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## Biofuels

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### Sorghum biomass: a novel renewable carbon source for industrial bioproducts

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# Sorghum biomass: a novel renewable carbon source for industrial bioproducts

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Sorghum (*Sorghum bicolor* [L.] Moench) biomass is considered as one of the potential renewable sources of energy for economic development and environmental sustainability, owing to its wide adaptability, C<sub>4</sub> photosynthetic pathway, and high nitrogen and water use efficiency. This plant could be effectively utilized as a source of food (grains), fodder (stem) and also as feedstock (lignin, cellulose and hemicellulose) for production of industrial solvents including biofuels. Genetic manipulation of sorghum has resulted in development of improved cultivars of sweet, high-biomass and low lignin sorghums (*bmr*) and so on. with increased productivity, palatability, along with reduced recalcitrance and enhanced tolerance to abiotic stresses, which can meet the diverse needs of population. This Review elaborates on recent developments in sorghum research towards conversion of cellulose and hemicellulosic components of sorghum biomass to biofuel and value added biochemicals by developing affordable processes at different sectorial levels.

There is a greater demand for alternative energy sources worldwide due to the rapid decrease of natural energy sources associated with drastic increase in the population and economic growth. In addition, constant use of petroleum products increases carbon dioxide emissions associated with climate change [1]. In this context, production and use of domestic energy resources including renewable assumes high priority to ensure energy security.

Of late, biomass feedstocks have significant potential to contribute to the biofuels production, and to decrease GHG emission and global warming [1]. This strategy fulfils the desire of most of the nations to become less dependent on imported fossil fuels and attaining energy security based on indigenous renewable sources. One of the prospects of improving rural economy is cultivation of bioenergy crops and conversion of their biomass to biofuels [2]. This created lot of interest among

researchers to strive for improvement of biofuel traits in biomass feedstocks all over the world [3–5]. The production of biofuels from biomass include ethanol from corn (*Zea mays* L.), sugarcane (*Saccharum* spp.) and sweet sorghum juice [6–8], biohydrogen from sugarcane (*Saccharum officinarum*), sweet potato (*Ipomoea batatas*) and sweet sorghum [9–11], methane from manure and biomass waste [12]. Currently, sugarcane (*Saccharum officinarum*) ethanol is produced through fermentation of sucrose rich juice, which can easily be extracted from stems.

The usage of crops such as sugarcane, corn and sorghum for biofuel production may be relatively easy compared with cellulosic or lignocellulosic processes. Ethanol produced as a by-product from sugarcane based sugar industry (molasses) may not be able to meet growing demand for energy. Consequently, efforts on alternate renewable sources for the production of ethanol

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## Key term

**Sorghum traits:** Various sorghum varieties developed through molecular ameliorating for grains, forage, second generation biofuels and so on.

increased. Processes for production of biofuels from the abundant lignocellulosic sugarcane residues will boost the ethanol output from sugarcane. Like sugarcane, sorghum residues, bagasse and straw could be the ideal feedstock for second-generation (2G) biofuel production. High biomass sorghum could be the best option mainly because of richness in carbohydrate content, wider adaptation to diverse agro-ecologies including marginal lands and, further, it does not compete with food crops for water or fertile land [2,13]. However, the literature on bioproducts from sorghum biomass is scarce; hence an attempt is made in this article to discuss in detail on the production potential of sorghum for 2G biofuel production *inter alia* other bioproducts from sorghum biomass.

### Biofuel & its importance

Use of fossil fuels is known to cause environmental damage due to the emission of GHGs leading to global warming. Hence, there is a great demand for alternative energy sources that are renewable and eco-friendly. Biofuel is defined as a type of fuel whose energy is derived from biological carbon fixation. Implementation of blending ethanol with petroleum has been in use in most of the countries worldwide to reduce the dependency on fossil fuels. Ethanol is being widely blended with gasoline in the USA, Brazil and China [1]. At present, global production of ethanol exceeds 85 billion liters per year [14,15] using sugarcane (contribute 60%), maize grain, sorghum grain, wheat grain and sugar beet (contribute 40%) as feedstocks [14]. However, the production levels could be increased to approximately eightfold with the use of lignocellulosic materials as a source of carbon for biofuel production [16]. With the use of sugarcane [15], and other crops such as corn, sweet sorghum and so on as substrate material for biofuel production [17], it is possible to produce the ethanol sufficient to blend gasoline to 10% as practiced in the USA.

According to International Energy Agency overviews of biofuels targets all over the world, this agency noticed that during 2005–2010, 27 million ha increase in harvested areas for 13 major crops, namely wheat (*Triticum aestivum*), rice (*Oriza sativa*), maize (*Zea mays*), soybeans (*Glycine max*), pulses (legumes), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), cotton (*Gossypium hirsutum*), rape seed (*Brassica napus*), groundnut (*Arachis hypogaea*), sunflower (*Helianthus annuus*) and sugarcane (*Saccharum officinarum*). This rapid expansion of biofuel crops indicates that there is a scope to expand their area globally to meet rising energy demand.

### Sorghum as biofuel feedstock

Sorghum (*Sorghum bicolor* L. Moench) is an important drought-tolerant cereal crop and ranks fifth among cereals in the world after wheat, rice, barley and maize. Sorghum is morphologically diverse, with grain sorghum being relatively short, for mechanical harvesting, and grown primarily for food and animal feed. Dual purpose sorghum can be grown for both grain and forage production, while forage sorghum varieties are grown exclusively for animal feed. Sweet sorghums have traditionally been grown for the production of sorghum syrup or molasses. Recently, a new class of sorghum called high biomass sorghum has been developed for feedstock production because of its high tonnage. It can be grown in tropical, subtropical and temperate zones, and uses relatively lower agronomic inputs such as irrigation and fertilizers compared with other crops. In addition, sorghum is more tolerant to drought than other crops and has been reported to possess high Water Use Efficiency (WUE) and Radiation Use Efficiency (RUE) [2]. Studies on sorghum, especially sweet sorghum, as a biofuel feedstock have increased and it is becoming a promising alternative 2G biofuel crop. Sweet sorghum is a high biomass and sugar yielding crop that has the potential to provide feedstock for renewable industries. Calculated ethanol yields from sweet sorghum stem juice are approximately 10,000 L of ethanol per ha [18], which may exceed that of sugarcane and sugar beet, depending on the genotype and growing environment (Table 1) [19–35]. Sweet sorghum generates high sugar yields over a wide range of geographical locations (from four t ha<sup>-1</sup> in cooler areas, up to 12 t ha<sup>-1</sup> of sugar in warmer climates) [36]. Ethanol yields from sugarcane and sweet sorghum (~8,000 L ha<sup>-1</sup>) exceed the ethanol yields from starch from grain crops (Table 1). Ethanol yields from corn starch are in the order of 1,140 [19] to 4 180 L ha<sup>-1</sup> [14,15]. However, food grain must be prioritized for human consumption rather than fuel production. In case of sorghum, sweet sorghum has become the preferred crop for biofuel production in China and the target is 10 billion liters in 2020. Realizing that using food crops for biofuel can contribute to increases in food prices, from 2007 onwards the Chinese government stopped new plans for grain based ethanol, and looked at cassava and sweet sorghum. In Latin America, in the wake of the price hikes in 2008–2009 and the food–fuel debates, the Inter-American Development Bank elaborated its scorecard for sustainable biofuels and turned its orientation towards crops such as *Jatropha* sp. and sorghum as 2G options. In African countries such as Mozambique and Angola, a number of foreign and few domestic investments on land deals for production of sweet sorghum as biofuel feedstock is underway [37]. Hence, sorghum is gaining as a promising crop with high biomass and sugars, and offers an option to produce biofuel without impacting food prices as juice is used for

