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Prospect of Sorghum as a Biofuel Feedstock

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ABSTRACT

In this chapter we review the genetic variability and related agronomic perspectives of sweet sorghum. These characteristics are mainly presented and discussed taking in mind quantitative and qualitative traits of sweet sorghum as a multipurpose feedstock. The recent remarkable expansion of bioenergy crops, encouraged by favorable biofuel policies, has boosted intensive research programs worldwide on the use of sweet sorghum as feedstock for food, fodder, energy and in other industrial applications. In energy terms, sorghum is the only feedstock where ethanol can be produced either through grain, sweet juice, syrup or biomass, in other words having relevance to first, second and third generation biofuels. As a row crop, management practices developed for other conventional crops under a wide range of agro-climatic conditions can be easily adapted to cultivate sweet sorghum, thanks to its versatility and low input requirements. However, harvesting, transportation from field to processor and processing remain as past and present unsolved problems. Moreover the large diversity in traits, important for biofuel production, opens up excellent opportunities for sweet sorghum improvement through traditional breeding and modern molecular tools. In general biofuel candidate

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traits present across the sorghum genus are governed by multiple genes, and both additive and dominance components of gene action can be exploited while breeding for high stalk sugar and juice yielding genotypes. In order to take full advantage of all carbohydrate forms it would be advantageous to develop specialized cultivars that allow a single process to utilize all plant components for liquid fuel production. However, more focused research in this area may aid in enhancing the economic viability and environmental sustainability of sweet sorghum value chain.

Keywords: sweet sorghum, biofuel, stalk sugar, juice content, breeding, genomics, improvement

13.1 Introduction

The world population is estimated to increase from 6.7 billion to 9.2 billion by 2030. On the other hand, global oil production is expected to decline from 25 billion barrels to 5 billion barrels by 2050 (Campbell and Laherree 1998). Thus the energy demands of the future are likely to play a key role in geopolitical economics. Given this reality, nations around the world are investing heavily in alternative sources of energy, including bioethanol from a diverse set of feedstocks. Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop, providing food, feed and fiber for the world and is currently grown in 35 m ha (FAOSTAT 2013) in over 104 countries. This crop is considered a new generation bioenergy crop owing to its multiple uses and wider adaptability to varied agroclimatic conditions. Further, it accumulates sugary juice in its stalks, yielding higher in addition to the biomass and grain yields. Sorghum being a C_4 species is more water-use efficient and can be cultivated in areas lying between 400 South and North latitudes of the equator (Rao et al. 2009). Among different biofuel feedstocks, sorghum is of particular interest because its biomass is variously used for the production of energy, fiber, building materials or paper, as well as for syrup and animal feed, while the grain is either used for human consumption or for ethanol production or as feed. This is the only feedstock where ethanol can be produced either through grain, sweet juice, syrup or biomass, in other words having relevance to first, second and third generation biofuels. Sweet sorghum (Fig. 13-1a) has many useful traits such as a drought resistance (Rao et al. 2012), water logging tolerance, salinity tolerance (Almodares et al. 2009) and with high biomass yield, etc. In recent years biomass sorghum (Fig. 13-1b) is gaining popularity as investments on efficient cost effective lignocellulosic biofuel production are increasing in many nations.

Many national agricultural research systems such as Brazil, the United States of America (USA), India, China, the Philippines, Mozambique and

Figure 13-1 Sweet sorghum cultivar ICSSH 58 (1a) and biomass sorghum cultivar ICSV 25333 (1b) grown during rainy season 2011 at ICRISAT, Patancheru, India.

Kenya have initiated long term multidisciplinary programs to explore the full utilization of genetic diversity to improve the biofuel related traits in this unique feedstock. International organizations like International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), International Center for Agricultural Research in the Dry Areas (ICARDA), Food and Agriculture Organization (FAO), Common Fund for Commodities (CFC), and Global Bioenergy Partnership (GBEP), etc. have either directly or indirectly promoted research and development on this feedstock without compromising food/fodder security. Recently many private sector players such as Ceres, Advanta, Dow Agrosciences, and Monsanto have made significant investments in this feedstock, either alone or in collaboration with research organizations. For example, Ceres, Inc. committed to a multi-year, joint research initiative with Texas A&M University's Agricultural Experiment Station (TAES) to develop biomass sorghums for biofuel production.

The purpose of this chapter is to give a brief account of the suitability of sweet sorghum as a multipurpose feedstock.

13.2 Sorghum Biomass and Sugar Production Potential

Among the several types of sorghum, their yield potential for biofuel production is highly variable depending mainly on the type of production

system and conversion process to be used. In agronomic terms, specific yield components of interest (i.e., stem's juice to produce ethanol and/or starchy and lignocellulosic components for second generation biofuels) can be maximized through the use of appropriate management practices. The recommended sowing density is variable, ranging from 12 to 20 plants per m^2 (Guiying et al. 2000; Barbanti et al. 2012; Zegada-Lizarazu and Monti 2012). However, according to several authors, planting density does not have any effect on yield and sugar concentration (Ferraris and Charles-Edwards 1986a,b; Lueschen et al. 1991; Wortmann et al. 2010). It follows then that higher planting densities with narrower than conventional row spacing could result in higher stalk and sugar yields and improved control of weeds (Broadhead and Freeman 1980; Lueschen et al. 1991). As a warm-season crop, the best time to sow sweet sorghum is spring, therefore, in most situations, except for equatorial latitudes, early spring or late winter sowing is not recommended as the crop does not tolerate cold and does not grow well under low temperatures (the minimum germination temperature is 10°C). Therefore, the best sowing and harvesting times should be determined according to local temperature and climatic conditions (Table 13-1).

Even though sweet sorghum can be cultivated under no-tillage conditions (Saballos 2008), a well-cultivated seedbed, timely thinning and appropriate weed control (Tsuchihashi and Goto 2004), during the establishment phase will favor the development of a full stand plantation and enhance yields. Sweet sorghum as a cultivated crop could be susceptible

Table 13-1 Effects of sowing dates, nitrogen rates, water availability and harvest times on yield and quality of sweet sorghum.

