Chapter 1
Sweet Sorghum: Breeding and Bioproducts

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Abstract Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop and is the dietary staple of more than 500 million people in over 90 countries, primarily in the developing world. However, sweet sorghum which is similar to grain sorghum except for accumulation of stalk sugars, is considered as a potential energy crop without impacting the food security of millions. Further, the sorghum stover is considered to be a potential lignocellulosic biofuel feedstock. Being a C4 plant, it has high photosynthetic rate, and several mechanisms are known to confer resilience that help produce higher yield in varied environmental conditions. This chapter not only discusses different breeding methodologies for improving candidate sugar and biomass traits but also the possible utilization of this smart feedstock for diverse biochemicals (lactic acid, xylitol, glycerol, etc.) and bioproducts (nanomaterials, anticancer and microbial compounds, adhesives, polymers, antidiabetic compounds, etc.) development.

Keywords Sweet sorghum • Biofuel • Stalk sugar • Genetic variability • Biochemicals • Bioproducts

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Introduction

The current scenario of declining fossil fuel reserves along with increased concerns on environment pollution and climate change is fundamentally responsible for greater interest in renewable energy sources globally. Sustainable availability of raw material for any economic and constant product production is one of the essential requirements. This has become more appropriate for the constant consumption products like biofuels, as the entire world economy is dependent on the availability of fuel resources. Interest in sweet sorghum (*Sorghum bicolor* (L.) Moench) in semiarid and rain-fed environments is increasing because of the multiple uses of this novel feedstock either for production of biofuels from stalk juice or for power generation from bagasse or for utilization in dairy industry as nutrient rich and easily digestible fodder [1, 2]. Additionally, sweet sorghum biomass is used for fiber, paper, syrup, and biopolymers. Sweet sorghum being a C4 crop has wide environmental adaptation, rapid growth, high grain and biomass productivity, suitability for marginal soils, and high concentrations of the easily fermentable sugars like sucrose, glucose, and fructose [3]. Drought and salinity are widely prevalent abiotic stresses that significantly lower the yields of various crops, and their frequency of occurrence is expected to increase due to climate change. Sweet sorghum grows in marginal areas because of its high tolerance to saline and drought conditions. Sweet sorghum has higher water-use efficiency than other summer crops under both well-watered and water-stressed conditions [4–6]. From the agronomic point of view, sweet sorghum is more environmentally friendly than maize because of its relatively low nitrogen needs and water requirements. It was reported that sorghum requires 310 kg of water to produce 1 kg of biomass, while maize consumes 23 % more water, i.e., 370 kg to produce same quantity of biomass [7]. Besides biofuel production from sweet sorghum, a plethora of food products such as beverage, cookies, syrup, sweets, chocolates [8], and bioproducts like biopolymer resin can be produced [9]. However, the commercialization of this smart feedstock primarily hinges on the national biofuel policy of respective countries besides identification of productive cultivars adapted to the targeted region owing to significant genotype × environment interaction [10].

This chapter will focus on genetic enhancement of sweet sorghum through conventional plant breeding and the production of various bioproducts based on this novel feedstock.

Food: Fuel Trade Off

It is often stated that sweet sorghum cultivars do not produce grain yield or the grain yield is very less vis-a-vis that of grain sorghum. Studies at the International Crops Research Institute for the Semiarid Tropics (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 [2, 3].

This is further corroborated by other published work [11] showing that there is significant soluble sugar content in the stems (79–94 %) during post-anthesis period, with the hybrids exhibiting significantly high soluble sugar content over varieties with 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 shows 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, selection programs can aim to improve both the traits simultaneously.

Climate Change

Global warming due to climate change will affect grain and stover yields in crops, more so in tropical Africa and Asia where sorghum is a major food crop. Most climate change models predict rise in air and soil temperatures and sea levels and increased frequencies of extreme weather events leading to unprecedented changes in agricultural production in the years to come. In the Intergovernmental Panel on Climate Change (IPCC), climate models predict an increase in global average surface temperature of between 1.4 and 5.8 °C from 2001 to 2100, the range depending largely on the scale of fossil fuel burning between now and then and on the different models used. At the lower range of temperature rise (1–3 °C), global food production might actually increase, but above this range, it would probably decrease [12]. However, broad trends will be overshadowed by local differences, as the impacts of climate change are likely to be highly spatially variable. In general, the sorghum maturity period of current varieties decreases with increased temperatures. Climate change effects in terms of high temperatures and erratic rainfall may drastically reduce sorghum yields in South Asia, Southern Africa, and West Africa [13]. Climate change will cause changes in the length of the growing period (LGP) in some regions. Cooper et al. [13] showed that the extent of global semiarid tropical (SAT) areas will be changed through (1) SAT areas being “lost” from their driest margins and become arid zones due to LGPs becoming too short or (2) SAT areas being “gained” on their wetter margins from

subhumid regions through the reduction in the current LGPs in those zones. It means sorghum could be grown in new areas of the currently humid tropics where sorghum is not grown at present. Therefore, development of crop cultivars with a maturity duration that suits the prevailing LGP will be one of the best options to cope with changes in LGP. ICRISAT and Indian National Agricultural Research System (NARS) have developed a wide variety of sweet sorghum female parental lines and restorers besides varieties with altered LGP that can play pivotal role in achieving the above said option.