**Table 1. Comparison of bioenergy sugar crops, and their sugar content and yields.**

Crop (species)	Genome size (Mb)	Growth period (weeks)	Propagation	Stover yield (t ha <sup>-1</sup> )	Brix (%)	Sugar yield (t ha <sup>-1</sup> )	Ethanol yield (sugar; L ha <sup>-1</sup> )	Ref.
Sugarcane ( <i>Saccharum</i> spp.)	~10,000	32–96	Vegetative, outcrossing	19 25–19 21	13.7	10.4–17.4 15 7–8	9950 7000 6280 5000 6000	[19–22]
Sorghum ( <i>Sorghum bicolor</i> L.)	740	12–16	Inbreeding, outcrossing	32.5 24.7 24–25	16–23 16–18 11–23	5.4–10.4 10.5 9 4–12	13,032 5414 2129–6388 10,000	[23–25]
Maize (corn) ( <i>Zea mays</i> L.)	2500	12–16	Outcrossing, inbreeding	20 16	ND	ND	3800 1500–2518	[16,21,26]
Switchgrass ( <i>Panicum virgatum</i> L.)	1372–1666	26	Outcrossing	6–7	ND	ND	1288–2851 555–3871	[14,18,27]
Miscanthus ( <i>Miscanthus</i> spp.)	4300–6800	52	Vegetative, outcrossing	27–44	ND	ND	4600–12,400	[21,28]
Cassava ( <i>Manihot esculenta</i> )	ND	36–96	Vegetative	20	ND	4–6 6–8	4500	[29]
Poplar ( <i>Populus trichocarpa</i> )	ND	ND	ND	5–11 6–17 5–11	ND	ND	1500–3400	[21,30]
Pearl millet ( <i>Pennisetum glaucum</i> L.)	2352	12–16	Outcrossing	19–26	ND	10–12	ND	[31]
Willow ( <i>Salix alba</i> )	ND	ND	ND	10 17–18	ND	ND	ND	[30]
Bamboo ( <i>Bambusea</i> spp.)	ND	ND	Vegetative	ND	ND	ND	2927	[32]
Giant reed ( <i>Arundodonax</i> L.)	ND	8–10	Vegetative	ND	ND	3–6	1790	[33]
Barley ( <i>Hordeum vulgare</i> L.)	5300	14–16	Outcrossing, inbreeding	ND	ND	4–6	402	[34]
Wheat ( <i>Triticum</i> spp.)	2500	12–14	Outcrossing	ND	ND	5.5	ND	[35]

ND: No data.

ethanol production. Sorghum also makes the economy of the industries viable due to its wider adaptability and productivity. In the USA, 30% of sorghum grain goes to ethanol production [2]. However, this approach is not suited to many developing nations as sorghum grain is a staple food for millions of people, particularly in Africa and South Asia.

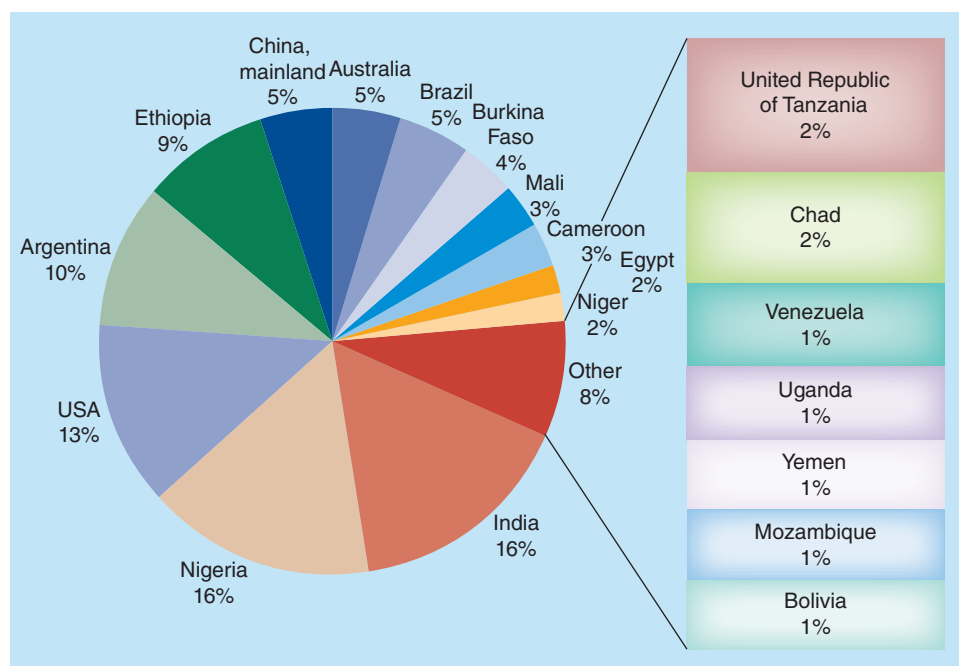
Sorghum genome is approximately 740 Mb in size, indicating an appreciably smaller and less complex genome than the maize, and the sequence of the grain sorghum BT × 623 has been released [38]. Sorghum as a member of the Saccharinae sub-tribe is an ideal model for sugarcane and *Miscanthus* sp., both of which are polyploids that do not succumb easily to genetic studies due to sterility issues.

#### ■ Geographical distribution, agronomy & production

Sorghum is a graminaceous, C<sub>4</sub> monocot originating from Africa, native to tropical and subtropical regions of all continents. Sorghum is grown under hot and dry

conditions. It is an important crop in Africa, parts of Asia and the USA. In addition to being a major source of staple food for humans, it also serves as an important source of cattle feed, fodder and is also used as raw material in various industries. It has potential to compete effectively with crops such as maize under good environmental and management conditions. Leading sorghum-producing countries include India, Nigeria, Ethiopia, the USA, Argentina and China (Figure 1) [39]. In India, it is one of the most widely grown crops in dry lands as a food crop. Sorghum is a dietary staple food of more than 500 million people in more than 30 countries. The annual cultivation of sorghum reported in 2011 was approximately 40.93 m ha in 107 countries.

A challenging task faced by the biofuel industry is the development of economically viable and sustainable biofuel refinery. Sorghum is becoming an efficient and sustainable alternative biofuel feedstock considering its tolerance to drought [41], increased WUE and other economically viable agronomic **sorghum traits** [2]. Yield



**Figure 1. Sorghum distribution.** Total world production of Sorghum biomass by 20 top countries is 42,765,025 metric tonnes. Data taken from [40].

is the basic and important parameter to be considered for development of energy crops. Sorghum is known for high yield potential within 90 to 140 days. It has an extensive and comparable fibrous root system such as that of rice, wheat and maize, and is able to penetrate a greater volume of soil to obtain moisture and other nutrients. However, the highest yields are obtained from varieties maturing in 100 to 120 days. For bioenergy crop, identification and development of high biomass, high yielding lines is very crucial and traits such as plant height, biomass, brix% (soluble sugars), flowering and early maturity, as well as grain yield, are important characteristics for potential industrial feedstocks.