	Treatments	Biomass yield (Mg ha^{-1})	Sugar yield (Mg ha^{-1})	Brix°
Sowing dates[1]	May 4	20.9	7.4	19.6
	June 3	14.9	4.6	18.3
	June 19	12.0	4.1	17.5
N fertilization (kg ha^{-1})[2]	0	11.9	1.6–2.2 (no response to N rate was found)	13.6
	101	14.5		13.3
	168	14.0		13.1
Irrigation[3]	Well watered	29.8	-	-
	Mid stress	24.8	-	-
	Severe stress	19.1	-	-
Harvest time[4]	Milk stage	17.1*	3.5	17.6
	Dough stage	16.6	4.0	20.0
	Ripe	16.6	4.2	21.0

[1]Data taken from Almodares et al. (2006).[2]Tamang et al. (2011). [3]Dercas and Liakatas (2007). [4]Broadhead (1972a). *Considering biomass humidity 70%.

to a series of pests and diseases such as aphids, lepidoptera, seed and stalk rots, anthracnose, Fusarium, maize dwarf mosaic and other viral diseases. Detailed information on pest control and management are given elsewhere (ICRISAT 1982; Fuller et al. 1988; Guiying et al. 2000; Saballos 2008).

Fertilization requirements of sweet sorghum depends on the fertility level of the field in which it is grown, but in general sweet sorghum requires almost 40% less nitrogen fertilizers than maize (Smith and Buxton 1993). Some reports suggest that for energy purposes the timing of fertilization is more important than the fertilization rate (Lueschen et al. 1991; Guiying et al. 2000; Almodares and Darany 2006). However, the reported effects of fertilization rates on yields are somewhat contradictory. For example, the added nitrogen fertilizers (0, 101 and 168 Kg ha^{-1}) in Texas, USA had little discernible effects on increasing fermentable sugar production (Table 13-1; Tamang et al. 2011). On the other hand, Wiedenfeld (1984), also in Texas, demonstrated that depending on the cultivars (MN 1500, Rio) the threshold for increased biomass yields (from 9.0 to 19.7 Mg ha^{-1}) and uptake rates (from 48 to 140 kg N ha^{-1}) changed with the fertilization levels applied (0, 112, 224 kg N ha^{-1}); but in general juice quality, expressed as total dissolved solids, decreased with the highest fertilization level.

Sweet sorghum produces best when adequate moisture is available (Table 13-1), but its real potential appears when it is grown under suboptimal conditions where the combination of its high radiation use efficiency and water and nutrient use efficiencies allow it to continue to produce when other energy crops would struggle (Woods 2001). Zegada-Lizarazu et al. (2012) determined that the water-use efficiency of sweet sorghum increased by 20% while that of maize decreased by 5% when these species were grown under limited water availability (Ψ= –868 kPa). From Table 13-1, it can be seen that yields up to 30 Mg ha^{-1} can be obtained when sweet sorghum plants are well watered. Mastrorilli et al. (1995) and Dercas and Liakatas (2007) indicated that such yields are reachable when about 554–657 mm of water are readily available to be consumed. However, when plants are stressed, yields are reduced accordingly and the degree of impact is also dependent on the plant growth stage when the dry period occurs. Dercas and Liakatas (2007) indicated that compared to well watered plants, yield reduction was only 1% when the drought stress occurred after anthesis. On the other hand, when the drought stress was throughout the vegetative growing period the yield reduction ranged from 25 to 36%. These results are in agreement with those of Mastrorilli et al. (1999), who indicated that the most sensitive growth period of sweet sorghum to drought is between 40 and 60 days after emergence. However, Zegada-Lizarazu and Monti (2013) determined that the most pronounced effects of drought on the photosynthetic apparatus are at later growing stages.

Although some irrigation trials indicated that the sugar concentration (glucose, fructose and sucrose) in sweet sorghum stalks did not change significantly due to the stress level and irrigation frequency (Curt et al. 1995; Miller and Ottman 2010), other studies indicated that sugar concentration in the stems follows an inverse pattern to that of biomass accumulation during drought and re-watering periods (Zegada-Lizarazu and Monti 2013). Miller and Ottman (2010) found that theoretical ethanol yields were similar across irrigation frequencies, while Sakellariou-Makrantonaki et al. (2007) indicated that the irrigation method had significant effects on ethanol yields. They found that subsurface drip irrigated plots produced up to 44% more ethanol than conventionally drip irrigated plots.

In quantitative and qualitative terms, the soft dough stage of grain filling has been considered the optimum harvest time for several sweet sorghum cultivars (Table 13-1). Broadhead (1969, 1972a) determined that sugar concentration and °Brix in the stem's juice increases from flowering to ripening, but due to the concomitant increase of starch in the juice, its quality is reduced when the plants are harvested after the dough stage. This determination of "quality", however, may have been based on the production of crystalline sugar, where starch is clearly a detriment. According to Tsuchihashi and Goto (2004) a practical method to determine the optimum harvest time is based on °Brix readings taken continuously from 30 days after anthesis until a peak period is reached. However, such optimum harvest period is short and moreover the fast degradability of the sugars in the stems remains a major bottleneck for harvesting large areas.

Silage harvesters, straw balers and sugarcane harvesters are being tested worldwide for harvesting sweet sorghum, but they still need to be improved/adapted before large-scale applications. A mobile field harvester that cuts, presses and collects the juice in a single pass has been experimentally tested with promising results (Kundiyana et al. 2006) but its applicability under real farming conditions is still unknown. Even though Broadhead (1972b) indicated that chopped stalks (20–40 cm) could be more easily handled and transported than whole-stems, the fast quality decay (in terms of °Brix and sucrose) limits its handling to about 48 hours following harvest; after that significant sugar quality losses are experienced. Eiland et al. (1983) showed that whole stalks were more stable than chopped stalks, where one week after harvest whole stems did not show significant signs of deterioration. These studies suggest that storage and transport issues are unsolved problems of both the past and the present. The short time available for transportation and processing are critical issues, especially in the case of large-scale production systems where large land areas must be harvested in a relatively short period of time.