Taxonomy

Sorghum was first described by Linnaeus in 1753 under the name *Holcus*. In 1974, Moench distinguished the genus *Sorghum* from genus *Holcus* [14, 15]. Subsequently, several authors have discussed the systematics, origin, and evolution of sorghum since Linnaeus [16–19]. Sorghum is classified under the family *Poaceae*, tribe *Andropogoneae*, subtribe *Sorghinae*, and genus *Sorghum*. The genus was further divided [20] into five subgenera: *Sorghum*, *Chaetosorghum, Heterosorghum, Parasorghum*, and *Stiposorghum*. Variation within these five subgenera except the subgenera *Sorghum* has been described [14]. *Sorghum bicolor* subsp. *bicolor* contains all of the cultivated sorghums. Harlan and deWet [20] 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, subsp. *bicolor* into five basic and ten hybrid races as depicted in Table 1.1. The 15 races of cultivated sorghum can be identified by mature spikelets alone, although head type is sometimes helpful. The Biodiversity International [formerly International Plant Genetic Resources Institute (IPGRI)] advisory committee on sorghum and millet germplasm has accepted and recommended this classification to be used in describing sorghum accessions.

Table 1.1 Classification of *Sorghum bicolor* (L.) Moench. subsp. *bicolor*

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)

Sweet Sorghum Distribution and Climatic Conditions

In simple terms, wherever sorghum is currently grown, sweet sorghum can also be cultivated commercially. Thousands of hectares are grown with sweet sorghum for biofuels production in Brazil, China, and the USA, while in the Philippines, it is grown for vinegar synthesis, and considerable areas in India, the USA, Indonesia, West Asia, and North Africa go for fodder production. In Western and Southern Africa, it is widely used for chewing purposes and local beverage production. This feedstock is well adapted to the SAT and is one of the most efficient dryland crops in converting atmospheric CO_2 into sugar [3]. 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 1,500 m asl. Most East African sorghum is grown between the altitudes of 900–1,500 m, and cold-tolerant varieties are grown between 1,600 and 2,500 m in Mexico.

Environmental Conditions

Sweet sorghum can be grown in the temperature range of 12–37 °C. The optimum temperatures for growth and photosynthesis are 32–34 °C, day length is 10–14 h, optimum rainfall 550–800 mm, and relative humidity between 15 and 50 %. However, the lower the diurnal and nocturnal temperature differential, the less stalk sugar accumulation observed is in tropical sweet sorghums.

Soil Conditions

Alfisols (red) or vertisols (black clay loamy) with pH 6.5–7.5, organic matter $>$0.6 %, soil depth $>$80 cm, soil bulk density $<$1.4 gcc, water holding capacity

>50 % field capacity, N ≥ 260 kg ha^{-1} (available), P ≥ 12 kg ha^{-1} (available), and K ≥ 120 kg ha^{-1} (available) are optimal soil conditions for sorghum growth.

Water

While sorghum will survive with a supply of less than 300 mm over the season of 100 days, sweet sorghum responds favorably with additional rainfall or irrigation water. Typically, sweet sorghum needs between 500 and 1,000 mm of water (rain and/or irrigation) to achieve good yields, i.e., 50–100 t ha^{-1} total aboveground biomass (fresh weight). The great advantage of this feedstock 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 favorable for growth again. Mid-season drought stops leaf development. Although this crop is susceptible to sustained flooding particularly at early vegetative phase, it tolerates water logging better than maize and sugarbeet [2].

Radiation

Being a C4 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 a better light penetration in the leaf canopy.

Photoperiodism

Most hybrids of sweet sorghum are relatively less photoperiod-sensitive vis-a-vis purelines. 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 rainy season.

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 in a crop, makes it possible to develop orderly and efficient breeding procedures.

Panicle Initiation

Sorghum blooming is hastened by short days and long nights. However, varieties differ in their photoperiod sensitivity [21]. Tropical sweet sorghum varieties initiate the reproductive stage when day lengths return to 12 h. Usually, the floral initial is 15–30 cm above the ground when the plants are about 50–75 cm tall [22]. 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 [2, 3].

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 [2, 3].

Panicle Structure

The inflorescence is a raceme, which consists of one or several spikelets. It may be short, compact, loose, or open and composed of a central axis that bears whorls of primary branches on every node. The spikelet usually occurs 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. 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. 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. Gynoecium is centrally placed and consists of two pistils with one ovule from which two feathery stigmas protrude. 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 pediceled spikelets, usually lanceolate in shape and possess only anthers, occasionally have a rudimentary ovary and empty glumes [9].