Another aspect of biomass feedstock production for biofuel is a function of RUE multiplied by light interception. This is defined on a land area basis by the architecture of the canopy and the planting density. In most highly bred crop species such as sorghum, maize, wheat and rice, leaf area distribution and angle have undergone extensive genotypic selection compared with biofuel feedstock crops, such as *Miscanthus* and switchgrass. These plants have an attractive advantage in terms of RUE [17]. Even the length of the growing season has a considerable advantage over dry harvestable biomass under their optimal growing conditions as in case of sweet sorghum. Within C4 species there is considerable variation in biomass production. Standing dry weight at harvest was 42 t ha<sup>-1</sup> for sorghum, 35 t ha<sup>-1</sup> for maize and

20 t ha<sup>-1</sup> for Napier grass between 100–200 days [17]. Peak dry matter yield of sorghum was 50 t ha<sup>-1</sup> compared with switchgrass that was less than 19 t ha<sup>-1</sup>.

Sorghum plants have higher WUE partly because of the ability to fix CO<sub>2</sub> with less open stomata, which enables the plant to maintain a lower intercellular CO<sub>2</sub> concentration and a greater diffusion gradient [42,43]. These grasses have the capacity to generate more biomass in conditions where the maximum daily temperature is higher leading to an increase in the incidence of drought [44]. Conditions of elevated temperature and CO<sub>2</sub> are reported to improve yields of sugar and biomass in sorghum [45,46]. The WUE of sweet sorghum increases, exceeding that of maize and grain sorghum, under water stress conditions [47,48]. Furthermore, the normalized leaf transpiration efficiency of sorghum was 10.5 μmol/mmol kPa, compared

with annual ryegrass or bamboo, which was approximately 4.8 μmol/mmol kPa [47]. Sweet sorghum plants grown in soil salinity of 3.2 dS m<sup>-1</sup> and limited (210 mm) irrigation water results in production of 27.1–33.5 t ha<sup>-1</sup> biomass, 2.6–3.86 t ha<sup>-1</sup> total sugar and 4926–7620 L ha<sup>-1</sup> theoretical ethanol yields [49]. Thus, this crop may yield sufficient sugar for ethanol production in areas that are not ideal for food production, such as arid environments where soil and water may be moderately saline. Sweet sorghum fresh biomass yields are quite high (up to 100 t ha<sup>-1</sup>), thanks to the efforts of research communities who are developing high yielding varieties and hybrids with improved biomass. This consequently increased the economic viability of these lines. Another important trait of biofuel crop is presence of soluble sugars (brix%). Brix% of sweet sorghum lines ranges from 6 to 30, which is comparable to sugarcane and is more than maize (sweet corn). Further efforts are underway for the development of sorghum lines with increased percentages of soluble sugars.

#### ■ Sweet sorghum

Sweet sorghum is a special type of sorghum that accumulates sugars in stalks to approximately 70–80% of total biomass similar to that of sugarcane, apart from yielding considerable amount of grain. In addition, juice of sugarcane contains 9.8% fermentable sugars while sweet sorghum possesses 11.8%. Sorghum has the



soluble sugars (brix%) up to 30, which is comparable to sugarcane and sweet sorghum lines with a high brix value (20–25) and are better suited for bioethanol production [50]. High biomass lines with more soluble sugars are highly suitable as multipurpose industrial feedstock. The sweet sorghum variety, keller, gained attention as a potential alternative feedstock for the renewable energy due to its high sugar content and biomass yields [50]. The production of biofuel from sugarcane bagasse, coupled with that from other sugar containing feedstocks such as sorghum may provide an opportunity to expand the operational season of sugar mills as well as to generate ethanol. Sweet sorghum with its high content of soluble stalk sugars and structural sugars  $C_5$  and  $C_6$  sugars (obtained from cellulose and hemicellulose components of the bagasse) could be used both for first- and second-generation biofuels. Ethanol is produced from stalk juice as similar to the molasses-based ethanol production process. In fact, sweet sorghum is an important crop grown around the world for syrup, ethanol, power, food and forage [51–53]. Agronomic-wise, sweet sorghum is also suitable for sugarcane-growing regions, whereby the sorghum can be planted prior to replanting sugarcane [54], or as an additional crop to be harvested in between sugarcane harvests, thus benefiting from an existing infrastructure (harvesting equipment and transportation), while increasing the output of sugarcane mills. Approximately 4000 sweet sorghum cultivars are distributed throughout the world [55], indicating a diverse genetic background to develop regionally specific, highly productive cultivars [42]. Furthermore, elucidating the genetic basis of stem sugar and stem juice accumulation, sorghum biomass can be processed more efficiently, maximizing biomass yield for a given geographic area and production system.

#### ▪ High biomass sorghum

Energy sorghum is a forage sorghum bred for high biomass production. Several biomass sorghum hybrids have been developed and improved for the production of lignocellulosic sugar and as starch feedstocks [56]. All types of sorghums produce lignocellulose that serves as feedstock for 2G biofuels.

The availability of whole-genome sequences, technology to perform high-throughput expression profiling and mapping populations that represent genetic diversity within a species, have enabled rapid advances towards the genetic improvement of bioenergy crops. Many different grass species such as corn (*Zea mays* L.), sorghum (*Sorghum bicolor* [L.] Moench) and sugarcane (*Saccharum* spp.) can be used for bioenergy production. The primary product of corn is grain, 47% of which is fed to animals, 40% is used for fuel ethanol and 12% is used for human consumption [56]. Moreover, maize

has a rich history as a genetic model organism, due to the release of the genome sequence of inbred line B73 [57], as such it deserves a major role in the bioenergy research portfolio. But the production of ethanol from maize grain is controversial in part because of the perceived competition between food and fuels [58], and in part because of high input requirements for water and fertilizer, leading to relatively small environmental benefits relative to the use of fossil fuels [59,60]. Hence, grain will remain as the major product of maize, genetic improvement of maize for bioenergy uses will need to focus on enhancing biomass quantity and quality without impacting grain yield and quality. Sugarcane has been deliberated as the classical feedstock material for biofuels, due to the success of sugar-based fuel ethanol in Brazil [61]. Sugarcane also has an impressive biomass yield potential, with average yields of 39 t ha<sup>-1</sup> per year and maximum commercial yields of 69 t ha<sup>-1</sup> per year [62]. It is, therefore, also an attractive lignocellulosic feedstock, either in the form of bagasse or as a dedicated biomass crop [63], but has a long harvest time of 8–24 months after planting, depending on the location and though ratooning is observed, yielding progressively less sugar. Moreover, the large genome size (~10 Gb) represents a major challenge in further improvement.

