documented in the Bioversity Germplasm Database (January 2006). Of the 167,890 accessions, the USDA germplasm collection maintains 42,614 sorghum accessions, of which more than 800 exotic landraces have been converted to day-length-insensitive lines to facilitate their use in breeding programmes. ICRISAT, for which sorghum is one of its mandated crops, is one of the major repositories, holding a total of 36,774 accessions (21.9%) from 91 countries (Reddy *et al.*, 2006). A preliminary survey indicated that the largest number of accessions (47,963; 28.6%) was held by gene banks in Asia, including China and India. An approximate total of 31,200 sorghum accessions (16.1%) were held in African gene banks, with East Africa holding larger collections of sorghum landraces than South and West Africa.

A set of mutation stocks, developed by the USDA Plant Stress and Germplasm Development Unit in Lubbock, Texas, USA (Xin *et al.*, 2008), is sufficiently extensive to allow identification of mutations in virtually every sorghum gene. Such genomic tools, already in place, will greatly facilitate the introduction of traits required to optimize sweet sorghum for bioenergy production schemes. The best known such mutations are the brown midrib (BMR). The brown midrib (*bmr*) mutations were first discovered in maize in 1926. Early studies revealed the trait resulted in lower fibre and lignin within the plant and could increase the conversion efficiency of sorghum biomass for lignocellulosic bioenergy. In sorghum, more than 19 *bmr* mutants were discovered by Porter *et al.* (1978). The *bmr* mutants are characterized by the reddish-brown coloration of the vascular tissue of the leaf blade, leaf sheath and stem, which is associated with alteration of secondary cell wall composition, especially lignin. Owing to the development of biocatalysts (e.g. genetically engineered enzymes, yeasts and bacteria), it is possible to produce ethanol from any plant or plant part containing lignocellulose biomass, including cereal crop residues (stovers). Sorghum stover also serves as an excellent feedstock for ethanol production. Stover contains lignin, hemicellulose and cellulose. Since the hemicelluloses and cellulose are enclosed by lignin (which is a phenolic polymer), it is difficult to convert them into ethanol, thereby increasing the energy requirement for processing. The *bmr* mutant sorghum, pearl millet (*Pennisetum glaucum*) and maize lines have significantly lower levels of lignin content (51% less in their stems and 25% less in their leaves). Purdue University research showed 50% higher yield of fermentable sugars from the stover of certain sorghum *bmr* lines after enzymatic hydrolysis. Therefore, the use of *bmr* cultivars would reduce the cost of biomass-based ethanol production. The *bmr* crop residues have higher rumen digestibility and palatability, making them good for fodder, too.

The availability of the genomic sequence for sorghum has made it possible to carry out genome-wide analyses. Whereas earlier studies on simple sequence repeats (SSR) marker development primarily utilized anonymous DNA fragments containing SSRs isolated from genomic libraries, more recent studies have used computational methods to detect SSRs in sequence data generated from genomic sequences projects (Manli *et al.*, 2009). In the sorghum genome, a total of 109,039 tandem repeats were detected, of which 15,194 were microsatellite (SSR) (Paterson *et al.*, 2009). Mining the frequency and density of the SSRs showed that the density was 154.98 counts Mbp^{-1} in sorghum genomes. Trinucleotide repeat (27.35%) motifs appear to be the most abundant type in sorghum, while the dinucleotide, tetranucleotide, hexanucleotide, pentanucleotide and mononucleotide repeats are 20.69%, 17.04%, 15.21%, 14.90% and 4.76%, respectively. The A-rich repeats are predominant in the most frequent SSRs. The SSR sizes are not evenly distributed, and hexa-motif gives the longest SSRs. This has provided a valuable resource to develop large numbers of SSR markers in sorghum. The genome sequence has also been used to investigate the diversity of *S. bicolor* resistance (R) genes and assess their importance in the mechanisms of disease resistance. The R genes were characterized based on their structural diversity, physical chromosomal location and phylogenetic relationships. Based on their N-terminal motifs and leucine-rich repeats (LRR), 50 non-regular nucleotide binding site (NBS) genes and 224 regular NBS genes were identified in 274 candidate NBS genes. The vast majority (97%) of NBS genes occurred in gene clusters, indicating extensive gene duplication in the evolution of *S. bicolor* NBS genes (Cheng *et al.*, 2010). Based on the location of individual NBS genes, 268 *Sorghum* NBS-encoding genes were mapped on the ten chromosomes.

Sorghum is extremely well-suited to association mapping methods (Hamblin *et al.*, 2005). Its largely self-pollinating mating system tends to preserve linkage relationships for longer periods than in largely outcrossing crops such as maize. Self-pollination also obviates the need to develop inbred lines. Hamblin *et al.* (2004) reported that linkage disequilibrium (LD) over very short distances in sorghum was more extensive than in maize, suggesting that sorghum may be suitable for LD mapping of genes underlying complex, agronomically important traits common to both species. More than 750 SSR alleles and 1402 single nucleotide polymorphic (SNP) alleles discovered in 3.3Mb of sequence (Gingle *et al.*, 2007) are freely available from the Comparative Grass Genomics Center relational database. In a grain sorghum panel of eight accessions, Nelson *et al.* (2011) reported 283,000 SNPs, which will greatly help in designing genotyping strategies. The sorghum SNP and indel data have been deposited in dbSNP under handle JCNLAB_KSU, with accession number ranges starting at 410962044 (SNPs) and 411578970 (indels).

Genetic synteny among C_4 grasses means that gene discovery in any one can readily be translated to genetic improvement of all bioenergy grass crops (Carpita and McCann, 2008; Vermerris, 2011). The diploid genomes of representative genotypes of sorghum (Paterson *et al.*, 2009) and maize (Schnable *et al.*, 2009) have been sequenced. Plant geneticists and breeders can now use genome-wide association studies (Gore *et al.*, 2009) to identify genes that are responsible for desirable phenotypes, and use genomic selection (Jannick *et al.*, 2010) to readily incorporate favourable alleles into breeding lines. Large panels of maize and sorghum accessions representing much of the worldwide genetic diversity for these species have been densely genotyped and offer powerful experimental systems for association genetics approaches to discover and validate key alleles relevant to biomass production (Buckler *et al.*, 2009; McMullen *et al.*, 2009).