13.3 Candidate Traits of High Biomass and Sugar

Although several biomass sorghum hybrids have been developed and improved through the years for the production of lignocellulose, sugar and starch (Rooney et al. 2007), breeding sorghum for biofuel purposes is largely based on methods that were developed for grain and forage production. Around the 70s, some sorghum populations were more or less improved for biomass and sugar production (Smith et al. 1987). In the following years these populations were selected for hybrid combinations and male-sterility (Petrini et al. 1995). Currently, promising populations and lines are being recombined in the search of the best ideotypes for multipurpose uses and adaptation to diverse environmental and stress conditions.

In addition to maximum biomass, high content of fermentable sugars, high germination capacity and early vigor of seedlings, another fundamental characteristic that modern biomass sorghum should have a wide range of maturity classes. This would allow staggered planting dates and extended harvesting periods, to better fit the requirements of a processing industry. For this purpose, especially in temperate climates, traits for low temperature tolerance that would allow early sowing must be selected. Even though there currently exists a considerable variation in low temperature tolerance among sorghum genotypes (Franks et al. 2006; Saballos 2008), the selection of cultivars with a high and uniform germination capacity and fast seedling emergence under low temperatures constitutes a prerequisite for sweet sorghum production in temperate climates.

Improving drought tolerance is an important trait to be considered in sorghum production because productivity and sugar concentration are adversely affected by drought. Very little is known about the genetic mechanisms that control drought tolerance in sorghum. The stay-green drought adaptation has been identified as a mechanism that allows sorghum plants to retain green leaves and maintain photosynthesis in a wide range of environments. The physiological basis of stay-green, however, remains unclear but its positive effect on yield under terminal drought has been confirmed and seems to be closely correlated with lodging resistance (Rosenow and Clark 1995). For biofuels production, this trait has the additional benefit to facilitate processing of stalks. Four major Quantitative Trait Loci (QTLs) have been identified to be involved in the stay-green trait of sorghum (Xu et al. 2000; Haussmann et al. 2002; Sanchez et al. 2002) and therefore, through their manipulation, drought tolerance of several biomass sorghum types could be enhanced.

Lodging of tall plants is also a common problem in sweet sorghum cultivars, especially when grown in high densities and windy areas. Selecting for lodging-resistant cultivars, i.e., developing plants with a good balance between tallness and increased stem structural and/or

morphological resistance (Hondroyianni et al. 2000), could be an important factor for the successful establishment of sweet sorghum as an energy crop. The interplay of the four major genes known to control plant height by affecting internode elongation (Saballos 2008) and stem morpho-structure are interesting traits to be investigated in the future. Selecting for large root systems or accentuated presence adventitious roots, balanced panicle weight, and stay-green may also influence the plants resistance to lodging (Saballos 2008).

Among other traits that can be manipulated through plant breeding, the photoperiod sensitivity of sorghum, mainly controlled by maturity genes, is an interesting one that could result in delayed flowering, increased tallness, and increased biomass production as the plants will continue to grow throughout the whole growing season in areas with more than 12 hours of daylight (Saballos 2008).

13.4 Genetic Improvement of Sorghum for Biofuels

Both the conventional and molecular breeding can be deployed to improve this crop for biofuels production.

13.4.1 Conventional Breeding

The nonsweet character is conferred by single dominant gene whereas stalk sugar is controlled by recessive genes with additive and dominance effects (Guiying et al. 2000). 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 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) (Rao et al. 2009).

The generation mean analysis of two crosses has shown predominant additive gene action for traits like sucrose and Brix of juice. However, for cane and juice yield, dominance gene action and dominance x 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 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 (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.

13.4.2 Molecular Breeding

Genetic mapping and characterization of QTLs is considered a valuable tool for trait enhancement. Plant breeders have investigated QTLs associated with the sugar components (brix, glucose, sucrose and total sugar content) and sugar-related agronomic traits (flowering date, plant height, stem diameter, tiller number per plant, fresh panicle weight and estimated juice weight), since the present attention is focused on identification/characterization of the molecular elements that influence the bioenergy-related traits. Previous studies showed a significant positive correlation between plant height (PHT), fresh total biomass yield, fresh stem yield and brix. Brix showed a positive correlation with sugar content and sucrose yield. Sugar yield in stems, the major factor influencing ethanol production potential of sweet sorghum, is determined by the combined effect of PHT, Stem and Leaf Fresh Weight (SLFW), brix and Juice Weight (JW) in the stalk. Thus, understanding the genetic control of these traits and the environmental effects would benefit in genetic improvement of sweet sorghum for ethanol production.

13.4.3 Agronomic Traits

Many QTLs affecting PHT are identified in sorghum (Lin et al. 1995; Ritter et al. 2008; Shiringani et al. 2010). Over 30 QTLs explained 7.0–62.5% of phenotypic variance. PHT of sorghum is controlled by four independently inherited genes: *Dw1*, *Dw2*, *Dw3*, and *Dw4* (Quinby and Karper 1954). *Dw2* is located on SBI-06 closely associated with DArT markers, sPb-7169 and sPb-1395 (Klein et al. 2001; Lin et al. 1995; Mace and Jordan 2010). *Dw3* is located on SBI-07 and *Dw3*, SbPGP1 colocalized with a height QTL on chromosome 7 (Brown et al. 2006). The *dw3* gene is Sb07g023730 flanked by the Simple Sequence Repeat (SSR) msbcir300 and Diversity Array Technology (DArT) marker M340509 and the RFLP marker SSCIR57 (Multani et al. 2003). The major QTL for height co-locates on genes, Sb.Ht9.1 in SBI-09 which is closely linked to the Restriction Fragment Length Polymorphism (RFLP) marker

txs307b (Lin et al. 1995; Brown et al. 2008). Three QTLs controlling PHT on SBI01, SBI-07 and SBI-09 were detected in four environments (Yan-and Guan et al. 2011). The QTL on SBI-07 is a major effect QTL controlling PHT localized in between the markers SbAGF06 and Xcup19. Similarly QTLs on SBI-09, is in between Sb5-206 and SbAGE0. Similarly 13 QTLs and one putative QTL influencing stem diameter were distributed over eight chromosomes. The QTL on SBI-03 and QTL on SBI-07 are major QTLs found to be stable across different environments.

