Advances and Challenges in Sugarcane Biofuel Development

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Abstract

Biofuel produced from the plant biomass shows greater alternative source of renewable energy and better than the fossil fuels in reducing the greenhouse gas emission from the burning of fossil fuels. Sugarcane is one of the best candidates for biofuel production which has been used successfully to produce bioethanol extensively in Brazil and also in other countries worldwide. Sugarcane is a perennial monocot with C4 photosynthesis, having a fast growth rate without any serious maintenance and can be harvested four to five times by multiplying using the ratoons. Sugarcane is one of the primary crops as a source for both food and bioenergy, with Brazil, India, and China contributing more than 60% of the world's total production. The diminishing resources of fossil fuel coupled with augmented research interest for an environmentally sustainable and renewable source of energy in the form of sugarcane. Industrial levels of biofuel production have been achieved in Brazil and the USA, however more concerted efforts needs

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to be directed towards deployment of second-generation biofuel production by utilizing lignocellulosic biomass.

Keywords

Sugarcane · Biofuel · Lignocellulosic biomass

11.1 Introduction

Exploration of alternative energy sources has shown renewed special research focus that could curtail or replace the usage of fossil fuels (Waclawovsky et al. 2010). The ever increasing energy demands can be supplanted by plant feedstocks, which are excellent sources of renewable energy resources. The availability of renewable bioenergy resources can go a long way in securing the energy needs of a country in sustainable economic manner, thereby diverting the precious resources from oil-based import economy towards a self-sustainable one. The encouraging trends in adoption of bioenergy-based utilization would go a long way in mitigating the adverse effects of greenhouse gases (GHGs). Besides, it offers socio-economic and environmental benefits. Several agricultural crops and plants have been explored and identified for biofuel purpose, like sugarcane (*Saccharum* spp.), maize (*Zea mays*), soybean (*Glycine max*), willow (*Salix* sps.), switch grass (*Panicum virgatum*), rapeseed (*Brassica napus*), wheat (*T. aestivum*), sugar beet (*Beta vulgaris*), palm oil (*Attalea maripa*), manioc (*Manihot esculenta*), miscanthus (*Miscanthus* sps.), potato (*Solanum tuberosum*), sweet potato (*Ipomoea batatas*), and barley (*Hordeum vulgare*); sorghum (*Sorghum bicolor*), cassava (*Manihot esculenta*), and hemp (*Cannabis sativa*) (Cho 2011; Davis et al. 2013; Balat 2010; Leite and Leal 2007; Solomon and Bailis 2014).

Sugarcane is one of the most energy efficient crops being grown in more than 100 countries (Fig. 11.1) having very wide adaptability range supported by its C4 photosynthetic system resulting into large biomass production per unit area having the desirable traits of high yield along with low input requirements and better processing capabilities (Verheye 2010) as well as reducing greenhouse gases (Matsuoka et al. 2009). Worldwide, it is grown on an area of 25.9 million ha, and its total production is ~1.84 billion tons with a fresh cane yield of 70.9 tons ha^{-1} (FAOSTAT 2019) (Fig. 11.2) The largest acreage of sugarcane lies in Brazil contributing 41% of world production (758 Mt), followed by India (306 Mt—16%) and China (104 Mt—5.6%) (FAOSTAT 2019) (Fig. 11.3). Sugarcane was originally domesticated around 8000 BC in Papua New Guinea. Commercial sugarcane is the cross of *Saccharum officinarum* with wild *Saccharum* spp., i.e., *S. spontaneum*, *S. robustum*, *S. barberi*, *S. sinense*, and *S. edule* (Talukdar et al. 2017; Allen et al. 1997; Jeswiet 1929). Commercial sugarcane suffers from high level of pollen sterility, and propagation through vegetative cuttings is the method of choice (Allsopp et al. 2000). Disaccharide sugar is the main product of sugarcane. Juice extracted by crushing of the canes is clarified at high temperature in the