Application of recent advances in phenotyping to the available populations for association genetics (Springer *et al.*, 2009; Springer and Jackson, 2010) can accelerate gene discovery (Tuberosa and Salvi, 2006; Famoso *et al.*, 2010), as demonstrated with the maize Nested Association Mapping resource. Such approaches are already in progress for traits important to bioenergy grasses. Spectroscopic and spectrophotometric screens offer a high-throughput method to characterize variation in biomass composition, even in the absence of visible phenotypes (McCann *et al.*, 2007;

Penning *et al.*, 2009). Grass species produce three primary forms of harvestable carbon: starchy grains, soluble sugar in the stem and lignocellulosic biomass. Molecular phenotyping has provided insights into the genetic basis for developmental and metabolic regulation of carbon partitioning and storage, which is essential to rapidly optimize feedstocks, either alone or in combination. Finally, detailed studies of root architecture help characterize novel traits such as root-system carbon mass, and provide greater insight into nutrient-use efficiency, drought tolerance and toxic-metal stress (Zhao *et al.*, 2004).

In a recent study, Murray *et al.* (2008b) have identified QTLs for grain and stem sugar composition and yield, and their results indicated that overall energy yields could be increased by concurrent improvement for both sorghum grain and sugar traits. Elucidating the genetic basis of stem sugar and stem juice accumulation, modifying cell wall composition so that sorghum biomass can be processed more efficiently, maximizing biomass yield for a given geographic area and production system, and understanding the different mechanisms underlying drought tolerance are the main focus areas among sorghum researchers who target bioenergy traits.

9.5.2 Genetic transformation

With the availability of the complete genome sequence in sorghum (Paterson *et al.*, 2009), it is also possible to explore functions of the reported genes using transgenic approaches. Transformation also offers a route to broaden the input and output traits for sorghum breeding programmes. The candidate traits for sweet sorghum improvement using genetic transformation are discussed briefly below.

Stalk sugar accumulation

Transgenic approaches to improve stem sugar accumulation have not been attempted in sweet sorghum. However, differential expression of some genes related to sucrose metabolism has been observed between sweet sorghum and grain sorghum (Qazi *et al.*, 2012). Further, mature internodes of sweet sorghum showed a lower expression of sucrose transporters suggesting that sucrose accumulation may result from lower transport of sucrose from sink tissues. These genes could serve as important candidate genes for transforming sorghum to achieve better stem sugar yields. However, genetic manipulation of some key enzymes involved in sucrose metabolism did not bring about greater sucrose accumulation in the mature internodes of sugarcane, suggesting their inadequacy in overcoming the osmotic limits of the sugar-storing vacuoles (Wu and Birch, 2010). Sugarcane transgenics expressing a bacterial sucrose isomerase gene or a sucrose–sucrose fructosyl transferase gene led to synthesis of isomaltulose and β-2,6-linked fructans, which are not normally found in sugarcane (Arruda, 2011). Synthesis of these less osmotically active carbohydrates in the vacuoles of storage parenchyma cells removed osmotic constraints and led to greater sucrose accumulation. A microRNA miR169 was recently shown to be involved in regulating sugar levels in sweet sorghum stems (Calvino *et al.*, 2011), suggesting epigenetic regulation of sucrose accumulation, which could be tackled using transgenic techniques.

Abiotic and biotic stress tolerance

The productivity of sweet sorghum is limited by water availability and the crop requires frequent irrigation during its growth period to achieve economically viable yields of grain and sugar. Stress-induced signalling intermediates and transcription factors are known to regulate expression of a large number of diverse downstream genes and have emerged as potential candidate genes for plant transformation. Transformation of sorghum with signalling intermediates such as calcium-dependent protein kinases did not help in improving abiotic stress tolerance, probably due to pleiotropic effects of this general signalling intermediate (Mall *et al.*, 2011). Transgenics expressing transcription factor coding genes *DREB* (Dubouzet *et al.*, 2003), *MYC, MYB* (Abe *et al.*, 2003) and *WRKY* (Wang *et al.*, 2007) were reported to show improved stress tolerance in crop plants, but no sorghum transgenics for these transcription factors has been reported so far. Cross-talk between drought and carbohydrate metabolism has been indicated by the discovery that promoters of drought-induced transcription factors (DREB proteins) show an over-representation of motifs related to sugar signalling (Srivastava *et al.*, 2010). Alteration in the expression levels of these transcription factors through genetic transformation may provide a useful means of improving drought tolerance and manipulating sugar metabolism in sorghum, which is yet to be tested.

Sweet sorghum plants are subjected to attack by a large number of pests, pathogens and parasitic plants such as striga (Aly, 2007; Maqbool *et al.*, 2001; Guo *et al.*, 2011). The success of the *Bt* technology in maize (Armstrong *et al.*, 1995; Barry *et al.*, 2000) and cotton (Cattaneo *et al.*, 2006) is a strong rationale for the evaluation of this technology in this feedstock as a means to combat specific target insect pests. Tolerance against insect pests (spotted stem borer) was reported in sorghum transgenics carrying the *Cry1Ac* gene (Girijashankar *et al.*, 2005). Methods to control fungal infestations such as anthracnose, including expression of chitinase genes from *Trichoderma*, have led to improved tolerance to this fungal disease of sorghum (Kosambo-Ayoo *et al.*, 2011). A number of viral agents have been shown to be capable of replication in sorghum (Jensen and Giorda, 2008), which includes sugarcane mosaic virus, maize dwarf mosaic virus and sorghum mosaic virus. Introduction of viral coat protein or replicase genes in transgenic plants has been shown to lead to virus resistance (Abel *et al.*, 1986; Nelson *et al.*, 1987; Stark and Beachy, 1989) and offers great potential for the introduction of durable virus resistance for sorghum. Striga is a parasitic plant species known to infect sorghum and other cereals (Aly, 2007). Silencing a critical gene in the parasitic plant's life cycle using RNAi technology has been successfully used in the *Orobanche aegyptiaca*/tomato host parasite interaction (Aly *et al.*, 2009), but such transgenic approaches have not been used in sorghum so far.