Eight QTLs controlling SLFW were detected in different environments, located on SBI-01, SBI-04, SBI-07, SBI-08 and SBI-09; four of which were detected on SBI-01. Location of QTLs on SBI-09 and SBI-07 were between markers Sb5-206 and SbAGE03 and markers SbAGF06 and Xcup19, respectively. For fresh biomass, seven QTLs were stable out of 10 detected and were distributed on six chromosomes, of which two were found on chromosome SBI-01. Similarly five QTL associated with fresh leaf mass were detected on SBI-02, SBI-03, SBI-04 and SBI-06 with significant phenotypic variation in different environments. And for stalk mass a total of 15 QTLs, distributed on all chromosomes except SBI-08, were detected. Ten of these QTL showed significant effects on the trait across different environments (Shiringani et al. 2011). A total of 16 QTLs associated with dry biomass were detected on eight chromosomes, with clusters of four QTL each found on chromosomes SBI-01 and with three QTLs found on SBI-02 (Shiringani et al. 2011). In case of dry stalk mass, these were influenced by 10 QTLs out of which six QTLs on five chromosomes were stable in different environments.

Six QTLs controlling juice weight were mapped on SBI-01, SBI-04, SBI-07 and SBI-09 across environments. The QTL on SBI-07 was located between markers SbAGF06 and Xcup19 and QTL on SBI-09, located between Sb5-206 and SbAGE03 markers, respectively. About 20 QTLs for brix were identified (Shiringani et al. 2010), four of which were detected on SBI-01, SBI-02, SBI-03 and SBI-07 with QTL on SBI-03 identified between markers Xtxp009 and Sb5-236 and QTL on SBI-02, between Xcup74 and Xcup29 respectively. Similarly three QTLs for grain yield were identified, two on chromosome 6 and one on chromosome 10. Along with them a minor QTL for increased grain yield under stress condition originated from Rio (the sweet sorghum parent) was identified on chromosome 4 (Ritter et al. 2008).

The gene orthologous to maize tillering gene, *Tb1* was identified in rice (Takeda et al. 2003), *Arabidopsis* (Finlayson 2007) and sorghum (Kebrom et al. 2006). Sequence mapping identified *Tb1* in sorghum as gene Sb01g010690, which is closely linked to the flanking SSR markers, txp302 and txp482 (Mace and Jordan 2010). This location also corresponds to major effect tillering QTL identified in three different studies (Paterson et al. 1995; Feltus et al. 2006).

In relation to maturity, six genes, *Ma1*, *Ma2*, *Ma3*, *Ma4*, *Ma5* and *Ma6* were identified in sorghum. The flowering time QTL was identified on chromosome 9 (Pereira and Lee 1995; Lin et al. 1995). The QTL on chromosome 1 is also consistent with flowering time (Crasta et al. 1999; Ritter 2007). The major flowering time QTL on chromosome 6 in CS05 was reported as *Ma1* (Lin et al. 1995; Brown et al. 2006). *Ma1* is known to be regulated by photoperiod and known to regulate the height and flowering time. *Ma1* has the largest impact on flowering date of all the maturity genes, and is flanked by the Amplified Fragment Length Polymorphism (AFLP) marker txa4001 and indel marker txi20 and RFLP markers pSB0189 and pSB0580 (Lin et al. 1995; Mace and Jordan 2010). The gene *Ma3* is located on SBI-01 and its locus on PHYB gene (Childs et al. 1997). Sequence mapping of the PHYB identified *Ma3* as gene Sb01g037340, closely linked to the flanking SSR markers txp229 and txp279 (Mace and Jordan 2010). The maturity gene *Ma4* has been reported to map near to txs1163 RFLP marker, however, no detailed genetic linkage mapping data has been reported for this locus. The projected location of this gene onto the consensus map was therefore based on the location of the RFLP marker, txs1163, together with the location of a closely linked major effect QTL for photoperiod sensitivity (Chantereau et al. 2001). The *Ma5* gene mapped to SBI-02 which, when present in the dominant form together with *Ma6*, very strongly inhibits floral initiation regardless of day length (Chantereau et al. 2001; Kim et al. 2004). The location of molecular markers flanking *Ma5* as determined by Fluorescence *In Situ* Hybridization (FISH) together with genetic linkage mapping are AFLP txa3424 and the SSR txp100 (Kim et al. 2004) and the location of *Ma5* on the consensus map was determined to closely linked to the SSR markers txp429 and txp431 (Mace and Jordan 2010).

13.4.4 Sugar-Related Traits

Production of biofuels from plant structural carbohydrates (the cellulose, hemicellulose and the lignin-containing portion of the stem, leaf and root tissue) is predicted to yield five times more net energy per unit land area than using grain starch and sugar while producing only a quarter of the greenhouse gases (Farrell et al. 2006; USDOE 2006; Somerville 2007). Many QTLs for structural and nonstructural carbohydrate yields are colocalized with loci for height, flowering time and stand density–tillering. Results of previously identified QTLs for grain and stem sugar composition and yield indicated that overall energy yields could be increased by concurrent improvement for both sorghum grain and sugar traits. Lignocellulosic leaf and stem structural biomass yield, composition and QTL can be used to improve sorghum as a biomass feedstock.

The QTL for grain starch was identified on chromosome 1, and sugar concentration on chromosome 3, which would be good breeding targets for improving energy content without physiological tradeoffs. A total of 10 QTLs and one suggestive QTL were detected for glucose content on seven chromosomes. For sucrose content, seven QTLs and two putative QTLs were pin-pointed on seven chromosomes. And for cellulose, which is a polymer of D-Glucose, a total of 16 QTLs were detected distributed on all chromosomes. The largest cluster was observed on SBI-06 (Shiringani and Friedt 2011). In the case of hemicellulose, eight QTLs were detected distributed across all chromosomes except chromosome 1 and 9. A total of 15 QTLs and two putative QTLs that control sugar content in stem juice were detected on seven chromosomes (Bian et al. 2006; Ritter et al. 2008; Shiringani et al. 2010). The QTL on chromosome 9 is colocalized with low grain yield and high stem sugar yield (WE05). With lignin, a total of 72 QTLs associated with fiber quality traits were detected on 10 chromosomes. A total of 17 QTLs were detected on all chromosomes associated with Acid Detergent Fiber (ADF). Higher additive effects among the detected QTLs were found on SBI-06, left flanked by E35M49-205 and SBI-07, left flanked by E31M59-202 respectively. And 14 QTLs distributed on all chromosomes, were detected associated with Neutral Detergent Fiber (NDF). The QTL on SB1-06 is flanked by Xtxp265. The QTL on SBI-07 is flanked by E31M59-202. For ADL, 15 QTLs were detected on SBI-04, SBI-06, SBI-07 and SBI-08 (Shiringani and Friedt 2011).