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 between the lemma and palea. 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 [22]. 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 up to 10:00 AM depending on the cultivar, location, and weather. Maximum flowering is observed between 6:00 and 8:00 AM. The anthers dehisce when they are dry and pollen is blown into air. The pollen remains viable several hours after shedding. The 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 [2, 3, 19].

Cytoplasmic male sterility has been found in sorghum (A_1-A_4 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-colored shriveled 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. There are two types of male sterility, viz., (a) genetic male sterility (GMS) and (b) cytoplasmic nuclear male sterility (CMS), both widely used in sorghum improvement programs [4].

Table 1.2 Genetic male sterility genes, their designated symbols, and mechanism of sterility

Gene symbol	Mechanism	Reference
ms_1	Normal pollen is dominant over aborted or empty pollen cells	[25]
ms_2	-do-	[26]
ms_3	-do-	[27]
ms_4	Empty pollen cells	[28]
ms_5	Aborted pollen	[29]
ms_6	Micro anthers without pollen	[29]
ms_7	Empty pollen cells	[30]
al	Antherless stamens	[31]

Genetic Male Sterility

Genetic male sterility is expressed in sorghum in many ways. Several sources of male sterility are identified. In all the cases, it was shown that a recessive allele in homozygous condition designated with a series of alleles, ms_1, ms_2, ms_3, ms_4, ms_5, ms_6, ms_7, and *al*, confers male sterility [19, 23, 24]. The genetic male sterility genes are represented in Table 1.2.

Cytoplasmic Nuclear Male sterility

The discovery of the male sterility resulting from the interaction of cytoplasmic and nuclear genes [32] laid the foundation and revolutionized the development of hybrid cultivar and hybrid seed production technology. The milo cytoplasm was from *durra* race, which induced male sterility in the nuclear background of *kafir* race, and this is designated as A_1 cytoplasm. Since then, several sources and types of male-sterile-inducing cytoplasms have been discovered and reported. In all these cytoplasms, recessive genes in the nucleus and sterile cytoplasm induce male sterility. These male-sterile cytoplasms have been differentiated based on the inheritance patterns of their fertility restoration. The inheritance of fertility restoration is not clear, as it is dependent on the specific cytoplasm and nuclear combinations. Fertility restoration is controlled by single gene in some combinations (e.g., A_1) but is controlled by two or more genes when the same nuclear genotype interacts with a different cytoplasm [33]. Although diverse male-sterile cytoplasms have been identified, by far, only the milo cytoplasmic male sterility system is widely used because the hybrids based on this cytoplasm produce sufficient heterosis (20–30 %) over the best available pure lines in sweet sorghum. In spite of A_2 cytoplasm being as good as A_1 cytoplasm for mean performance as well as heterosis for economic traits such as stalk yield, juice yield, grain yield, days to 50 % flowering, and plant height, it is not popular as the anthers in A_2 male steriles, unlike the A_1 male steriles, mimic the fertile or maintainer lines and lead to

difficulties in monitoring the purity of hybrid seed production, and also the restoration frequency is low. ICSSH 58 (ICSA 738 × ICSV 93046) is the first A_2-based sweet sorghum hybrid in the world bred at ICRISAT and reached the farmers' fields. Other alternate sources like A_3, A_4, A_{4M}, A_{4VZM}, A_{4G1}, A_5, A_6, 9E, and KS are not useful primarily because (1) restorer frequencies are low (restorer frequency: $A_1 > A_2 > A_4 > A_3$) and (2) male steriles cannot be readily distinguished from male fertiles. There is a need to search for more useful form of male sterility yet different from milo (A_1). Milo restorers need to be diversified in guinea background to further enhance the yield advantage in hybrid development. Restorer frequency is very low on non-milo cytoplasms. So, there is a need to identify and breed for high-yielding non-milo cytoplasm restorers [2, 34]. The high Brix% possessing (>14 %) female hybrid parents are not available in plenty on sweet sorghum breeding programs across the globe to exploit the potential heterosis for stalk yield and juice yield [2].

Breeding Sweet Sorghum

Breeding Behavior

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. Hand pollination should begin around 9:30 or 10:00 AM and can be extended up to 11:30 AM to 12:30 PM on a foggy morning [22].