Delayed flowering

Delayed flowering enables sufficient biomass to be built before transition to the reproductive phase and therefore correlates to improved yields. MicroRNAs (miR172) that regulate flowering time have been identified in sorghum and over-expression of these miRNAs could be a useful strategy to delay flowering in sorghum (Calvino *et al.*, 2011). A PSEUDO RESPONSE REGULATOR 37 (SbPRR37) gene has been identified in sorghum, which inhibits floral induction through the suppression of expression of various floral activators. The expression of SbPRR37 was found to be light-dependent and under control of the circadian clock and provides the possibility of regulating flowering time through manipulating its expression (Calvino *et al.*, 2011). Genes associated with reproductive transition/inflorescence branching (*Sb-lfy* and *Sb-tfl*) and spikelet determinacy (*Sb-bd1* and *Sb-ids*) have been identified in sorghum, which also may serve as important candidate genes in manipulating flowering (Dwiwedi *et al.*, 2008).

Digestibility of sorghum grains

The grain-mould infected sorghum grain can be used for conversion to ethanol. The prolamin seed storage proteins of sorghum, called kafirins, are assembled into protein bodies, with a very defined pattern, which influence digestibility of sorghum protein. Oria *et al.* (2000) described a high digestible, enhanced lysine sorghum mutant in which the protein bodies were highly folded, with a redistribution of the different kafirins, which translated to a phenotype with increased digestibility (Duodu *et al.*, 2003). Like the maize *floury-2* and *opaque-2* mutants, the high digestible, enhanced lysine mutant of sorghum has value in both food and feed applications. However, there is a tendency of these altered prolamin grains to have reduced agronomic properties, and postharvest issues (Huang *et al.*, 2004). A transgenic approach to modulate the seed storage proteins, without negatively altering the endosperm characteristics, has emerged as an important target for improving digestibility of sorghum.

9.5.3 Genetic transformation methods

Genetic transformation of sorghum has been attempted using *Agrobacterium*-mediated

(Zhao *et al*., 2000; Howe *et al*., 2006; Arulselvi *et al*., 2010) and particle bombardment methods (Girijashankar *et al*., 2005; Raghuwanshi and Birch, 2010; Liu and Godwin, 2012). The explants used for transformation include immature embryo tissues (Howe *et al*., 2006; Gurel *et al*., 2009; Liu and Godwin, 2012) and shoot meristems (Devi *et al*., 2004; Pandey *et al*., 2010). Use of explant tissues having young cells, whose cell walls have a higher number of sites for *Agrobacterium* attachment (Verma *et al*., 2008), heat shock treatments to the explants (Gurel *et al*., 2009) and addition of polyvinyl pyrrolidone to the regeneration media on which the explants were cultured (Lu *et al*., 2009) were shown to bring about more efficient transformation. Safer selectable markers for transformation have been used such as the bacterial phosphomannose isomerase (*Pmi*) gene, which enables the transformed but not the non-transformed plants to metabolize mannose (Gurel *et al*., 2009). Methods to obtain marker-free transgenics of sorghum have also been developed (Lu *et al*., 2009). Native sorghum promoters that would be more effective in driving the expression of transgenes have been identified, which include the sucrose synthase gene promoter (Sivasudha and Kumar, 2008), a meristem-specific promoter (Verma and Kumar, 2005) and a wound-inducible promoter (Girijashankar *et al*., 2005). Transformation efficiency reported in sorghum ranges from 0.01 to 1.3% using biolistic transfer and from 0.8 to 7.6% using *Agrobacterium*-mediated transfer (Raghuwanshi and Birch, 2010), which is however still far lower than that achieved in other crops such as maize and rice.

With efficient sorghum transformation systems now being available, transgenic approaches to improve agronomic traits in sweet sorghum are not too distant a goal.

9.6 Breeding Sweet Sorghum

9.6.1 Breeding behaviour

Sorghum is basically a self-pollinating crop but natural cross-pollination varies from 0.6 to 6% depending on the cultivar. Sorghum has the advantage of possessing complete self-pollination due to its floral biology, cleistogamy and genetic and cytoplasmic genetic male sterility. Breeding methods relevant to self- as well as cross-pollinated crops are therefore applied to breed pure line varieties, hybrids and populations in sorghum. Stigmas exposed before the anthers dehisce are subjected to cross-pollination. Hand pollination should begin around 9:30 or 10am and can be extended up to 11:30am to 12:30pm on a foggy morning (House, 1980).

9.6.2 Candidate traits and variability

The major characteristics that a sweet sorghum cultivar should possess are:

1. High biomass productivity (45–80 t ha^{-1}).
2. High Brix% (18–20%).
3. Thick stems and juicy internodes with maintenance of stem juiciness until maturity.
4. Photo- and thermo-insensitivity so that it can be grown throughout the year and fit into diversified cropping systems.
5. Tolerance to shoot pests and diseases.
6. Good digestibility of residues when used as forage or for lignocellulosic ethanol production.
7. Tolerance to mid-season and terminal drought.
8. High water and nitrogen-use efficiencies.
9. Suitability for specific conversion technologies (*bmr*).
10. Grain yield (3.0–5.0 t ha^{-1}).

Ayyangar *et al*. (1936) suggested a single dominant gene conferring the non-sweet character. Guiying *et al*. (2000) reported that stalk sugar is under the control of recessive genes with additive and dominance effects. On the contrary, later studies provided support for the existence of multiple genes with additive effects. Continuous variation in the amount of extractable juice was observed in juicy genotypes and inbred progeny of juicy × dry lines, suggesting multiple genes may be involved in controlling the trait (Saballos, 2008).