The brown midrib (*bmr*) mutants of sorghum have brown vascular tissue in the leaves and stem as a result of changes in lignin composition. There are about 29 mutants with altered lignin biosynthesis (monolignol) pathways categorized into four allelic groups, viz. *bmr*2, *bmr*6, *bmr*12 and *bmr*19 (Porter 1978; Saballos et al. 2008). *Bmr*6 and *bmr*12 represent the mutant forms of Cinnamyl Alcohol Dehydrogenase (CAD) and Caffeic Acid O-Methyltransferase (COMT) genes of the monolignol pathway, respectively.

SBI-04 contains *bmr*6 gene. *Bmr*6 results in altered lignin composition and affects cinnamyl alcohol dehydrogenase activity (Saballos et al. 2009). Using a sequence mapping the *bmr*6 gene was determined as Sb04g005950, linked to the SSR marker gpsb050 (Mace and Jordan 2010). The *bmr*12 gene is present on SBI-07 (Bout and Vermerris 2003). The *bmr*12 allelic group contains six known alleles (*bmr*12-ref, *bmr*12-7, *bmr*12-15, *bmr*12-18, *bmr*12-25 and *bmr*12-26) of the gene encoding the lignin biosynthetic enzyme caffeic acid O-methyltransferase (Bout and Vermerris 2003). Sequence mapping determined *bmr*12 gene as Sb07g003860 which is co-located with the SSR marker txp312 and the DArT marker sPb-6942 (Mace and Jordan 2010).

13.5 Biofuels from Sweet Stalk Sugars

In tropical, subtropical and arid regions from the USA, Mexico, China, India, southern Africa and other developing countries, where agronomic harsh conditions prevail, one of the most promising crops for fuel is sweet sorghum (Reddy et al. 2005; Rao et al. 2009; Zhang et al. 2010). This feedstock offers food-feed-fuel security as ethanol is produced from fermentation of sugary juice extracted from the stalks while grain is used for food or feed. This is a highly efficient photosynthetic crop that reached a worldwide production of 56 million tons of grain in 2009 (FAOSTAT 2011). Sorghums can be grouped as, grain, forage, high biomass or sweet, all of which are used for bioethanol production. In the USA only a small percentage of fuel ethanol (around 2–3%) is obtained from grain sorghum (RFA 2010; Turhollow et al. 2010), but in 2009 about 30% of the US grain sorghum was used for ethanol production (Blake 2010). An average yield of 390 L of ethanol from 1 ton of sorghum grain was obtained, but yields as high as 400 L/ton with fermentation efficiencies of more than 90% has been achieved and reported (Chuck-Hernandez et al. 2009). On the other hand, forage sorghum is characterized as a high biomass crop, and could be a valuable dedicated energy crop for lignocellulosic ethanol production. Its capacity has been boosted by intensive research programs worldwide, focused on the design of new varieties tailored for ethanol production (Rooney et al. 2007).

Sweet sorghums have generated interest as a feedstock for ethanol production since the 1970s. The main product of focus obtained is sugar (14% soluble sugars) rich juice that can be directly fermented into ethanol with efficiencies of more than 90%. Approximately 50–85 tons/ha of sweet sorghum stalks with juice extraction of 39.7 to 42.5 tons/ha led to 3,450 to 4,132 L /ha ethanol production has been reported (Serna-Saldívar et al. 2012). Other studies have shown similar ethanol production results including with production of 3,296 L/ha (Kim and Day 2011) and in the range of 4,750 to 5,220 L ethanol/ha were reported (Wu et al. 2010). In addition to the juice, the sorghum bagasse or residue, can also be converted to ethanol in a lignocellulosic conversion process. And with sorghum bagasse of 15.3 to 42.5 ton/ha, ethanol production of 2,400 to 6,375 L/ha was observed. By fermenting hemicellulose hydrolysate from sweet sorghum bagasse as the sugar source overall yields were high (>80 gal/US ton) and the ethanol titres ranged from 24 g/L to 32 g/L, with bagasse concentration of 10% dry matter (Geddes et al. 2012). Altogether with the juice, residue or bagasse can be converted to ethanol or used for other traditional applications. But sweet sorghum varieties typically have low grain yield, but recently varieties with more balanced grain/sugar production have been developed in China and India for ethanol production. These varieties can be used as a dual-purpose

crop, where the grain is harvested for human or animal consumption and the sugars are fermented to ethanol. Alternatively, these varieties can be used as dedicated bioenergy crops, where both the sugars, and the grain, and the bagasse are used for ethanol production (Vermerris 2011). Sorghum yields a better energy output/input ratio compared to other feedstocks such as sugarcane, sugar beet, maize and wheat (Almodares and Hadi 2009).

Hence there is a considerable elation for the use of sweet sorghum as an alternative feedstock for ethanol production due to the following benefits: 1) high yield potential and composition, 2) water-use efficiency and drought tolerance, 3) established production systems, 4) potential for genetic improvement using both traditional and genomic approaches, and 5) successfully grown ability to grow on clay, clay loam or sandy loam soils and can tolerance tote salinity and alkalinity to a large extent (Reddy et al. 2008; Rao et al. 2009). Public and private entities continue to perform research to maximize sugar content, increase or diminish its grain production capacity and increase production yields.

Even with these positive attributes, the use of sweet sorghum has been slow to develop. Some of the impediments to its commercialization are the ones facing all new technologies. Even though sweet sorghum harvesting and processing is similar to sugarcane, it is considered a new technology by many. That is because it has never been produced in large commercial scale. To produce the crop in large scale, several issues need to be addressed, which are important but not insurmountable.