Sorghum is a versatile species that is produced as a source of grain, forage, sugars and biomass. Grain sorghums tend to be short whereas forage sorghums tend to be tall and slender, which facilitate combined harvesting. Sweet sorghums are very tall (4–6 m) and accumulate sugars in their stems just like sugarcane. Biomass sorghums have the potential to produce high tonnages of  $C_5$ ,  $C_6$  sugars and lignin. Genetics, coupled with photoperiod sensitivity, can make sorghum produce high biomass yields. These can be grown outside of traditional sugarcane production regions because of their adaptability [64]. Sorghum biomass yields vary between 15 and 25 t ha<sup>-1</sup>, but have been reported to be as high as 40 t ha<sup>-1</sup> [64,65]. Moreover, sorghum is a highly promising bioenergy crop because of its low input requirements (water, radiation, nitrogen), wide adaptability and abundant genetic diversity [66], as well as no trade-off between grain and sugar production [67].

Understanding the different mechanisms underlying drought tolerance enables the sorghum researchers to target and enhance bioenergy-related traits [2]. Natural variation within species for biomass production, cell wall composition, sugar yield, nitrogen and WUE may reveal species with useful traits. Moreover related sorghums, Sudan grass and sorghum × Sudan grass may also have a role as short growing season crops or crops allowing two cuttings in one growing season. Generation of feedstock crops that are optimized for

## Key terms

**Pretreatment:** Technology regularly employed to destruct the polymeric lignocellulosic composite structure to dislocate the lignin barrier from the polymeric carbohydrates, cellulose and hemicelluloses, and thereby enhancing the accessibility of holocellulolytic enzymes that depolymerize the polymeric sugars to soluble fermentable sugars.

**Brown midrib:** Hybrid trait of sorghum produced by using *brown midrib-6*, *-12* and *-18* genes.

biofuel production may be achieved by selective breeding for natural differences, or by genetic modification. Developing sorghum as an alternative biofuel feedstock with all the desired sorghum traits is one of the ideal ways to meet the requirements of energy.

#### ■ Biomass composition

Sorghum crop is known for its efficient production of dry matter among cereals. Being a  $C_4$  plant,

sorghum has a high photosynthetic potential producing fresh biomass up to  $100 \text{ t ha}^{-1}$ , and 30–55 tons of fresh stalk yield  $\text{ha}^{-1}$ . High tonnage biomass sorghum can play a significant role in cellulosic ethanol production compared with other grasses, wood and nonedible parts of plants. Cellulosic ethanol may be produced from lignocellulose, a structural chemical compound that makes up most of the mass of the plants. Cellulose has been exposed to chemical/biological saccharification and subsequent biological conversion of the monomeric sugars to ethanol [68]. Sweet and forage sorghums have high yield potential of up to 20–40 t/ha dry biomass and above 100 t/ha fresh biomass, and they are good sources of cellulose and hemicelluloses with important features required to be a potential industrial feedstock. Stems of sorghum contain large quantities of soluble and insoluble carbohydrates that include sucrose (soluble), cellulose and hemicelluloses (insoluble), which make it a potential industrial crop. Developing cultivars for high stalk yield, biomass, sugar content and bioethanol yields combining tolerance to shoot pests (shoot fly, stem borer, shoot bug and so on) and improved crop production practices contribute to economically viable sorghum feedstock. The various sorghum biomass conversion routes employed for the generation of sugars, subsequent fermentation into ethanol and their yields were shown in Table 2 [69–73].

The second factor that influences biofuel production is the ratio of biomass composition for structural carbohydrates. In general, any lignocellulosic biomass consists mainly of cellulose, hemicellulose and lignin, and these components of the cell wall differ depending on crop (Table 3) [27,74–86]. Chemical composition of sugarcane bagasse was determined to be 42% cellulose, 25% hemicellulose and 20% lignin, and that of energy cane was 43% cellulose, 24% hemicellulose and 22% lignin [19–22]. Sweet sorghum (normal) has 45% cellulose, 27% hemicellulose and 21% lignin [27]. The higher percentage of cellulose and hemicellulose contents of sorghum makes it as a more suitable 2G biofuel crop [23–25]. Cellulose content in the high biomass sorghum

lines can range from 27–52%, while the hemicellulose (17–23%) and lignin content ranges from 6.2–8.1% [13]. Thus, high biomass sorghum lines are promising as potential industrial feedstocks.

Lignin is a noncarbohydrate polymer that encompasses the cellulose through the covalent connections with hemicellulose and, thereby, hinders the release of glucose from cellulose during the hydrolysis reaction. Lignin content and composition may vary due to natural mutation in the genes. Sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose and arabinose. With the suitable technological developments, some of above sugars can be used for the production of bioethanol. Theoretical ethanol yields would be 3609 kg per ha from sugarcane, 12938 kg per ha from energy cane and 5804 kg per ha from sweet sorghum [16].

#### ■ Brown midrib sorghum

The major impediment of converting biomass to biofuels is high pretreatment costs for removal of lignin, besides high cost of enzymes used for saccharification. Part of the problem could be solved by developing low lignin biomass. **Brown midrib** (*bmr*) mutations are novel mutants in phenylpropanoid pathway [66]. *bmr* forages usually contain less lignin (as low as below 6% in some lines) because of modifications in their lignin biosynthesis pathway. In sorghum, *bmr* mutants were first developed at Purdue University via chemical mutagenesis and these *bmr* mutants with altered lignin content are characterized by brown vascular tissue [87]. Introgression of several *bmr* alleles into high biomass and stay green lines was done and characterized [88], and most of the *bmr* mutants resulted in increased yields of fermentable sugars followed by enzymatic saccharification, albeit with varied background effects [89]. Alleles of *bmr12*, *bmr18* and *bmr26* [90] and *bmr6* [91,92] have been characterized at molecular level. Allelic genes *bmr12* and *bmr18* decrease caffeic acid *O*-methyl transferase activity and *bmr6* has been linked to a decrease in cinnamyl alcohol dehydrogenase activity [93].

A higher incidence of stalk breakage at maturity in *bmr* sorghum compared with normal lines was reported [70]. However, an increase in lodging attributable to *bmr* was not detected in several other studies, possibly due to overriding effects of genetic backgrounds. The genetic background in which *bmr* genes are deployed is critically important regarding lodging [26,94–96]. Results of replicated field studies with *bmr* genes deployed in isogenic sorghum lines showed obvious line effects, but no significant differences attributable to *bmr* genes [21]. A dual-purpose bioenergy crop that supplies fodder and fermentable

sugars from the lignocellulosic biomass can be developed by introducing the *bmr* trait in high biomass sorghum, which may contribute to the development of industrially suitable and economically viable biomass production.