Candidate Traits and Variability

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

1. High biomass productivity (75–100 t ha^{-1})
2. High Brix% (20–23 %)
3. Thick stems and juicy internodes
4. Photo- and thermo-insensitivity aids to fit into diversified cropping systems
5. Tolerance to shoot pests and diseases
6. Good digestibility of residues when used as forage
7. Tolerance to mid-season and terminal drought
8. Salinity and heat tolerance
9. High water, nitrogen, and radiation use efficiencies
10. Juice quality and quantity sustenance during post-harvesting
11. Grain yield (4.0–7.0 t ha^{-1})

Ayyangar [35] suggested that a single dominant gene confers the non-sweet character. Later, it was reported that stalk sugar is under the control of recessive genes with additive and dominance effects [36]. On the contrary, subsequent 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 [8, 37, 38]. There was also a report suggesting the involvement of several genes affecting the biofuel traits in 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 [39]. 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) [2]. Knowing general (GCA) and specific (SCA) combining ability effects of genetic materials is of practical value in breeding programs. 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 nonadditive 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 produce high sugar yields in post-rainy season. There is an indication of existence of transgressive segregation for sugar yield that can be exploited [39]. The heritability for traits such as stem juice content, stem sugar concentration, total stem sugars, juice glucose, juice fructose, and juice sucrose was low [40, 41]. The predominant role of nonadditive gene action for plant height, stem girth, total soluble solids, millable stalk yield, and extractable juice yield and substantial magnitude of standard heterosis for candidate sugar traits (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 %) indicate the importance of heterosis breeding for improving ethanol productivity of cultivars [42]. The significant positive correlation of general combining ability (GCA) effects with per se performance of parents in sweet sorghum facilitates quicker identification and development of sugar rich, high biomass yielding hybrid parents [2, 43]. The generation mean analysis of two crosses has shown predominantly additive gene action for traits like sucrose% 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 overdominance inheritance, utilization of hybrid vigor by developing sweet sorghum hybrids is an attractive option. Also one of the parents with high sucrose content will suffice in getting good hybrids with high sugar and juice yield [44].

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 dominant components of gene action have to be exploited while breeding for high stalk sugar and juice-yielding genotypes. It was demonstrated that the improved hybrids top ranking for grain and sugar yields in rainy season are not top ranking in the post-rainy season and vice versa. It is important to breed for rainy and post-rainy seasons separately [2–4]. The selections for post-rainy season adaptation should be made in post-rainy season only, and for rainy season adaptation, selections can be made in both rainy and post-rainy seasons.

Breeding Objectives

In general, the sweet sorghum breeding programs aim to develop parents and hybrids which can address both first and second generation (lignocellulosic feedstock development) biofuel production issues. The breeding objectives are:

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

Breeding Methods

The most commonly used programs in sweet sorghum improvement are short-term programs (pedigree method and backcross) and long-term programs (population improvement methods). The most common approach in sweet sorghum breeding has been elite × elite crosses followed by pedigree selection. Breeding new female lines, B and R lines have 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 programs and requires new traits, especially resistances, to be brought in by pre-breeding and often backcrossing. The success of a backcrossing program depends on the precision with which the desired trait can be identified and thus introgressed into the recurrent parent through backcrossing.

Fig. 1.1 Comparison of grain sorghum (front) and sweet sorghum crop (rear) at flowering

Pedigree Method

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 which 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 other individuals. The population size becomes crucial for the success of recovering desirable genotypes, when several genes are involved. In this method (Fig. 1.1), 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 the best F_1 plants produce better yielding F_4 progenies. The selection in segregating generations should be based on (1) performance of the families of the selected cross on the whole and (2) the individual plants performance within the selected family. Selection for many of the per se selection criteria encompassing various traits like tallness, stem thickness, and juice yield 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 per se values. Once the promising lines have been identified, they can be test crossed onto 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 pedigree method of selection are SSV 74, SSV 84, CSV 19 SS, and CSV 24SS [45].

Backcross Method

This method does not offer an opportunity to provide new recombinants as hybrids are crossed back to either of the parents and thus they cannot be fixed. However, it can be utilized to incorporate brown-midrib (*bmr*) or specific defense-related alleles (e.g., stem borer resistance) or improve other traits like seed size, seed shape, and cold tolerance through repeated backcrosses. The backcross method has also been successfully employed in the Indian and ICRISAT breeding programs for transfer of BMR genes and genes which confer high digestibility into elite dual-purpose varieties. Several *bmr* lines in sweet sorghum background, stacked *bmr* mutants, stem borer tolerant lines, etc. have been developed through this method. Several stay-green QTLs (*stgB, stg2*, *and stg3*) are being introgressed into elite sweet sorghum cultivars by deploying this method.

Population Improvement

This method provides 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. 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 like grain mold, good grain, photo-insensitive, and early dual purpose were developed and maintained. Recently, ICRISAT has started developing sweet sorghum population with ms_3 gene for applying recurrent selection. While population improvement programs are not the most common in sweet sorghum breeding, they are an important source of genetic variation and improved traits.

Genomics

The availability of genomic sequence for sorghum has made it possible to carry out genome-wide analyses. Whereas earlier studies on simple sequence repeat (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. In the sorghum genome, a total of 109,039 tandem repeats were detected, of which 15,194 were microsatellite (SSR) markers [46]. In a recent studies, several major 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 [37, 40, 41]. 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.

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 [47]. 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 [48]. A microRNA miR169 was recently shown to be involved in regulating sugar levels in sweet sorghum stems suggesting epigenetic regulation of sucrose accumulation [49]. Similarly, a wide hybridization is another useful approach to transfer biotic and abiotic stress tolerance conferring gene transfer from tertiary gene pool *sps* to sweet sorghum cultivars exploiting *iap* (*i*nhibition of *a*lien *p*ollen) lines like T × 3361, Nr481 [50].