Internationally, sweet sorghum projects are proceeding slowly. The most active countries with strong biofuels programs include Brazil and the Philippines. As in the USA, projects are still in a pre-commercial scale, usually incorporating sweet sorghum into existing sugarcane operations. In the case of Brazil, Monsanto is expected to sell enough sweet sorghum for about 20,000 hectares in 2013, which is enough to produce about 80 million liters per year of ethanol (21.1 million gallons). Last season, Brazilian mills planted Ceres sweet sorghum on more than 3,000 hectares. The trials demonstrated large increases in biomass, extractable juice volume and total harvestable sugar, with hybrids averaging 80 or more metric tons per hectare. Subsequent field evaluations in Southeast USA have confirmed similar results. Similarly various projects are developed in the Philippines, such as in San Carlos, a pilot trial of 1,000 hectares is planned for conversion to syrup. The plantation would supply feedstock for 2.5 million liters annually. In San Mariano, the Isabella plant has a production capacity of 52,840 gallons of bioethanol per day. A total planting of 400 hectares of sweet sorghum is planned by June, 2012 (Nieves 2012). In the USA, the focus is largely on research and development although the Mississippi Agricultural and Forestry Experiment Station (MAFES) and the United States Department of Agriculture (USDA) have developed

several sweet sorghum varieties. In some cases, the projects are performed in conjunction with agriculture departments in universities and the private sector. At Texas A&M University, hybrid sweet sorghums varieties are being developed for biomass and energy production. And study conducted in four different areas of Texas; Moore, Hill, Willacy, and Wharton counties, showed that ethanol production using sweet sorghum and corn is the most profitable. And also stated that sweet sorghum ethanol supplemented by grain is more economical (Morris 2008). In Tennessee, Delta BioRenewables delivered its first-ever commercial-sized batch of sweet sorghum juice to the Commonwealth Agri-Energy plant in Kentucky. Delta BioRenewables is looking to supplement corn with sorghum, which is drought tolerant and a good rotation crop. After the successful test batch, the company hopes to use sweet sorghum for approximately 5% of its annual ethanol production (Sapp 2012). Similarly in Oklahoma, Oklahoma State University is developing biofuels from sweet sorghum with high-energy content, drought resistance and adaptation to multiple climates and soil conditions (Sapp 2013). In Georgia, the USDA's Agricultural Research Service is looking at 117 different genotypes of sweet sorghum that could prove to be a key feedstock for biofuels in southern USA. In the USA, sweet sorghum can be grown in the same areas as grain and forage sorghum, making it a viable energy crop for regions that currently do not participate in corn ethanol production, including the southern Great Plains, mainly Kansas, Nebraska, and Texas (NASS 2007). The possible growing area in the USA makes sweet sorghum a potentially viable energy crop.

Because sweet sorghum can be used as either an energy crop or sold as forage for livestock, sweet sorghum has different markets that make it more secure for farmers to grow versus biomass crops that will only have one market option. The potential food versus fuel conflict, from the diversion of crop land for its cultivation is allayed as sweet sorghum meets the multiple requirements of food, fuel and fodder (Basavaraj et al. 2012). In view of the potential benefits of sweet sorghum as a feedstock for bio-ethanol production, a value chain approach model of sweet sorghum as a food-feed-fodder-fuel is being tested on a pilot basis in Andhra Pradesh, India to augment incomes of farmers while promoting a sustainable sweet sorghum–ethanol value chain. The farmers cultivating sweet sorghum around the distillery are directly linked for supply of sweet sorghum stalk, and the distillery entered into a buy back agreement with farmers to purchase the stalks at an agreed price prior to sowing of the crop (Basavaraj et al. 2012). Although efforts to commercialize sweet sorghum are slowly developing, opportunities to integrate this crop's unique qualities into the nation's and the world's biofuels industry are real. As efforts to integrate sweet sorghum into new and existing processes continue, establishment

of large commercial plantations will require investment capital from well-informed investors, experienced agricultural specialists and diligent planning.

13.6 Lignocellulosic Ethanol Production—The Status

Governments around the world have recognized the role that biofuels play in a renewable energy portfolio and have introduced targets for their implementation in the future (US Congress 2007). Although currently most of the ethanol produced from renewable resources comes from sugarcane and starchy grains, significant efforts are being made to produce ethanol from lignocellulosic biomass such as agriculture residues. Production of renewable fuels, especially bio-ethanol from lignocellulosic biomass, holds remarkable potential to meet the current energy demand and serve as a safer alternative to the common additive, Methyl Tertiary Butyl Ether (MTBE), in gasoline (Scott-Kerr et al. 2009). The technological advances in recent years are promising to produce ethanol at low cost from lignocellulosic biomass (Joshi et al. 2011).

The leading nations in bioethanol production are the USA and Brazil, with the US being the world's largest producer (Carere et al. 2008). Asian countries altogether account for about 14% of world's bioethanol production. Production of bioethanol largely depends on sugarcane and/or starch based grains and tubers (mainly corn, potatoes) and is considered a first generation process but extensive use of grain crops for fuel has become controversial. These first generation crops cannot sufficiently meet the needs of global energy, especially today when the world population has reached 7 billion people (Serna-Saldívar et al. 2012). Over all US energy consumption is growing at an average annual rate of 0.3% from 2010 to 2035 (AEO 2012). The use of these crops cannot support the ambitious objectives of renewable fuel legislation in countries like the USA, where a target of 36 billion gallons of liquid biofuels have been established for 2022. Therefore, second generation processes which utilize lignocellulosic materials to produce bioethanol are gaining momentum.