#### ■ Pretreatment & enzymatic conversion of sorghum biomass

The efficiency of lignocellulosic biomass pretreatment and enzymatic conversion has long been deliberated as a crucial factor in determining the economic practicability of industrial scale biofuels production [97,98]. Effective lignocellulosic bioenergy conversion requires appropriate pretreatment to liberate the plant cell wall polysaccharides (cellulose + hemicellulose) from its lignin seal (Figure 2) [99] and to disturb its recalcitrant crystalline structure prior to subjecting it to enzymatic hydrolysis [100]. The overall economics of fermentation of sugars to ethanol is mainly dictated by the cost of the pretreatment and the enzymes used for saccharification. The pretreatment process employed determines the structural disturbances to the material and improvement of the accessibility of enzymes to biomass. The degree of crystallinity of cellulose has a significant effect on the ratios of enzymes required for the effective release of fermentable sugars [101,102]. The type of pretreatment employed should be selected based on the chemical linkages and lignin content of the material. The regular softwood biomass including sugarcane bagasse, rice straw and so on include Guaiacyl-lignins (Figure 3) [103], while the hardwood biomass contains Guaiacyl-Syringyl-lignins (Figure 4) [104]. The Guaiacyl-lignin units contain a free C-5 position for C–C inter unit bonding, which makes them equitably resistant to lignin depolymerization, whereas S-lignin is relatively unbranched and has a lower degree of condensation, and hence is easy to delignify [105]. Sorghum belongs to the family *Gramineae* and its lignin comprises high contents of *p*-Hydroxybenzoic acids and guaiacyl units, and to a little extent of *p*-coumaric acids which are predominantly connected through  $\beta$ -O-4' aryl ether linkages together with slight amounts of  $\beta$ - $\beta'$ ,  $\beta$ -5',  $\beta$ -1' and  $\alpha$ ,  $\beta$ -diaryl ether linkages [106]. Therefore, the type of pretreatment and the process conditions should be selected based

Table 2. Bioethanol yields from different routes of processing sorghum (*Sorghum bicolor* L. Moench) biomass.

Biomass type	Pretreatment and conditions	Sugar substrate	Conditions	Sugar yield	Organism for ethanol production	Ethanol (product) on sugar utilization (g/g)	Yield of biomass on sugar utilization (g/g)	Ethanol efficiency (%)	Ref.
Sorghum (brown midrib) stalks	Alkaline treatment (15% w/w solids, 4.0% ammonium hydroxide, 170°C and 20 min)	Solid	Enzyme saccharification	88%	<i>Saccharomyces cerevisiae</i>	ND	ND	ND	[69]
Sorghum (brown midrib) stalks	Dilute-acid pretreatment (1.75% w/v, 15% w/v solid loading, 121°C, 1 h)	Solid	ND	77.4%	<i>S. cerevisiae</i>	ND	ND	ND	[70]
Sorghum stalks	Acid hydrolysis (93% acid solution, 121°C for 30 min)	Hydrolysate	ND	18.8%	ND	23.93 g/l	0.14	94.45	[71]
Sorghum bagasse	Dilute-acid pretreatment (210°C, 4 g acid/100 g sugarcane bagasse)	Hydrolysate	ND	20 g/l	<i>Neurospora crassa</i>	27.6 g/l	ND	84.7	[72]
Sorghum juice	ND	ND	ND	35.09%	<i>S. cerevisiae</i>	10.7 g/l	0.11	38.81	[71]
Sorghum grains	Acid hydrolysis	Hydrolysate	ND	29.32%	ND	12.4 g/l	0.21	97.39	[71]
Sorghum grains	ND	ND	Enzyme saccharification	50 g/l	<i>S. cerevisiae</i>	40.11%	0.0657	163.50	[71]
Miscanthus	Alkali treatment (145.29°C, 28.97 min and 1.49 M)	Solid	Enzyme saccharification	83.92%	<i>S. cerevisiae</i>	59.20 g/l	ND	ND	[73]

ND: No data.



Table 3. Composition of lignocellulose biomass crops.

Crop	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ref.
Sweet sorghum	26.30	20.00	07.10	[27]
Corn stover	45.00	35.00	15.00	[74]
Switchgrass	33.75	27.04	05.80	[75]
Miscanthus	50.54	24.83	12.02	[76]
Willow	42.80	26.20	27.50	[77]
Poplar	42.20	16.60	08.20	[78]
Cassava	15.60	04.60	02.80	[79]
Napier grass	42.00	19.50	11.10	[80]
Pearl millet	24.70	29.60	17.80	[81]
Purple guinea	33.40	31.20	03.55	[82]
Giant reed	32.93	28.48	21.31	[83]
Barley	42.00	28.00	10.20	[84]
Wheat	38.00	29.00	15.00	[85]
Rice	39.00	35.00	19.00	[86]

on the composition and lignin content of the biomass. Apart from this, enzyme formulations should contain enough holocellulolytic activities with low protein content to release the sugars. There are reports on assorted pretreatments including physical (ball milling), chemical (acid, alkali, cryo and inorganic salts) and biological processes, which have been developed to release the polymeric sugars [107,108].

For *bmr* sorghum material (Sorghum Project Variety-2017) optimized dilute acid pretreatment with 0.45%  $\text{H}_2\text{SO}_4$  load at 121°C for 20 min resulted in 77% of cellulose yield [RADHIKA K, UMA A, UMAKANTH AV *ET AL.* CHARACTERIZATION, OPTIMIZATION OF DILUTE ACID PRE-TREATMENT AND ENZYMATIC SACCHARIFICATION OF LOW LIGNIN BMR MUTANTS OF SORGHUM (CSV 15 × IS 21891) FOR SECOND GENERATION BIOFUELS (2013), SUBMITTED], better than the yields reported for switch grass (52.3%) at 4%  $\text{H}_2\text{SO}_4$  load [109]. Lignin negatively impacts the enzymatic degradation of lignocellulosic biomass by hindering the holocellulolytic enzymes activity through nonspecific adsorptions. Apart from this, during the pretreatment process it could lead to the generation of aromatic compounds and their derivatives including monomeric phenols, syringones, syringaldehydes and so on, which can inhibit the efficiency of fermenting microbes through

specific mechanisms [110]. Hence *bmr* feedstocks having high biomass with low lignin content would significantly improve the biomass conversion efficiency over the wild type counterparts. However, very limited literature is available on the usage of *bmr* material for biofuels production [111]. Some of the recent reports by Saballos *et al.* observed 17, 20, and 21% improvement in the enzymatic saccharification of sorghum *bmr2*, *bmr6* and *bmr12* stover, respectively, over the wild type [89]. Similarly, Corredor *et al.* achieved 79% of hexose yield by enzymatic hydrolysis of a *bmr* forage sorghum stover and, in this case, the hexose sugars from wild type was 48% of yield only [112]. Two quantitative trait loci (QTLs) (QStCI\_10\_2A and QStCI\_10\_3A) were found on chromosomes 2 and 3, accounting for 22.35% of the phenotypic variation in biomass crystallinity index in the recombinant inbred population of *S. bicolor* × *S. propinquum* [113]. In this study, a total of 49 QTLs (20 leaf, 29 stem) were associated with enzymatic conversion efficiency. The identification of these QTLs would aid in identifying specific genes relevant to increasing conversion efficiency of sorghum in particular and other related feedstocks in general.