Recent studies suggest the involvement of several genes affecting the biofuel traits in the sweet sorghum background. The evaluation of four promising sweet sorghum lines (‘Keller’, ‘BJ 248’, ‘Wray’ and ‘NSSH 104’ (‘CSH 22SS’) along with the check ‘SSV 84’ indicated substantial genotypic differences for extractable juice, total sugar content, fermentation efficiency and alcohol production (Ratnavathi *et al.*, 2003). An analysis of 53 ICRISAT-bred elite hybrids in both the rainy and post-rainy seasons showed that the correlation and regression coefficients are significantly high for all the component traits of sugar yield (Brix%, stalk yield, juice weight and juice volume) (Srinivasa Rao *et al.*, 2009). Knowing general (GCA) and specific (SCA) combining ability effects of genetic materials is of practical value in breeding programmes. GCA effects represent the fixable component of genetic variance, and are important to develop superior genotypes. SCA represents the non-fixable component of genetic variation and it is important to provide information on hybrid performance. The line × tester analysis of 171 hybrids along with their parents in both rainy and post-rainy seasons showed that the magnitude of SCA variance was higher, suggesting the importance of non-additive gene action in inheritance of sugar yield-related traits though both additive and dominant genes controlled overall sugar yield during both rainy and post-rainy seasons in tropical sweet sorghums. Hence, selection in early generations would be ineffective and recurrent selection with periodic intercrossing is advocated. However, breeding for good combining restorer parents can result in high sugar yield in the post-rainy season. There is an indication of existence of transgressive segregation for sugar yield that can be exploited (Reddy *et al.*, 2011). The heritability for traits such as stem juiciness, sugar concentration in stems, total sugar, juice glucose, juice fructose and juice sucrose was low (Murray *et al.*, 2008a).

The predominant role of non-additive gene action for plant height, stem girth, total soluble solids, millable stalk yield and extractable juice yield, substantial magnitude of standard heterosis for all the traits related to ethanol production (stem girth: up to 5.3%, total soluble solids: up to 7.4%, millable stalk yield: up to 1.5% and extractable juice yield: up to 122.6%) indicates the importance of heterosis breeding for improving these traits (Sankarapandian *et al.*, 1994). The significant positive correlation of GCA effects with per se performance of parents in sweet sorghum facilitates quicker identification and development of sugar rich, high biomass yielding hybrid parents (Selvi and Palanisamy, 1990). Makanda *et al.* (2009) and Srinivasa Rao *et al.* (2009) reported significant GCA effect for stem Brix and associated traits, implying the importance of additive gene action.

The generation mean analysis of two crosses has shown predominant additive gene action for traits such as sucrose percentage and Brix% of juice. However, for cane and juice yield, dominance gene action and dominance × dominance gene interaction were of higher magnitude in both the crosses. Since the traits important for high sugar content have dominance and over-dominance inheritance, utilization of hybrid vigour by developing sweet sorghum hybrids is an attractive option. Also one of the parents with high sucrose content will suffice in obtaining good hybrids with high sugar and juice yield (AICSIP, 2007).

From these studies, it is quite evident that significant diversity exists in traits important for biofuel production and this opens up excellent opportunities for sweet sorghum improvement. Biofuel traits are governed by multiple genes and both additive and dominance components of gene action have to be exploited while breeding for high stalk sugar and juice-yielding genotypes.

9.6.3 Breeding objectives

In general, the sweet sorghum breeding programmes would aim at development of hybrid parents and hybrids which can address both first and second generation (lignocellulosic feed stock development) biofuel production issues. The breeding objectives would be:

1. To develop sweet sorghum female parents with high stalk sugar and grain yield apart from brown midrib trait.
2. To develop restorer lines/varieties with high sugar content, brown midrib trait and resistance to stem borer and shootfly.
3. To develop and identify sorghum hybrids (amenable for mechanical harvesting) with high biomass suitable for use in bioethanol and bioenergy production.

9.6.4 Breeding methods

The most commonly used programmes in sweet sorghum improvement are short-term programmes (pedigree method and backcross) and long term programmes (population improvement methods).

Short-term approach

The most common short-term approach in sweet sorghum breeding has been elite × elite crosses followed by pedigree selection. Breeding new female lines, B and R-lines has increasingly become dependent on crossing elite by elite lines, B × B and in some cases such as improving for resistance B × R lines. In case of male lines (R-lines) improvement, it is R × R crosses. This process progressively narrows the genetic base of breeding programmes and requires new traits, especially resistances, to be brought in by pre-breeding and often backcrossing. The success of a backcrossing programme depends on the precision with which the desired trait can be identified and thus introgressed into the recurrent parent through backcrossing.

PEDIGREE METHOD. The pedigree breeding method is the most commonly used method of breeding in sorghum, where the selection begins in the F_2 generation targeting superior plants that are expected to produce the best progenies. Hybrids between diverse parents segregate for a large number of genes and every F_2 individual is genetically different from each other individual. The population size becomes crucial for the success of recovering desirable genotypes, when several genes are involved. In this method (Fig. 9.3), superior individual plants are selected in successive segregating generations from the selected families and a complete record of parent progeny relationship is maintained. Identifying a potentially good cross is essential since best F1 parents give better yielding F_4 progenies. The selection in segregating generations should be based on: (i) performance of the families of the selected cross on the whole; and (ii) the individual plant's performance within the selected family.

Selection for many of the specific selection criteria encompassing various traits such as tallness, stem thickness, juice yield, etc. can be rapidly applied in the first two or three segregating generations since crosses between elite lines produce a high proportion of progeny with desirable specific values. Once the promising lines have been identified, they can

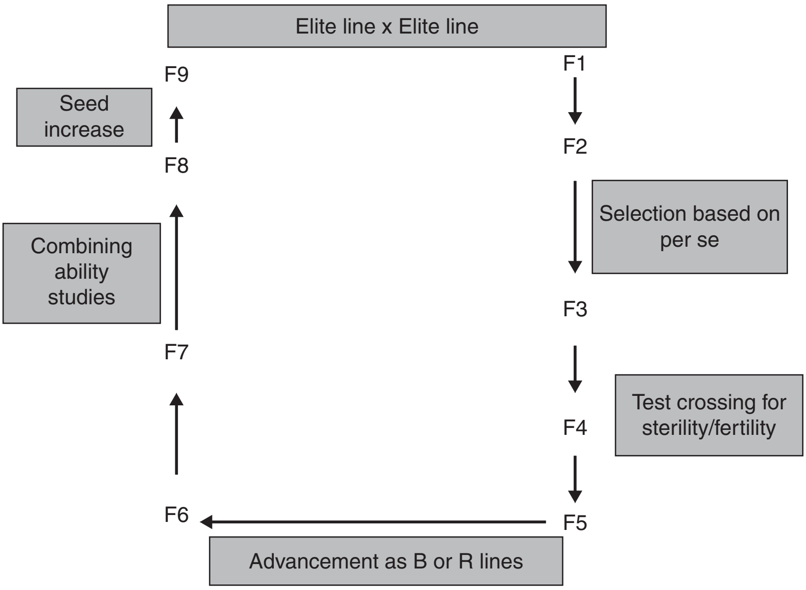

Fig. 9.3. Development of new sweet sorghum genotypes by the pedigree method.

be test crossed on to male-sterile lines for checking fertility restoration and may be classified as B or R lines. Lines with high biomass yield and other desirable agronomic characters can be released as varieties. The pedigree method has been utilized to create new recombinants, transfer of few to many genes governing resistances to various insects, diseases, cold tolerance etc. in sorghum. In India, the important sweet sorghum genotypes released through the pedigree method of selection are 'SSV 84' and 'CSV 19SS'.