The production of ethanol from lignocellulosic biomass (corn and sorghum stover, wheat straw, sugarcane bagasse, rice straw, rice hull, corn cob, oat hull, corn fiber, woodchips and cotton stalk; energy crops and various weeds, etc.) has become one of the best alternatives as these sources are abundant and the cost of their procurement is often low. Energy crops of greatest interest include perennial grasses as switchgrass (*Panicum virgatum*), energy cane (*Saccharum* spp.), sweet and forage sorghum (*Sorghum bicolor*) and Miscanthus (*Miscanthus* spp.) and giant reed (*Arundo donax*) (Serna-Saldívar et al. 2012). Many countries are moving towards developing or have already developed technologies to exploit the potential of lignocellulosic

materials for the production of bioethanol. Lignocellulosic feedstocks not only include agricultural residues, wood, dedicated energy crops but also municipal solid waste, which has significant advantages over first generation feedstocks for ethanol production. The development of new and improved bioprocesses and feedstocks could lead to cost reduction from an estimated of 0.69 cents to below 0.51 cents/L (Kim and Day 2011). The net energy balance of lignocellulosic ethanol, in terms of energy in/energy out, has been shown to be significantly lower than ethanol produced from sugarcane and starch feedstocks (Hayes 2009). Additionally, emissions of greenhouse gases are reported to be 50–85% lower for lignocellulosic ethanol than those from gasoline, with corn ethanol providing a 25–40% reduction (IEA 2004; Hayes 2009). Extensive research has been completed on conversion of lignocellulosic materials to ethanol in the last two decades (Dale et al. 1984; Wright 1998; Azzam 1989; Cadoche and Lopez 1989; Reshamwala et al. 1995; Duff and Murray 1996). Lignocellulosic materials are often hard to dispose off and cannot be digested by humans but are rich in sugars that can be fermented into ethanol. Marginal land can be used, with less intensive use of water and fertilizers. Production of cellulosic ethanol can also utilize "waste materials" such as agriculture and forest residues as feedstocks.

Lignocellulosic biomass consists of lignin, cellulose, hemicellulose, pectin and other components. Cellulose is the principle component typically ranging from 30 to 50% of dry weight. Cellulose is a homopolysaccharide composed of repeating β-D-glucopyranose units (Zhang et al. 2004). Hemicellulose is less complex, is 25 to 35% of dry biomass and easily hydrolysable, composed of pentoses (D-xylose and D-arabinose), hexoses (dmannose, D-glucose and D-galactose) and sugar acids (Balan et al. 2009). Lignin is the third major component, ranging from 20 to 35%. It is a complex polymer of phenyl propane (p-coumaryl, coniferyl and sinapyl alcohol) acting as a cementing agent and an impermeable barrier for enzymatic attack (Howard et al. 2003). Lignocellulosic biomass can be converted to ethanol using either a biochemical or thermochemical platform. In biochemical conversion the plant fiber is separated into its component parts; cellulose, hemicelluloses and lignin. The cellulose is then further broken down to simple sugars that are fermented to produce ethanol. Thermochemical conversion transforms the lignocellulosic feedstock into carbon monoxide and hydrogen (syngas) by partial combustion. These gases can be converted to liquid transportation fuels or commodity chemicals by catalytic or biological pathways. Though the lignocellulosic biomass is abundant, the commercialization of potential processes to produce ethanol from biomass is limited due to high capital costs, insufficient research and the associated risks.

Bioethanol production from lignocellulosic materials relies on technologies that will efficiently hydrolyze cellulosic biomass to fermentable sugars. Although several detoxification methods have been devised, an appropriate strategy for efficient hydrolysis of cellulose to fermentable sugars is lacking (Alvira et al. 2010; Geddes et al. 2011). The current status of technologies and technical challenges involve cost effective pretreatments to liberate the cellulose from the lignin/hemicellulose matrix and reduce its crystallinity. Similarly, research to reduce costs to produce high sugar yields at accelerated rates is under way. The improvements in pretreatment processes, improvement in efficacy of enzymatic hydrolysis via the development of more efficient enzymes, improvement in fermentation process efficiency, and the development of improved technologies to recover ethanol and removal toxic by-products will decrease the operating and capital costs. Integrated fermentation technologies for lignocellulosic materials such as Simultaneous Saccharification and Fermentation (SSF), simultaneous saccharification and cofermentation (SSCF), consolidated bioprocessing (CBP) and genetic engineering are currently evolving, and could potentially provide technologies that will lead to efficient, commercial production of bioethanol from lignocellulosic material. Based on the current state of technology, capital costs for biochemical cellulosic ethanol are estimated to be between US$4.03 and $5.60 per US gallon of annual capacity. Operating costs are estimated to be between US$1.34 and $1.69 per US gallon, depending upon the assumptions made about feedstock costs, enzyme costs, and the kind of pretreatment to be employed (Scott-Kerr et al. 2009). Projected capital costs for future plants employing anticipated improvements in biochemical conversion are estimated to be US$3.33–4.44 per US gallon ethanol annual capacity with operating costs dropping to US$0.40–0.89 per US gallon of ethanol (Scott-Kerr et al. 2009). Utilization of lignocellulosic materials can replace the equivalent of 40% of the gasoline in the US market (Wheals et al. 1999). It was predicted that the use of higher carbohydrate content materials combined with the improvement of conversion technology could reduce the cost of ethanol (Sun and Cheng 2002).

In USA the Energy Independence and Security Act (EISA) of 2007 mandates that the nation need to produce 30 billion gallons of biofuel by 2020. About 16 billion gallons need to be from cellulosic biomass. Geopolitical and national security reasons have contributed to the inevitability of seeking alternative energy, especially from renewable and sustainable sources. According to Bloomberg New Energy Finance there is enough biomass available to produce 93 billion US gallons of cellulosic ethanol in 2030 (BNEF 2012).

Some of the developing countries such as Nepal, India with rich biodiversity and renewable resources have never utilized these resources to

their full potential given the social and economic challenges the countries face. The increasing consumption pattern coupled with rising populations and increasing per capita demand for energy has placed an unsustainable burden on the environment of these countries. Though the Indian government has a policy on hand to blend a certain amount of (10%) ethanol into gasoline (petrol), this has never been implemented due to unsettled disputes over ethanol prices and other vested interests. Currently there are no visible commercial applications of biodiesel or bioethanol in such countries (Joshi et al. 2011). India has 0.5% of the oil and gas resources of the world (Sukumaran and Pandey 2009). The demand for motor gasoline has been growing at an average annual rate of 7% during the last decade (MPNG 2009) and it shows an increasing trend. India is one of the largest producers of ethanol and currently all commercial ethanol production uses molasses as feedstock. The demand for ethanol is projected to be 2.2 billion liters by 2017. Consequently, sourcing of ethanol from renewable feedstock resources other than molasses is imperative for meeting this increased demand. Hence lignocellulosic biomass is an important potential resource that can be used since India does not have surplus grains or other starchy biomass to spare for fuel applications. Presently, eight strong players are setting to unlock the full potential of lignocellulosic ethanol (2011–2030) including the USA, Mexico, EU-27, Brazil, Australia, China, Argentina and India (BNEF 2012). The race is on to commercialize this second generation ethanol by reducing the costs of the lignocellulose-to-ethanol process. Production requires significant cost reductions and at least the same level of financial support that was given to the first-generation systems if second-generation ethanol is going to be fully competitive by 2020 (Stephen et al. 2011).