#### ■ Sorghum-based biochemicals

Biomass represents a renewable, versatile and abundant resource in nature that has multifunctional utilities [114]. There are significant advances in the conversion of cellulose and hemicellulose into chemicals recently and sorghum, as a cheap source of cellulose and hemicellulose, has found its role in the production of various value-added chemicals. For example, sorghum can be converted into sugars, polyols, levulinic acid (LA) and furan derivatives, apart from producing biofuels [115]. The conversion overview is as shown in

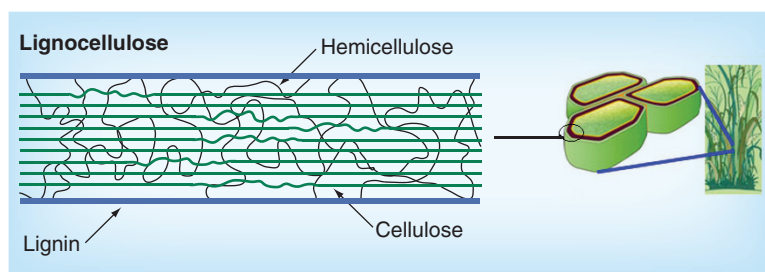
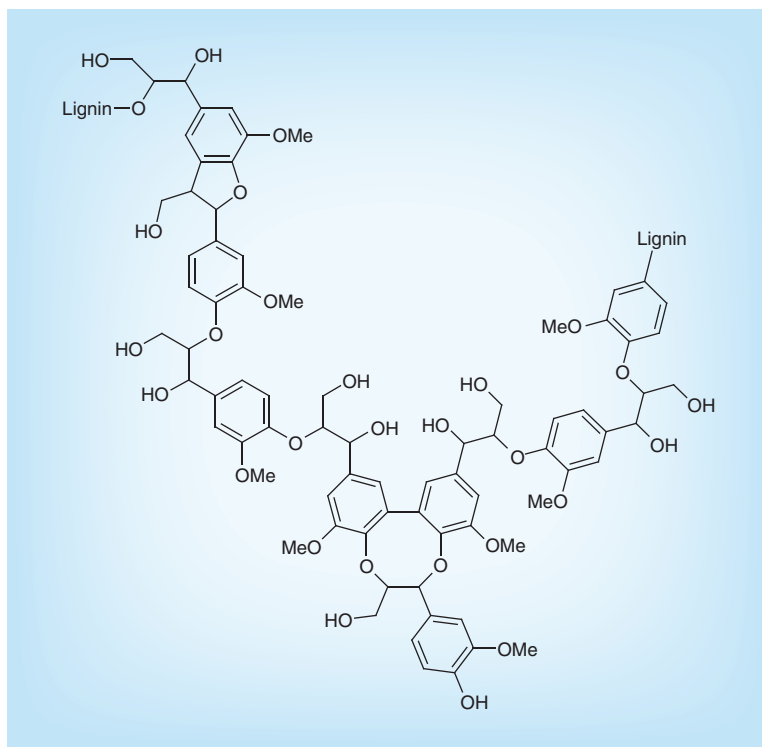


Figure 2. Lignocelluloses in plant cell walls.

**Figure 5.** Hydroxymethyl furfural (5-HMF) is one of the furan derivatives [110]. Sorghum could also be used as a feedstock to provide these platform chemicals such as 5-HMF, which may be important in the future of biorefinery.

The hydrolysis of sorghum has been investigated extensively, resulting in the production of oligomers, monomeric sugars and furfural. The conversion of sorghum straw for xylose production has been investigated by using hydrochloric acid [116,117]. The hydrolysis reaction was conducted at 100 and 122°C with 6% hydrochloric acid at a reaction time of 83 and 70 min, respectively. Xylose, glucose, acetic acid and furfural were found in the hydrolytes with xylose being the major product. In a later work, the same group investigated the hydrolysis of sorghum straw using phosphoric acid to produce furfural [118]. Optimal conditions for furfural production were 6%  $\text{H}_3\text{PO}_4$  at 134°C for 300 min, which yielded a solution with 13.7 g furfural/L. Sulfuric acid has also been used for the hydrolysis of sorghum straw with the maximum glucose and xylose yields of 0.234 and 0.208 g/g dry substrate at 120°C with 3%  $\text{H}_2\text{SO}_4$  for 10 min [119]. The kinetic model analysis of using sulfuric acid for the hydrolysis has been reported by Liu *et al.* [120]. The results indicated that elevated reaction temperatures promote the hydrolysis of hemicellulose and the degradation of xylose. Besides the use of acids, a more environmentally friendly method for the hydrolysis of sweet sorghum bagasse to hemicellulose is by using hot water [121]. The primary products are xylose, arabinose, glucose and their oligomers. Degradation products such as furfural, 5-HMF and acetic acid have also been found.

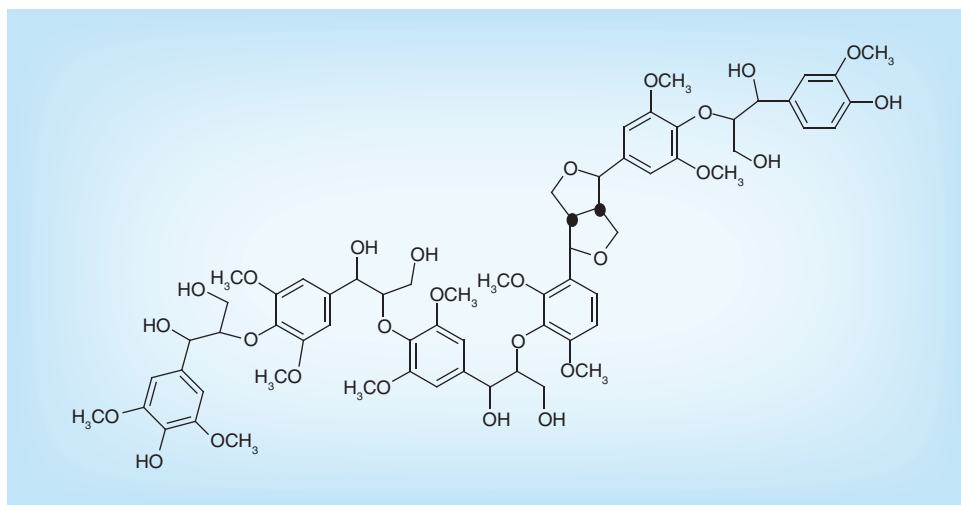
Ethylene glycol is an important chemical in the petrochemical industry. After the first conversion of cellulose into hexiols [122,123], ethylene glycol was produced from cellulose with a high selectivity over nickel-promoted tungsten carbide catalysts [124]. The conversion routes could be as shown in Figure 6 [124]. Carbon-supported tungsten carbide ( $\text{W}_2\text{C}$ /activated carbon) can effectively catalyze cellulose conversion into polyols. However, by adding a small amount of nickel, the yield and the selectivity of ethylene glycol and sorbitol can be significantly increased. A synergistic effect between nickel and  $\text{W}_2\text{C}$  has been proposed and assumed to be responsible for the high selectivity. After demonstrating the reaction with cellulose, an attempt to obtain ethylene glycol from sorghum straw has been tested [125]. The sorghum straw was pretreated with steam explosion, alkali and hydrogen peroxide, and then catalysts such as Co, Ni, Ru, Rh, Pd, Pt and  $\text{W}/\text{WO}_3$  have been used for the hydrogenation and hydrogenolysis of cellulose materials in the sorghum straw.