Bioproducts of Sweet Sorghum

A profile of different biomass and grain-based bioproducts derived from sweet sorghum is represented in Fig. 1.2. The following section details these bioproducts.

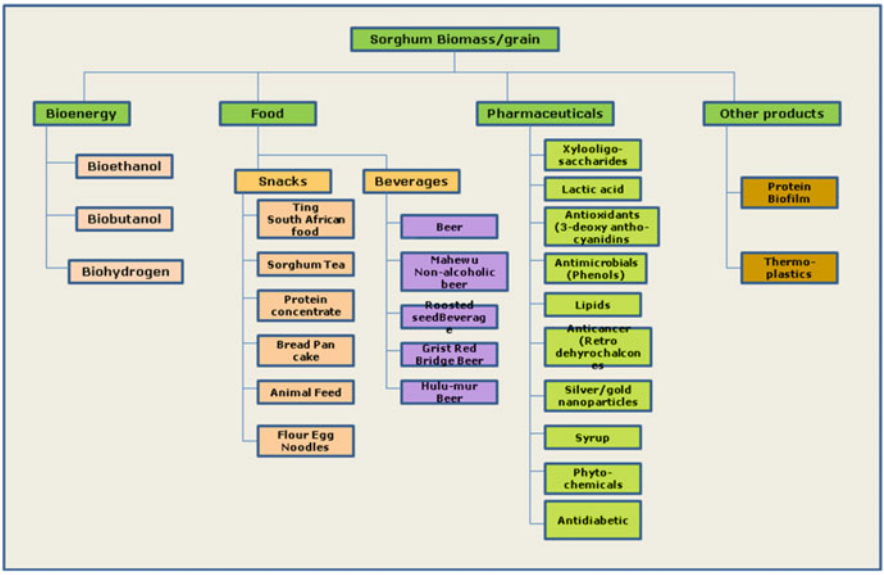

Fig. 1.2 Sorghum-based bioproducts profile

Beverages

Sorghum grain-based beverages are consumed in Africa [51] and known by different names across Africa, including *burukuto* (Nigeria), *pombe* (East Africa), *bjala* (Northern Sotho), and *bil-bil* (Cameroon). African sorghum grain-based beer is produced by lactic acid as well as alcoholic fermentation to achieve distinct sour taste. The souring process is initiated using yogurt, sour dough starter cultures, or by spontaneous fermentation. Opaque sorghum beers are also popular alcoholic sorghum-based beverages in Africa which is known by *tchoukoutou* (Benin) in West Africa, *dolo* (Burkina-Faso), *pito* (Ghana), and *burukuto* or *otika* (Nigeria) [52]. These lager beers are characterized by sour taste with relatively high dry matter content (5–13 g 100 ml^{-1}) and low alcohol content (2–3 ml 100 ml^{-1}) [53]. These beers are mostly prepared with Guinea corn (*Sorghum bicolor*) along with other cereals such as millet or maize as adjuncts or substitutes [52]. The manufacturing process consists of malting, brewing, and fermentation steps. Depending on the geographical location, variations have been observed in the production process [54].

In China, sorghum is fermented to produce distilled beverages, like *baijiu* (sorghum white wine), *maotai* (sorghum liquor), and *kaoliang* (sorghum wine). In USA, two sorghum-based beer products "New Grist" and "Redbridge" have been marketed since 2006, which are gluten-free and hence preferably consumed by people suffering with celiac disease and also popularized among health-conscious drinkers due to its low-carbohydrate content [54]. The nonalcoholic fermented African sorghum beverages like *kunun-zaki* (Nigeria), *hulu-mur* from sorghum malt and flour (Sudan), and *motoho-oa-mabele* from sorghum meal (South Africa) involve some form of lactic acid fermentation [55]. *Kunun-zaki* is a highly perishable product and has a short shelf life (24 h) under tropical ambient conditions; however, the shelf life may be extended under refrigerated conditions [56] or by using 0.1 % sodium benzoate or sodium metabisulfite in combination with pasteurization at 60 °C for 1 h by more than 3 weeks [57]. *Hulu-mur* is a traditional Sudanese nonalcoholic beverage made from a fermented mixture of unmalted flour of sorghum (*Sorghum bicolor*) and malt [58, 59].