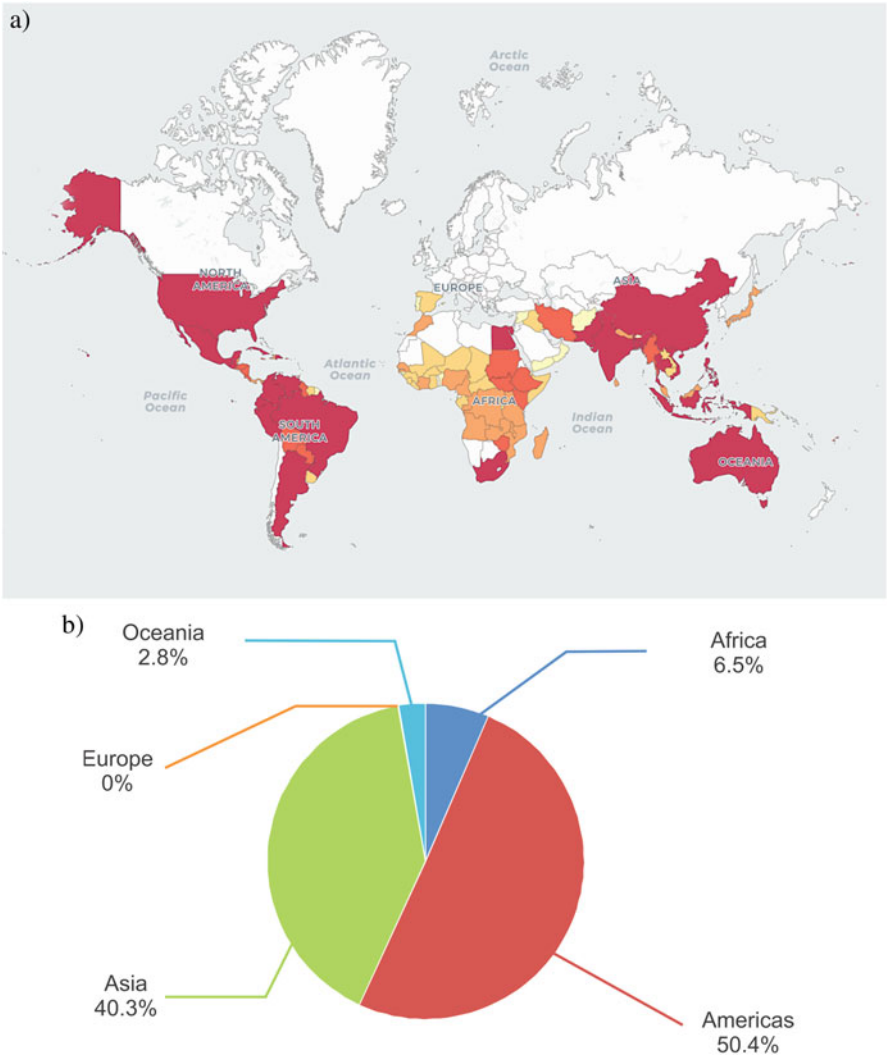

Fig. 11.1 (**a**) Major sugarcane-producing countries. (**b**) Global distribution of sugarcane

presence of lime, which forms complexes with phosphorus in juices, precipitating with impurities supported by flocculants (Mackintosh 2000).

Bioethanol obtained through sucrose fermentation of sugarcane (*S. officinarum*) often referred as "noble cane" is referred to as "first-generation" bioethanol production. Production of biofuel through fermentation of the lignocellulosic plant cell wall biomass of sugarcane is referred to as "second-generation" bioethanol production. Third- and fourth-generation bioethanol are derived from algal sources and