**Figure 3.** The model of softwood lignin and types of linkages involved.

The possible dimer structures formed during the breakdown of soft wood lignin are: arylglycerol-β-aryl ether (β-O-4'); noncyclic benzyl aryl ether (α-O-4'); phenylcoumaran (β-5'); biphenyl (5-5'); diaryl ether (4-O-5'); 1,2-diaryl propane (β-1'); and pinoresinol/lignin type (β-β').

The conversion of cellulose into HMF has become a research focus since 2009. Lewis acids such as  $\text{CrCl}_3$ ,  $\text{CrCl}_2$ ,  $\text{CuCl}_2$  and  $\text{SnCl}_4$  have been proven effective for this reaction [126–128]. Metal-free catalysts such as boric acid have also been reported for the conversion of cellulose into 5-HMF [110]. The catalysts are proposed to have interaction with the hydroxyl groups along the cellulose chain. Apart from the use of pure cellulose, some researchers have used sorghum biomass as their starting material. Corn straw and sorghum straw have been pulverized and converted under the catalysis of  $\text{Cr}(\text{NO}_3)_3$  with the additive LiCl in 1,3-Dimethyl-2-imidazolidinone solvent [129]. The reaction is conducted at 140°C for 4 h. After the reaction, 5-HMF was obtained by solvent extraction. In this report, the conversion could reach 60% with a 30% yield of 5-HMF. Another example of sorghum straw conversion into 5-HMF utilizes Bronsted acids,  $\text{CrCl}_2$ ,  $\text{CrCl}_3$  or a combination of them as catalysts in  $\text{ZnCl}_2$  aqueous solution [121]. The reaction condition was 140°C and 20 min for sorghum with a 5-HMF yield of 20%. Supercritical carbon dioxide has been employed for the conversion of sorghum straw into 5-HMF at 120°C [130]. The raw material is treated with acid/alkali and



**Figure 4. Hard wood lignin.** The possible dimer structures formed during the breakdown of hard wood lignin are: phenyl coumaran ( $\beta$ -O-4/ $\alpha$ -OH); syringresinol ( $\beta$ -O-4/ $\alpha$ -CO); spirodienoneguaicyl/syringyl ( $\beta$ -1/ $\alpha$ -OH); O-methyl (O-CH<sub>3</sub>); and biphenyl (5-5').

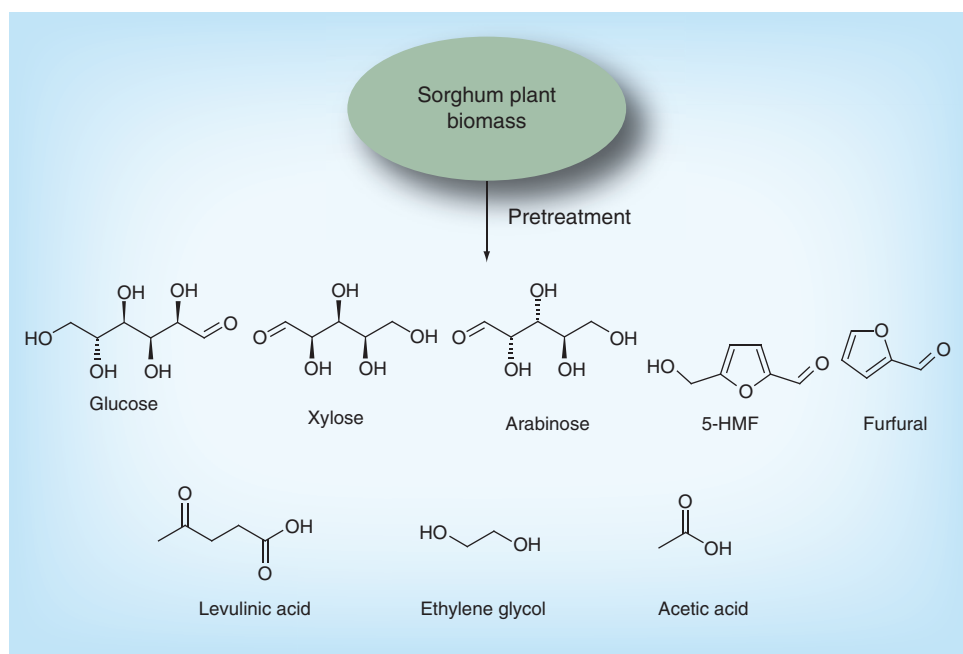
#### Key term

**Lignin-based co-products:** Including renewable biopolymers, adhesives, resins, phenolic-based chemicals, vanillin, bio-oils, bio-char and so on.

filtrated, and then liquid carbon dioxide and water were added into the autoclave. LA is usually observed in biomass conversion, forming via the rehydration of 5-HMF. LA can be produced in a biorefinery process at 50–70% yields from cellulosic feedstocks using dilute acid hydrolysis. Using sorghum straw as the starting material, LA with a yield of 55% was obtained by using 4% sulfuric acid as catalyst at 230°C for 10 min [131]. Another work

describes the employment of solid superacid catalyst with a formula of  $S_2O_4^{2-}/M_XO_Y$  or  $S_2O_8^{2-}/M_XO_Y$  [132,133]. The reaction was conducted at about 200°C for 10 min, reaching a yield of about 30%. The production from whole kernel grain sorghum has been investigated by using sulfuric acid [132]. A stepwise heating step scheme improved the yield of LA to a maximum of 33% at 200 °C with 8% acid concentration [134].

As sorghum is a low-cost, abundant and sustainable resource, it provides a promising way to produce a series of value added biochemicals, potentially at an affordable price. The direct production of monosaccharides, 5-HMF, furfural and LA from sorghum have been demonstrated, which is highly encouraging, although extensive further research to optimize the process is still required [134]. In addition, it appears that only the cellulosic component has been utilized for chemical production whereas lignin is largely ignored. Considering that lignin is an aromatic polymer with high energy content, presently it could be used for the production of a variety of **lignin-based co-products**, future research efforts are required to be directed to the conversion of lignin component in the sorghum straw.



**Figure 5. Sorghum conversion into various chemicals.**

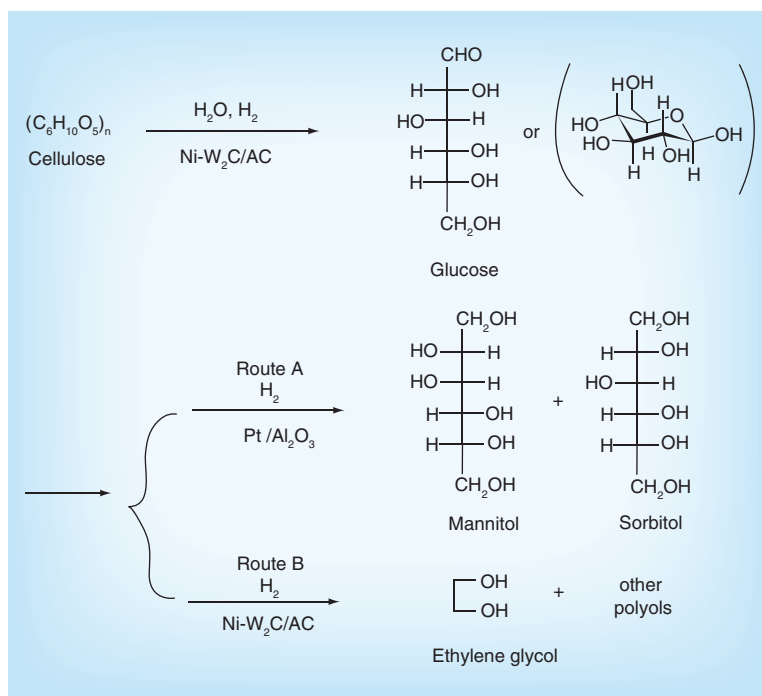
#### Future perspective

There is a rise in demand for renewable energy sources for biofuels. In order to meet the demand, there is a need to develop sorghum cultivars that produce high stalk yield per unit time, input energy and land areas in different agro-climatic areas. These cultivars should also be photo- and thermo-insensitive with desired levels of resistance/tolerance to various stresses and should be of different maturities to widen the harvest window, which thereby ensures a continuous supply of feedstock to the industry.