Foods

Nearly 30 different fermented sorghum/sweet sorghum-based food products are consumed in Sudan. *Injera* is a leavened, spongy, and sour thin flat, round, staple fermented Ethiopian traditional bread prepared with flour from either of different cereals, water, and starter (*ersho*, a liquid saved from the previously fermented dough). Injera prepared using tef [*Eragrostis tef* (*Zucc*) Trotter], a tiny millet-like grain, is the most popular and preferred cereal ingredient, although other different types of cereals, including sorghum, tef, maize, wheat, finger millet, and barley, are used [60]. The white tannin-free sorghums are preferred due to the light *injera* color

or because of relative brittleness and dryness of sorghum injera after storage [60]. Kisra (aseeda or aceda) is a traditional bread for Arabian Gulf, Sudan, and Iraq which is similar to injera. It is made from the fermented dough of sorghum (*Sorghum bicolor*) or pearl millet (*Pennisetum typhodium*) grains. The fermented dough is baked into thin sheets and consumed along with stew prepared from vegetables and meat. *Lactobacillus* sp., *Acetobacter* sp., and *S. cerevisiae* were the main microflora isolated from kisra and responsible for the fermentation process [61]. Studies on kisra preparation indicated that the fermentation of kisra enhanced riboflavin and significantly decreased thiamine, without any change in the mineral contents [62]. Sweet sorghum porridges produced by hard grain and cysteine addition to wheat flour accelerated the stress and structural relaxation [63] and reduced the mixing time by facilitating the breakdown of the wheat proteins by splitting the disulfide bonds rapidly thus aiding faster dough development [64]. Addition of L-cysteine hydrochloride (0.1 %) increased water absorption, but decreased dough development time and dough stability [65]. The Pampanga Agricultural College, Philippines, has pioneered in this work and published a compendium that enlisted a huge array of products such as cakes, cookies, biscuits, rice, porridges and beverages.

Bioethanol

Sorghum-based ethanol production is a very recent area (two decades old) which involves preprocessing steps like harvest approaches [66], juice processing techniques [67], as well as fermentation and depends on yeast strain used and yield ranges from 78 to 90 % [68, 69]. Biomass-based solid-phase fermentation and juice-based liquid batch fermentation [70] and fed-batch fermentation [71] were also been investigated. Application of immobilized yeast in a fluidized bed reactor [72], gelatin bead-packed bed reactor [73], stirred tank, and tubular bioreactors [74] as well as application of statistical approaches [72] and use of very high gravity (VHG) [75] shortened the fermentation time significantly and increased the conversion efficiency. Higher ethanol yields were reported by fermenting 30 % sulfuric acid-treated sorghum [76] and from sorghum fibers pretreated with dilute ammonia followed by enzymatic hydrolysis and fermentation [77]. Specifically, the energy yield from ethanol obtained from the above-referenced studies ranged between 6,500–8,900 kJ/kg dry and 1,400–2,700 kJ/kg fresh sorghum biomass, respectively (assuming that the energy yield from ethanol is 26,500 kJ/kg). To date, ethanol and methane are the well-known microbial-derived products from sweet sorghum [78]. The data indicates that from a metric ton of sweet sorghum having 18 % Brix, 350–450 L of juice can be realized and up on fermentation 45–55 L of transport grade ethanol can be realized [2–4]. The utilization of bagasse has a most promising future for its conversion to ethanol or butanol, while the residual solids (mainly lignin) can be incinerated to cogenerate heat and power [2]. Other byproducts of sweet sorghum ethanol value chain are vinasse and furnace oil. Vinasse can be converted into a valuable fertilizer.

Biohydrogen

Among different biofuels, hydrogen is widely acknowledged as an attractive candidate for replacement of fossil fuels as it is a clean and renewable energy carrier with high energy yield as compared to other biofuels and emits water as an end product upon consumption. Several approaches such as thermochemical (gasification, pyrolysis, supercritical conversion, etc.) and biotechnological (photofermentation, water–gas shift reaction or uptake hydrogenase, dark fermentation, direct and indirect biophotolysis, etc.) have been evaluated [79–82] for hydrogen production. Evaluation of different resources and available processes suggests the use of renewable energy material with high carbohydrate content which would offer better solution. Rich fermentable carbohydrate containing energy crops such as *Miscanthus*, sugarcane, and sweet sorghum is advantageous over other biomass resources. Thermodynamically, simple sugars are advantageous over complex carbohydrate substrates for microbial metabolism and also known to influence the biohydrogen yields. Sweet sorghum biomass has high concentration of soluble sugars 18–22 % on dry weight basis [83] predominantly sucrose with variable levels of glucose, starch, and fructose depending on genotype and has an edge over other biomasses [84]. The fermentation-based production of biofuel is mainly regulated by the structural complexity of substrate material, microbial nature, and other physiological factors [82, 85]. A significant negative correlation between lignin content and fermentative biohydrogen production was reported by Prakasham et al. [80] while working with low lignin containing brown-midrib sorghum mutants.