BACKCROSS METHOD. This method does not offer an opportunity to provide new recombinants and thus they cannot be fixed. However, it can be utilized to incorporate BMR or specific defence (e.g. stem borer resistance) (Fig. 9.4) or improve other traits such as seed size, seed shape and cold tolerance through repeated backcrosses. The backcross method has also been successfully employed in the Indian and ICRISAT breeding programmes for transfer of BMR genes and genes that confer high digestibility into elite dual-purpose varieties. Several BMR lines in sweet sorghum backgound, stacked BMR mutants, stem borer-tolerant lines etc. have been developed through this method. Several staygreen QTLs (*stgB, stg2 and stg3*) are being introgressed into elite sweet sorghum cultivars by deploying this method.

Long-term approach

When the objective is to introgress new desirable genes distributed in many source lines, population improvement methods are used. Population improvement methods, besides offering greater opportunities for recombination to break linkages between desired and undesired traits, provide scope for increased utilization of biotic and abiotic stress resistant, but agronomically non-elite source germplasm lines. The population improvement provides a long-term breeding strategy to derive diverse and broad genetic-based superior varieties/hybrid parents. Therefore, a comprehensive crop improvement strategy has to combine both short- and long-term progress for continuous improvement of economic traits (Reddy

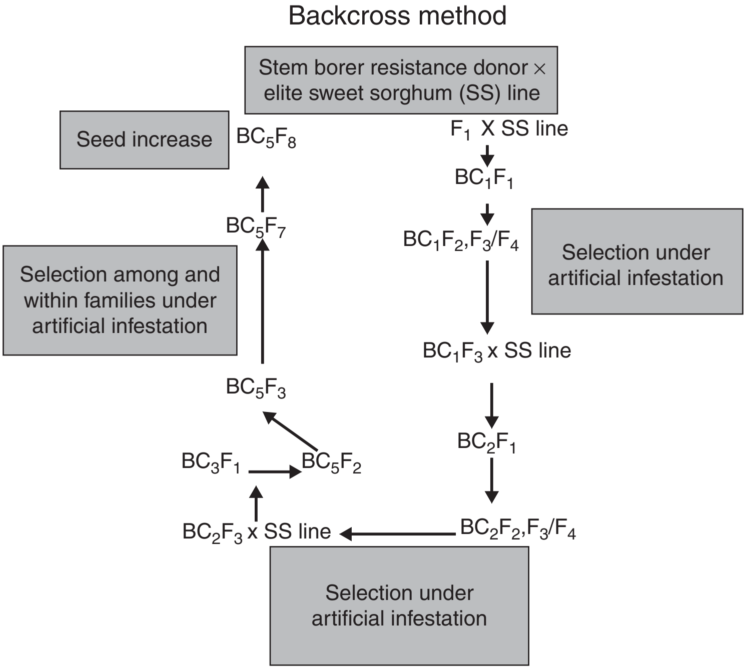

Fig. 9.4. Transfer of resistance genes by the backcross method into elite sweet sorghum lines.

et al., 2006). The population improvement procedure involves selection of component parents with high GCA, incorporation of genetic male sterility, intercrossing and random mating among parents and applying appropriate recurrent selection schemes. At ICRISAT-Patancheru, 24 sorghum populations encompassing characters such as grain mould, good grain, photoinsensitive, early dual-purpose, etc. were developed and maintained. Recently ICRISAT has started developing a sweet sorghum population with *ms*$_3$ gene for applying recurrent selection. While population improvement programmes are not the most common in sweet sorghum breeding, they are an important source of genetic variation and improved traits (Rooney and Smith, 2000).

9.7 Crop Agronomy and Value Chain Integration

9.7.1 Crop agronomy

The already standardized agronomic practices for grain sorghum are not entirely applicable to sweet sorghum because sweet sorghums produce more biomass along with sugars. Developing improved ecoregion-specific agrotechnology and pre- and post-harvesting stalk juice quality studies are the urgent priority. Moreover, the commercial viability of industry hinges upon raw material (sweet sorghum) availability for most part of the year. The adaptation (general and specific) of improved cultivars to different regions and seasons needs to be identified owing to high genotypic main effect plus genotype-by-environment (GGE) interaction of sugar yield (Srinivasa Rao *et al*., 2011a) and its competent traits as described earlier. Standardization of optimized spacing (45 × 15 cm/60 × 15 cm/75 × 15 cm), fertilizer application (80–100 kg N ha^{-1}, 30–50 kg P_2O_5 ha^{-1}), intercultural operations (thinning, weeding, soil mulch), irrigation schedule (both alfisols and vertisols are rain fed in the rainy season and require up to five irrigations in the post-rainy season), harvesting timing and methodology will greatly enhance the productivity of sweet sorghum. In some areas response to micronutrients (B, Zn and S) in juice yield and quality was observed (Srinivasa Rao *et al*., 2011b). Even though cultivars differ in biomass and grain production and hence differ in N uptake, the crop seems to be insensitive to the mineral nitrogen supply and seems to have a great potentiality in semi-arid environments in terms of yield production (Cosentino *et al*., 2012). The grain and sugar yields are highest in the rainy and summer seasons, whereas in the post-rainy season the grain yield is high, but with less stalk and sugar yield. However, the results from tropical and temperate crosses have helped in deriving few post-rainy season cultivars at ICRISAT. In Brazil efforts are being made to grow sweet sorghum in a period where stalks are harvested before and after the sugarcane season so as to extend the period of operation of the distillery. The present-day multi-feedstock distilleries can successfully run on a variety of feedstocks. Therefore, studies on intercropping or relay cropping with cassava, sugarcane, sugarbeet, soybean, jatropha, pongamia, etc. are required to enhance the period of raw material availability. Agronomic and physiological measures aiding in increasing the period of industrial utilization (PIU) of sweet sorghum (e.g. customized fertilizer application, irrigation at physiological maturity, spraying gibberellic acid (GA), ethrel, solubar, etc., or soil application of micronutrients or other amendments to delay maturity, etc.) will further strengthen sweet sorghum as a biofuel/industrial crop. Rapid sugar accumulation immediately after flowering and its retention for a longer period for staggered feedstock supply is another area of research that deserves immediate attention.