Future research should also address the optimization of sorghum as an energy crop through exploration of the available genetic resources through plant breeding with the aid of molecular tools. This

could dramatically increase biomass yields of sorghum under marginal and less favorable crop ecologies, and adaptation to drought, colder, arid, saline and alkaline conditions, thus may be suited to meet the demand of feedstocks for biofuel production without a significant impact on our food supply and natural environment. Focus on development of sorghum cultivars of high biomass with desired levels of fermentable sugars either or in combination of introgression of *bmr* genes, identifying candidate traits important for bioenergy and characterizing sorghum collections for these traits, selecting for major QTL to regulate the total nonstructural carbohydrate yield, development of appropriate harvest, storage and fermentation technologies.

Despite extensive development in biofuel/ethanol production from lignocellulosic biomass materials from the past few decades, the cost of the 2G ethanol is still at its apices and this high cost is because of some technological obstructions encountered during the extrusion of lignin barrier from lignocellulosic composite, which need to be addressed with economically and ecologically sustainable strategies. Pretreatment accounts for approximately 30–35% of the total cost of bioprocess and the current leading pretreatment technologies for lignocellulosic materials are energy intensive, which need to be addressed efficiently with industrial practicability. Sorghum cultivars with reduced lignin can pave a better path to increase cellulosic ethanol production as compared with other crop residues and also improve process economics targeting higher conversion efficiency. Reduced lignin content will be highly beneficial for improving biomass conversion yields through biomass pretreatment with dilute acid and also for the production of 5-HMF, ethylene glycol, LA, beside pentose and hexoses. Further research efforts are required in identifying linked genes in regulation of G and S lignin, which would be beneficial in reduction of pretreatment and saccharification processes – major impediments in lignocellulosic based 2G bioethanol production. Although the utilization of cellulose and hemicellulose in sorghum is relatively well explored for production of high value chemicals, more emphasized research activities should be directed at reducing the cost for the production of these chemicals and biofuels, and the development of new reaction efficient pathways for other value added compounds including effective ways to transform lignin component into high value chemicals. In addition, efforts are also needed on understanding the relationship between the genes associated with the QTLs for crystallinity index in biomass trait and lignocellulosic enzyme associated QTLs in fungi that produce lignocellulosic enzymes. Understanding the relationship of genotype to phenotype especially with reference to cell wall components and its inter- and



**Figure 6. Catalytic conversion of cellulose into polyols.**

intra-structural relationship will be the day of research that could be useful in marker-assisted breeding programs aimed at increasing overall bioenergy yields concomitant with the selection of high total biomass genotypes with selective lignin type, more amorphous cellulose polymer.

Several leading institutions and industries are actively engaged in the development of biorefinery concepts for the efficient utilization of lignocellulosic biomass materials with economical practicalities. Understanding the rich diversity of the lignocellulase gene pool from microbial sources and the development of a suitable enzyme cocktail could be significant breakthrough in the lignocellulose conversion process. However, engineering inputs at reactor development to sustain high solid loadings without disturbing the effectiveness of enzymes on the target material is the need of the hour. Overall, an integrated bioprocess lignocellulosic-based biofuel will gain potential to overcome the regularly employed gasoline in forthcoming future.

#### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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## Executive summary

## Preamble

- Biomass feedstocks have significant role in future biofuel program, energy security, global warming, reduction of green house gas emission and dependence on fossil fuels.
- Sugarcane juice and sugar industry byproduct, molasses were the major sources of first while lignocellulosics feedstocks for second generation biofuels.
- International Energy Agency reported that scope is enormous for rapid expansion of biofuel crops because of renewable nature and ever increasing energy demand.

## Crops for lignocellulosic biofuels

- Traditional crop maize considered as a genetic model organism in the bioenergy research portfolio, however production of ethanol from maize grain is controversial because of perceived competition between food and fuels
- Next alternative crop sugarcane has an impressive biomass yield potential ( $69 \text{ t ha}^{-1} \text{ year}^{-1}$ ) has large genome size ( $\sim 10 \text{ Gb}$ ) represents a major challenge in further improvement.
- Sorghum, alternatively emerging as ideal feedstock for the second generation biofuel because of richness in carbohydrate content, wider adaptation to diverse agro-ecologies including marginal lands, and its noncompetitiveness with food crops for water/fertile land.

## Sorghum as novel feedstock

- Sorghum is a gaminaceous, C4 monocot, grown as an important crop on 51 m ha in 107 countries with higher harvestable biomass of  $42 \text{ t ha}^{-1}$  compared with other bioenergy crops maize, napier grass and switch grass.
- Genotypic selection (high-throughput expression profiling, and mapping populations) resulted in development of sweet-, high biomass- and Brown Midrib (*bmr*)-sorghum, and so on.
- Sweet sorghum possess high content of soluble stalk sugars and structural sugars is considered as an alternative for first and second generation biofuels.
- Genetic manipulation at sugar yield, nitrogen, water and radiation use efficiency traits coupled with photoperiod sensitivity approach resulted in development of high biomass sorghum producing high tonnage of C5, C6 sugars, an ideal bioenergy crop.
- Theoretical ethanol yields would be 3609 kg per ha from sugarcane, 12938 kg per ha from energy cane, and 5804 kg per ha from sweet sorghum.

## Developments in sorghum

- *bmr* sorghum emerged based on introgression of *bmr* alleles and stay green lines resulted in development low lignin containing biomass could be the choice for economize the biofuel process.
- Biomass to biofuel process economics are mainly governed by cost share of pretreatment and enzymatic saccharification which in turn dictated by degree of crystallinity.
- Two quantitative trait loci observed on chromosomes 2 and 3, accounting for 22.35% of the phenotypic variation in biomass crystallinity index in the recombinant inbred population of *S. bicolor* x *S. propinquum*.
- Softwood contains Guaiacyl-lignin (resistant to lignin depolymerization) while hardwood contains Guaiacyl-Syringyl-lignin hence, pretreatment selection should be based on chemical linkages and lignin content.

## Lignocellulosic feedstock based co-products

- Sorghum can be used as a feedstock to provide platform chemicals such as sugars, hexiols, levulinic acid, furan derivatives and so on by selective process parameters.

## Prospective outlook

- Future directive research focused on development novel genetic variants with high S-lignin and high amorphous cellulose along with biomass and high sugar containing juice could be the best feedstock choice for bioenergy sector.

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