Multi-substrate utilizing microbial strains would offer edge over single carbohydrate metabolizing strains as conversion yields improve substantially. Rumen bacteria has such potential, and it was reported that when grown on different subparts of sweet sorghum like sorghum stalks and sorghum water extract, the biohydrogen yields were comparable [83]. The biohydrogen production was from xylose, cellobiose, arabinose, formic acid, etc. besides glucose. In a mixture of cellobiose, arabinose, xylose, and glucose, glucose was most preferred and arabinose was least for microbial metabolism and differed with microbial genetics as the initial enzymatic conversion of these carbohydrates to intermediates of glycolysis played a significant role. Glucose gets metabolized via EMP, while xylose enters only after the conversion to xylulose and subsequently to xylulose-5-phosphate by the sequential catalysis of xylose isomerase and xylulose kinase [86]. Irrespective of microbial strains and biomass material, all biohydrogen processes are regulated by hydrogen-producing enzymes [79, 87, 88] and associated with CO_2 production as well as may be combined with other gases like methane and hydrogen sulfide depending on the biological source and substrate. In fact, studies on hydrogen production inhibition indicated that higher hydrogen gas concentration shifts microbial metabolic pathways to produce more lactate, ethanol, acetone, butanol, or alanine [89]. In addition, biohydrogen yield is regulated by different bioreactor conditions such as pH, microbial consortia, structural complexity of biomass,

Table 1.3 Comparison of biohydrogen yield with different plant biomasses

Biomass type (treatment conditions)	H_2 yield (ml H_2/g TVS)	Reference
Corn stover (220 °C for 3 min)	49.00	[90]
Corn husk	62.30	[82]
Corn straw	9.00	[91]
Corn stalk	3.00	[92]
Corn stalk waste	149.69	[92]
Corn cobs (1 % HCl + 100 °C for 30 min)	107.9	[93]
Maize leaves	18.00	[94]
Rice husk	40.38	[82]
Wheat straw	6.40	[95]
Wheat powder	281.00	[96]
Sugarcane bagasse (130 °C for 30 min)	19.70	[94, 97]
Groundnut shell	44.12	[82]
Sweet sorghum plant (130 °C for 30 min)	32.40	[94]
Whorled Rosinweed leaves	10.30	[94]
Switchgrass	27.10	[98]

hydraulic retention time (HRT), and hydrogen gas partial pressure during anaerobic fermentation [79, 80] irrespective of the biomass used. A comparative account of biohydrogen yields using different plant biomasses is shown in Table 1.3.

Lipids

Sweet sorghum extract was also evaluated for production of lipid using *Chlorella protothecoides*. This microalga exhibited dry cell yield and lipid content of 5.1 g L^{-1} and 52.5 %, respectively, when sweet sorghum extract was used as carbon source. However, when the sorghum extract was supplemented with yeast extract, the dry cell yield and lipid productivity of the microalga reached to 1.2 g L^{-1} day^{-1} and 586.8 mg L^{-1} day^{-1}, respectively [99]. Similarly, another heterotrophic thraustochytrid, *Schizochytrium limacinum* SR21, was explored for lipid production using sweet sorghum juice [100]. Semi-solid-state fermentation of crushed sweet sorghum has been reported to produce single cell oils (SCO) using the oleaginous fungus, *Mortierella isabellina*. The sugars and nitrogen present in sweet sorghum were used by the fungus for oil accumulation, and the maximum oil efficiency of 11 g/100 g dry weight of substrate was observed [101].

Nanomaterials

Sweet sorghum syrup-based facile, easy, reproducible, stable, spherical, and rapid synthesis of stable gold and silver glyconanoparticles was demonstrated at room temperature without the use any surfactants [102, 103]. Glucose and fructose present in the syrup were responsible as capping ligands along with sucrose

resulting in the formation and stabilization of nanoparticles with unique H-bonding capabilities for building smart nanomaterials which find application in biomedicine as probes of carbohydrate–carbohydrate interactions and carbohydrate–protein interactions, anti-adhesive therapy, biolabels, bioamplification strategies, antimicrobial agents, and in material science for microstructure manipulation, quantum dots, and magnetic bioconjugation [102, 104].

Xylooligosaccharides

Xylooligosaccharides (XOS) have a great prebiotic prospective and their production on an industrial scale is carried out from lignocellulosic materials (LCMs) rich in xylan by chemical and enzymatic methods, and the latter is preferred in food industry because of lack of undesirable side reactions [105]. XOS seems to exert their nutritional benefits in relation to human health exhibiting excellent physiological properties including improvement in decreasing cholesterol, bowel function, calcium absorption, and lipid metabolism [106]. Furthermore, they can promote a favorable intestinal environment by selectively enhancing the growth of colonic microbiota such as *Bifidobacterium* and *Lactobacillus* [107]. In the recent years, enzymatic production of xylooligosaccharides has attracted more industries in order to make the conversion process economical and also for the effective utilization of renewable plant-based biomasses [108]. In view of this fact, sorghum grain or sorghum bagasse after pretreatment can be used as excellent sources for XOS production as its hemicellulose content varies based on the genotype of cultivar. Four types of oligosaccharides were reported from alkali-extracted sorghum glucurono-arabinoxylan by digestion with a combination of (1- > 4)-β-D-arabinoxylan arabinofurano-hydrolase (AXH) and endo-(1- > 4)-β-D-xylanase (Xyl I), both from *Aspergillus awamori* and were purified by size exclusion chromatography followed by preparative high-performance anion-exchange chromatography [109].