As the global climate is gradually changing to higher temperatures and sweet sorghum is bound to grow in new areas, thermo-photoinsensitive non-lodging cultivars that are resistant to multiple pests and diseases will need to be developed. Breeding of short, mid–late and late maturing genotypes is necessary in order to have a broad harvest window in sweet sorghum, and thus providing raw material to the distillery over a long period. Proper planning of sowing a mix of these cultivars in the catchment area of a distillery would help to achieve more commercial stalk sugar/ethanol. When cultivars with different maturity groups are grown in an area, pests such as shootfly and midge may be likely to infest late-maturing cultivars. Therefore, breeding for tolerant cultivars for these insects is needed. The sorghum crop is traditionally challenged by marginal lands with poor fertility status and poor moisture-holding capacity and sweet sorghum also encounters similar problems. Sporadic water inundation due to excessive rains/ floods also becomes an unforeseen constraint. The self-fermentation of juice inside the stalk prior to juice extraction is a major concern, particularly when juice extraction is delayed after harvest due to the long distance between factory and the field. Preliminary results indicated that there will be reduction of sugar yield by 16.8% if the juice extraction is delayed by 48 h (Srinivasa Rao *et al.*, 2012). Research should address the postharvest losses in terms of juice quality and quantity.

9.7.2 Value chain integration

The sweet sorghum feedstock supply chains have two primary models of operation.

1. The centralized model: the sweet stalk is directly supplied to the plant from the farmers' fields, and the juice is extracted and fermented to ethanol and allied co-products. Its operational area is generally limited to a 40–50 km radius around the plant owing to high transportation costs involved in bulky raw material supply. Examples of such centralized plants include Tata Chemicals Ltd, Nanded, Maharashtra, India and ZTE Ltd, Inner Mongolia, China. A few of the sugar mills in Brazil started utilizing the sweet sorghum for bioethanol production in 2011/12.

2. The decentralized model: the decentralized crushing units (DCU) are key to centralized distillery as supply of syrup during the off season will augment the longer period of operation and this is viable in case of crop production >50 km away from the central distillery. In simple terms a DCU comprises the crusher and boiling unit, and essentially crushes the stalks to extract juice. The extracted juice is either concentrated to syrup or fermented *in situ* to alcohol. Sweet sorghum is a seasonal crop that in India can be cultivated in three seasons a year (rainy, post-rainy and summer) to supply raw material for 3–4 months annually for ethanol production (Kumar *et al.*, 2010). The grain and sugar yields are best in the rainy and summer seasons, whereas in the post-rainy season the grain yield is high, but with less stalk and sugar yield. A commercial ethanol distillery requires feedstock year round – for at least 10 months annually – for economical operation. However, in regions with short harvest windows, smaller hectarages or with low plantation densities, a typical centralized model with a 30 kilolitres (kl) day^{-1} processing plant dedicated to sweet sorghum ethanol production could operate only seasonally, requiring a high capital investment that might not be cost effective. In areas with low plantation densities, the transportation costs associated with supplying the plant with sweet sorghum feedstock become prohibitive. Transportation costs are a significant cost factor in all sweet sorghum models studied, with costs ranging from US$34 to US$107 t^{-1} fermentable carbohydrates (Bennett and Anex, 2009). In view of the need for a regular supply of feedstock to the distillery, it is widely believed that DCUs help in sustainability of the supply chain by feeding syrup (60% Brix) to the distillery in the off season. The following gaps were identified in DCU sustainability.

- At present, there is a very limited period of operation of the crushing unit (less than 20–25 days) as the cultivar maturity window is not large. Research should aim at developing sweet sorghum genotypes with adaptability across seasons and months of the year.
- DCUs are being operated only for the rainy season crop (June–September). The post-rainy and summer season crops require an assured irrigation source, thereby increasing the cost of cultivation. Hence, the need for developing post-rainy season-adapted cultivars with drought tolerance.
- Juice extraction efficiency and syrup conversion efficiency are low. A scenario analysis conducted at ICRISAT showed that improving these even by 5% has significant bearing on the economics of the whole value chain.
- As syrup is the main product of a DCU, its quality parameters need to be improved to

meet the requirements of diverse end users (such as suitability for use in food, beverage and pharmaceutical industries). Research also needs to focus on improving organoleptic characteristics.

- Commercial dairies are increasingly using the fresh bagasse, after chopping, to feed cattle. Education and training is needed for farmers to raise awareness of the multiple uses of bagasse, such as for feed block making, ensiling or biocomposting.
- Little or no information is available on the utilization of co-products such as vinasse, steam, foam and froth. Therefore, efforts are needed in using steam for heating or boiling the juice, and in exploring the use of nutrient-rich vinasse, foam and froth as livestock feed and biofertilizers.
- Capacity building of staff at every step – not only syrup production, but also co-product utilization – would go a long way toward improving the operational efficiency and economic viability of DCUs.
- The varied products and co-products of the DCU need to be positioned to exploit locally existing market opportunities, i.e. an inclusive market-oriented development (IMOD) approach, as this brings the DCU closer to the rural farming communities.