The next five years, the often scoffed mantra of cellulosic ethanol developers is getting whittled down to the next year or two. A milestone reached in 2013 when Blue Sugars Corp. got the first cellulosic Renewable Identification Number (RIN) issued by the US EPA. Another notable event happened was when Ineos Bio began commissioning its plant in Florida. Similarly Chemtex International Inc. announced a new 20 million gallon per year (MMgy) project in North Carolina, even as it is commissioning its first, similarly sized plant in Crescentino, Italy. A 6.25 MMgy of cellulosic capacity in the US and Canada at nine demonstration plants and more than 104 MMgy under construction are coming online in 2013–2014. Some 20,000 gallons of cellulosic ethanol was produced at the Upton, Wyo., plant operated by Blue Sugars Corp. The company announced partnership and the first commercial licensing agreement with Brazil's big oil company, Petrobras SA. Since 2010, the two have been collaborating on Blue Sugar's technology, using bagasse as the feedstock. During this course a large reduction in the use of enzymes was achieved in the hydrolysis process. Internationalization is apparent in Florida as well, where the 8 MMgy

Ineos New Planet BioEnergy LLC plant is being commissioned. Ineos purchased its cellulosic ethanol technology from Bioengineering Resources Inc. in 2008, along with BRI's research facility in Fayetteville, Ark. More than 40,000 hours of run time have been chalked up in the pilot facility based on the microbial conversion of syngas into ethanol just in 10 minutes from when the feedstock enters the gasifier until it exits as ethanol. In Italy commissioning of Chemtex's commercial-scale plant has also been under way. Chemtex received a conditional USDA loan guarantee for a 20 MMgy project in Sampson County, N.C., with a 2014 start-up. Further south, a developer announced a 20 MMgy plant in Lenox, Ga., is expect to be completed in late 2013, focusing on both cellulosic ethanol and fuel pellets using paulownia tree. Another ethanol industry, Abengoa BioEnergy is a familiar player in the U.S., operating six first-generation plants with a total capacity of 374 MMgy. It is a subsidiary of Spain-based Abengoa, a big player in the renewable energy sector. Its first cellulosic ethanol facility is under construction in Hugoton, Kans. The company expects to require less than 15% of the available biomass (corn stover and switchgrass) from a 50-mile radius. In the same way BlueFire Renewables Inc.'s 19 MMgy plant in Fulton, Miss., is negotiating with China Huadian Engineering Co., a unit of China Huadian Corp., which is China's fourth largest utility, to invest in the Fulton facility, in return, thereby gaining BlueFire technology. The company also formed a new subsidiary, SucreSource LLC, to market its front-end process for sugar production, GS Caltex, Korean oil and petrochemical company has a professional services agreement with SucreSource for pilot testing of its process for chemical production operational. It is said that some publically traded companies keep their investors well-informed, with US. Securities and Exchange filings are available for the public to read. Some companies are quite aggressive in telling their stories as they seek to attract investors, while others, illustrated by World Ethanol Institute, lay low until concrete progress is reported (Schill 2012).

13.7 Food-Fuel Tradeoffs

It is often stated that sweet sorghum cultivars do not produce grain yield or the grain yield is low compared to that of grain sorghum. Studies at ICRISAT during 2007–08 showed that sweet sorghum hybrids had higher stem sugar yield (11%) and higher grain yield (5%) compared to grain types and 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 during post-rainy season, both sweet sorghum hybrids and varieties had higher stalk sugar yields (50 and 89%) and lower grain yields

(25 and 2%). Thus, there is a tradeoff between grain and stalk sugar yields in the sweet sorghum hybrids, about 25% in post-rainy season and the tradeoff being less in both hybrids and varieties in the rainy season (Rao et al. 2010). This is further supported by other published work (Zhao et al. 2009) showing that there are significant soluble sugars in the stems (79–94%) during the post-anthesis period, and the hybrids exhibited significantly higher soluble sugars than varieties with the same maturity period; and the effects of year, harvest time and genotype on calculated ethanol yield 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. Therefore, selection programs can aim to improve both sugar and grain yield traits simultaneously.

13.8 The Future

Sorghum will play an important role in the agricultural systems of the future. Its high photosynthetic efficiency, adaptability to various climates and conditions, high carbohydrate production potential, low input requirements and efficient use of water make it both versatile and sustainable.

One of the unique benefits of sorghum is its ability to produce carbohydrates in several different forms, including grain, directly fermentable sugar and lignocellulosic biomass. The various carbohydrate forms in grain, stalk juice and biomass can be used for manufacturing varied bio-products, including food, fuel, feed, fodder or fiber. In order to take full advantage of all carbohydrate forms, processing and harvesting equipment must be developed for maximizing yields of each component. To date, equipment exists for either harvesting grain from grain sorghum, or for extracting liquid sugar from sweet sorghum, but there is no existing equipment for efficiently harvesting all three components from a single crop.

When lignocellulosic ethanol production reaches full commercial scale, forage and sweet sorghums will be sought after as highly productive biomass feedstocks in diverse agro-climatic conditions. In a scenario where biofuels are the main product of interest, it would be advantageous to develop a single process that could utilize all plant components for liquid fuel. This would require simultaneous hydrolysis of the starch and cellulose components, and conversion of all plant carbohydrates to ethanol (or another biofuel like butanol). While more research is required in this area, it is a worthwhile goal.

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