Antidiabetic Compounds

The extracts of sorghum contain various phytochemicals like tannins, phenolic acids, phytosterols, and policosanols. Phenolic extracts of some varieties of sorghum exhibited antidiabetic effects by increasing serum insulin in diabetic rats, and the effect was comparable with glibenclamide, a powerful antidiabetic drug [110]. Sorghum tea made from roasted grains is rich in procyanidins which exhibited stronger α-glucosidase and α-amylase inhibitory activities [111]. Commonly, acarbose, a commercially available drug, is used as an alpha-glucosidase inhibitor which reversibly and competitively inhibits the digestion of oligo- and disaccharides at the brush border of the small intestine and helps to keep blood sugar levels within a target range. This effect controls diabetes and also the development of obesity [112]. In this view, efforts can be made to validate the clinical role of procyanidins for the treatment of alpha-glucosidase inhibition.

Antioxidant Compounds

Antioxidant activity of various foods is significantly correlated with total phenols and tannins and based on this feature; sorghum foods were shown to possess antioxidant activity [113], hence prevents a plethora of physiological complications like cancer, early aging, diabetes, and cardiovascular diseases [114]. The quantity of antioxidant activity is based on the processing of sorghum samples and decorticated sorghum; cooking based on extrusion was shown to reduce the phenol content and accordingly the antioxidant activity [115]. To obtain the whole health benefits of sorghum, it is better to select a process which retains its total phenolic contents. Pigmented testa contains condensed tannins composed of flavan-3-ols which are excellent antioxidants [116]. The tannin sorghum contains high dietary fiber content which slows the hydrolysis of food in the GI tract and the calorific availability which may be responsible for reduced weight gain (antiobesity effect) in animals. Pigmented sweet sorghums have high concentration of 3-deoxyanthocyanins (luteolinidin and apigenidin) [117].

Antimicrobial Compounds

Studies conducted on the antimicrobial properties of sorghum extracts showed strong inhibitory activity against *Escherichia coli* [118]. The antimicrobial property of sweet sorghum against a specific microorganism is based on the type of cultivar as the antimicrobial property of a plant extract is based not only on the phenolic content but also on the presence of various secondary metabolites [119].

Cytotoxicity Against Cancer Cell Lines

Sorghum grain contains retrodihydrochalcones, 3-(2,4,6-trihydroxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one, and 3-(2,6-dihydrox-4-methoxyphenyl)-1-(4-hydroxyphenyl)-propan-1-one which are cytotoxic in nature against various human cancer cell lines [116]. However, future studies are required to evaluate the cytotoxic effects of retrodihydrochalcones from sweet sorghum.

Polylactic Acid

Polylactic acid (PLA) is a biodegradable thermoplastic resin that can be substituted for petroleum-based thermoplastics, reducing environmental pollution and other problems associated with petroleum-based plastics [120]. Lactic acid can be produced either through chemical synthesis or through a fermentation process [121]. Agro-based materials such as cereal grains like corn, sorghum, and sweet sorghum bagasse are major potential sources to produce lactic acid through

fermentation [122]. There are reports on the production of lactic acid monomer from different varieties of sorghum wherein the whole ground sorghum grain was liquefied and fermented to lactic acid using *Rhizopus oryzae* NRRL 395 and the efficiency of saccharification was dependent on the native glucoamylase [123]. Sweet sorghum bagasse residue after alcohol fermentation can also be used for the preparation of biodegradable PLA with a tensile strength of 49.5 M and a flexible strength of 65 MPa [124].

Protein-Based Films and Adhesives

Sorghum grain has an average protein content of 11 % and its proteins are classified as prolamins (kafirins) and non-prolamin proteins. Kafirins constitute 77–82 % of the endosperm proteins and are involved in intermolecular cross-linking. Kafirin was reported to have potential in biofilm-forming applications. Its mechanical, water-vapor barrier and color properties of free-standing films from laboratory-extracted kafirin were comparable to those of zein films of commercial importance [125]. Sorghum flour as such can be used as protein extender in phenol-formaldehyde-based plywood adhesive for sprayline coaters or foam extrusion. The sorghum-based plywood glue had a viscosity of 1,104 cP and adhesion strength of 1.37 MPa which was comparable with the industry standard glue [126].

Summary

Sweet sorghum is the only first generation feedstock that provides both food and fuel besides fodder with relatively high RUE, WUE, and NUE with greater adaptation to semiarid regions [127]. Though it has gained importance as a stable food and fodder crop, recently it is increasingly viewed as a viable feedstock for the production of various bioproducts ranging from biofuels, beverages, food, pharmaceuticals, antioxidants, antimicrobial, and antidiabetics. Hence, focused research on its production and processing is required for efficient exploitation of polymeric carbohydrates, fermentable sugars, and biomass for varied needs of the society.

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