9.8 Commercialization – Status and Bottlenecks

Experiences with sweet sorghum in India, the USA and China have shown that the crop has high potential as a bioenergy feedstock, with several opportunities for immediate use as a complementary feedstock and seasonal low-cost feedstock (mould-affected grain). Regions with a warm climate, large tracts of land and a system similar to sugarcane processing should work well for sweet sorghum. Further, the crop can be grown in regions of the world where sugarcane cannot be cultivated. Government policy support is necessary for utilization of this novel feedstock for commercial bioethanol production. Since 2007, a handful of distilleries across the world have started using sweet sorghum for ethanol production on a commercial scale. M/s Rusni distillery, the first sweet sorghum distillery, was established in 2007 at Sangareddy, Medak district of Andhra Pradesh, India, which is amenable to multiple feedstock. It has a capacity of 40 kilolitres (kl) day^{-1} and produces fuel ethanol (99.6% alcohol), Extra Neutral Alcohol (ENA) (96%) and pharma alcohol (99.8%) from agro-based raw materials such as sweet sorghum stalks (juice), moulded grains, broken rice, cassava and rotten fruits. This is not under operation currently for different reasons. Another, 30 kl day^{-1} Tata Chemicals Limited distillery in Nanded, Maharashtra, India, started operations in 2009 solely based on sweet sorghum and produced 90 kl of transport grade ethanol in 2010. Several companies in China (including Liaoning Guofu Bioenergy Development Company Limited, Binzhou Guanghua Biology Energy Company Ltd, Jiangxi Qishengyuan Agri-Biology Science and Technology Company Ltd, Xinjiang Santai Distillery, Jilin Fuel Alcohol Company Limited, Heilongjiang Huachuan Siyi Bio-fuel Ethanol Company Ltd, ZTE Agribusiness Company Limited and Fuxin Green BioEnergy Corporation) have conducted large-scale sweet sorghum trials. In 2010, ZTE Agribusiness Company Limited, Wuyuan County, Inner Mongolia and Fuxin Green BioEnergy Corporation, Heishan County, Shenyang province used sweet sorghum as feedstock to produce ethanol. The Chinese government is encouraging sweet sorghum processing industries by offering a subsidy to both growers and processors. In the Philippines San Carlos Bio-Energy Incorporated, Negros produced ethanol from sweet sorghum. In 2010, grain sorghum production in the USA was 11.6 Mt (http://faostat.fao.org), equivalent to approximately 457 million bushels, and 20% of those were used for ethanol production (http://www.sorghumgrowers.com). EnviroFuels LLC in the USA is currently in the process of developing a 20 million gallon (75,708,235 l) per year sugar-based ethanol plant in Highlands County, Florida using sweet sorghum as the primary feedstock. Sugarcane will be used to supplement the feedstock base in the winter months when sweet sorghum is not available. The facility will be designed for a future expansion to 40 million gallons (151,416,471 l) per year. In Tennessee (USA), BioDimensions Industrial Sugar Platform Development Company planted and harvested about 75 ha of sweet sorghum. A portion of the sugars was fermented to ethanol, while the bagasse was used for both fuel pellets and animal feed. The group expects to distill about 5000 gallons (18,927 l) of hydrous ethanol, much of which will be used in industrial ethanol engines. In Brazil, seed company Ceres Inc. has established a subsidiary focusing on developing sweet sorghum as a feedstock for the ethanol industry. The company's goal is to be the first supplier of new hybrids with high levels of sugar. The company is currently working with multiple ethanol mills, technology providers and equipment companies to facilitate the introduction of sweet sorghum hybrids into existing ethanol mills.

To make the value chain sustainable, some of the major challenges to be addressed include the following:

1. Convincing and educating farmers on the cultivation of sweet sorghum for higher returns.
2. Mechanization in sweet sorghum cultivation and crushing activities.
3. Supply chain innovations to reduce the time lag between the harvesting and crushing of stalks both in centralized and decentralized areas and continuous supply of sweet sorghum stalk and syrup to the distillery to run at optimum capacity.
4. Increasing the juice storability and fermentation efficiency, increasing process efficiency and reduction in investment and operational costs of syrup and ethanol production.
5. Exploring alternative markets for syrup such as food additives, pharmaceuticals, beverages, bakery and confectionery units.

9.9 Sustainability and the Way Forward

9.9.1 Sustainability

The major components of the biofuel life cycle are feedstock production, transportation to refinery, processing and conversion and distribution to retailers and customers (Fig. 9.5).

The amount of greenhouse gas (GHG) emission reduction and how this is calculated is also a matter of much debate. Authors such as Searchinger *et al*. (2008) and Fargione *et al*. (2008) argue that life cycle studies have failed to factor in land use change effect, such as carbon storage and sequestration sacrificed by diverting land from its existing uses for biofuels harms the environment rather than helping it. It is obvious that the above parameters of life cycle assessment (LCA) vary widely with respect to the location,

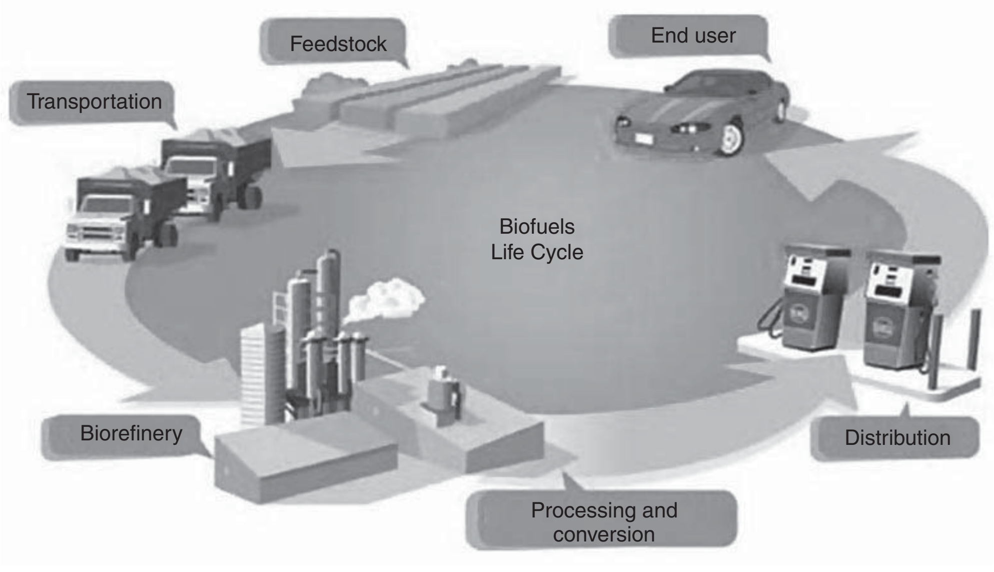

Fig. 9.5. Biofuel life cycle (US Department of Energy).

technology, methodology and several other factors either directly or indirectly affecting the value chain. Net energy ratio and GHG balance primarily decides the benefits of an energy value chain. However, it was reported that sweet sorghum has a higher net energy balance (3.63) compared to grain sorghum (1.50) and maize (1.53) (Wortmann *et al*., 2008). Another report estimated an energy balance of 8 and carbon emission reduction by 86% (CII-DBT study, 2010). First- and second-generation bioethanol from sweet sorghum can contribute significantly to the conservation of fossil resources and to the mitigation of GHGs. If the crop is used for the production of ethanol (from grains and sugar) and green electricity (from surplus bagasse), 3500 l crude oil equivalents can be saved per hectare cultivation area. If both food from grains and ethanol from the juice are produced, 2300 l crude oil equivalents can be saved per hectare cultivated area. Regarding GHGs, between 1.4 and 22 kg CO_2 equivalents can be saved depending on yield, production methods and the land cover prior to sweet sorghum cultivation (Köppen *et al*., 2009). For both categories, the exact values vary greatly with specific scenarios and local conditions. In general, the following parameters determine the results: type and efficiency of conversion technology, the use of byproducts (e.g. bagasse), the crop yield per cultivation area, land-use changes, as well as the type of fossil energy carriers that are replaced. Even if the seeds were used as food, bioethanol from the stem's sugar juice still shows clear advantages over fossil fuels. If both sugar and seeds were used as food, the respective conversion related energy and GHG expenditures could be compensated by producing second-generation ethanol from the bagasse. Even though the ethanol yield per unit weight of feedstock is lower for sweet sorghum compared to sugarcane, the much lower production costs and water requirement for this crop more than compensates for the difference, and hence it still returns a competitive cost advantage in the production of ethanol in India (Farrell *et al*., 2006).

9.9.2 Way forward

Sweet sorghum has a low water demand and is especially advantageous in areas with water shortage (Srinivasa Rao *et al*., 2012). It has a lower nitrogen fertilizer demand possibly due to traits such as BNI-capacity, reduces the risk of nutrient leaching and thus soil and water pollution, as well as making it well suited for small-scale farming. Its relatively short vegetation cycle allows sweet sorghum to be grown in double-cropping systems based on water availability, which in turn can lead to greater agrobiodiversity and a reduced demand for fertilizers and pesticides. Under intensive production practices, sweet sorghum production risks similar disadvantages as other intensive monocultures, such as soil degradation and loss or soil and water pollution due to more fertilizer and pesticide use. Establishing new sweet sorghum cultivation sites instead of integrating the crop into existing agricultural systems may lead to a loss of biodiversity, which is more detrimental for species-rich ecosystems. Like many other biofuel feedstocks, the sweet sorghum-based bioethanol value chain has some limitations with regards to location-specific LCA, certain emissions compared to its fossil equivalent due to poor productivity owing to poor management of the crop or submarginal ecologies where the crop is cultivated.

A limiting factor for its widespread cultivation is the limited availability of varieties/hybrids adapted to different agroclimatic conditions resisting both biotic and abiotic stresses, including colder climate. Consequently, research should address the optimization of sweet sorghum as an energy crop through breeding for enhanced productivity under limited available resources. Genetic improvement should focus on stalk sugar, biomass quantity and quality and general agronomic traits (such as water and nutrient use efficiency) and, in particular, adaptation of sweet sorghum to colder, arid saline, and alkaline conditions. Further improvement in Brix%, juice volume and stalk yield (≥45 t ha^{-1} with hybrids) should be targeted in sweet sorghum to help improve the benefits to the industry and farmers without any detrimental effect on grain yield. The juice volume should not be compromised while increasing the Brix%. The best way of selecting genotypes will be based on sugar yield per ha (a function of juice yield and Brix%) with 18–20% Brix as the base level in the restorers and 14–17% in the female parents in the rainy season, and 15–17% in R-lines/varieties and 12–16% in the female parents in the post-rainy season. Going by the high variability present in sorghum germplasm for Brix% (up to 23%) with a low Brix% observed in female (seed) parents (12–15%), there is an urgent need to improve the sugar content (Brix%) in seed parents through genetic enhancement. There is also a need to develop and evaluate cultivars producing high stalk yield per unit time, inputs, energy and land area in different agroclimatic areas of the country. Other research areas on quality and processing that need immediate attention include high ethanol yield, fermentation efficiency, diffusion, and diversified products from bagasse (power, pulp, biomanure, cattle feed, etc.).

Bioethanol from sweet sorghum (sorganol) is potentially a win–win solution. Sorganol will not be the unique solution, but will compliment other renewable sources of energy and contribute to addressing some of the problems, e.g. reduction of GHG emissions, improving air quality in large cities, reducing dependency on imported oil, creating jobs in rural areas and improving quality of life in developing countries. From an industry viewpoint, it is imperative to improve field and distillery integration as well as significant enhancement in processing and fermentation efficiency. From the experience gained over the last 3–4 years, a viable agro-enterprise of this massive scale will be commercially viable if it is a multi-feedstock distillery backed up by government policy support.

As the demand for biofuels rapidly expands, its associated production systems and supply chains are consolidating. Forward-thinking management systems could significantly enhance ecological sustainability and livelihood development, particularly for poor farmers in the developing world. International trade will be crucial to enlarge the share of bioethanol in future transport energy demand. All nations, irrespective of the development index, should join hands in formulation of policies that target the entire innovation chain to ensure that the development and use of biofuels in general and sorganol in particular follow an integrated pathway, which simultaneously targets climate change mitigation and adaptation, energy security and all-round sustainable economic development. The potential for significant genetic improvement of sorghum as a biofuel crop is excellent. Full exploration of the available genetic resources through plant breeding with the aid of molecular tools could dramatically increase biomass yield of sorghum and thus meet the demand of feedstocks for biofuel production without a significant impact on our food supply and natural environment.

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