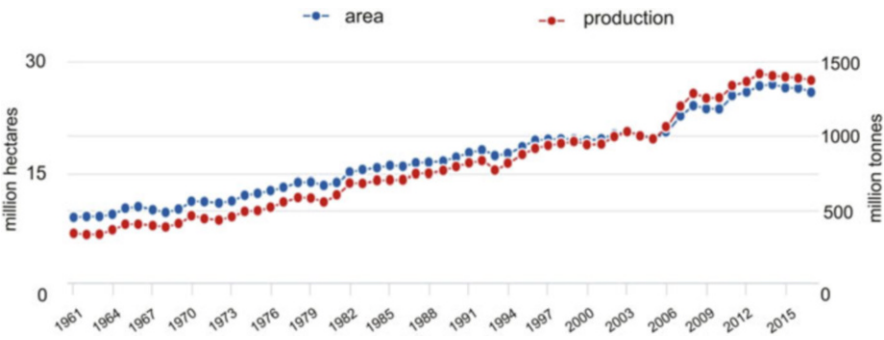

Fig. 11.2 Sugarcane area and production around the world (1961–2017) (FAOSTAT 2019)

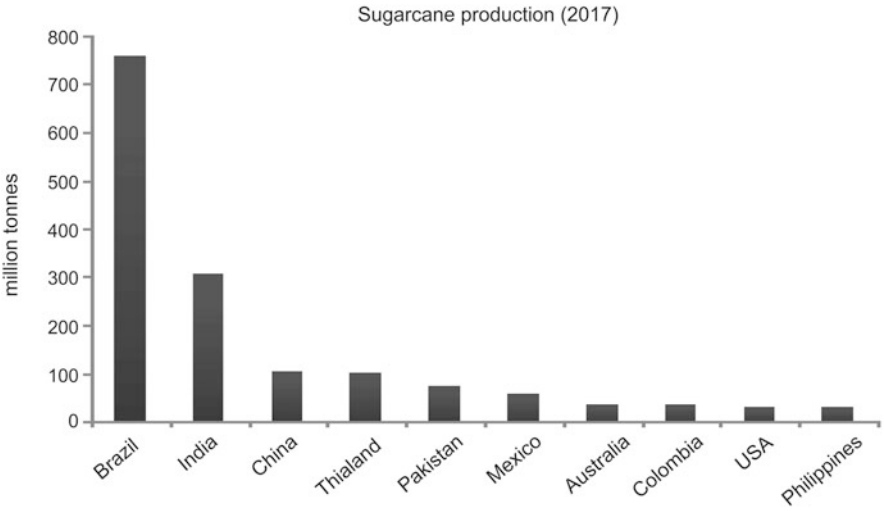

Fig. 11.3 Leading sugarcane-producing countries (FAOSTAT 2019)

genetically modified microalgae, respectively (Buckeridge et al. 2010; Carvalho et al. 2013).

11.2 First-Generation Bioethanol Production

The first-generation bioethanol is sourced from easily extractable sugar or starch sources. Here, sugarcane offers an obvious advantage with ~20% juice content with production levels of 8000 L/ha which is twice that of maize, thereby requires half the land requirement (Lima and Natalense 2010). Sugarcane undergoes chopping and shredding in traditional mills to extract the broth. First-generation bioethanol produced from the sugarcane by fermentation of sugar obtained from its juice and left-

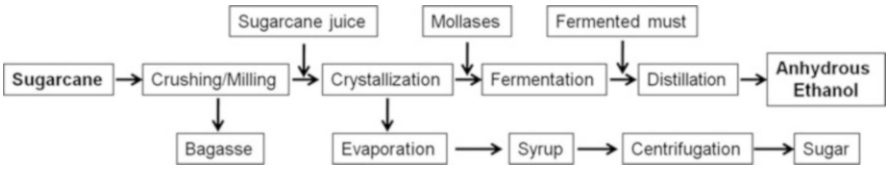

Fig. 11.4 Block flow diagram of a sugarcane-based first-generation bioethanol production

over plant material after extracting the juice (bagasse) is burned to produce steam for electricity generation, to produce fertilizers, or to produce heat in the sugar mills (Pandey et al. 2000) (Fig. 11.4). The impurities and contaminants laden extracted broth are removed as bagasse with aid of filters (physical treatment), and clear broth undergoes chemical treatment wherein soluble impurities are coagulated using CaO and phosphoric acid with pH7.0, followed by decantation and concentration to 20–22° brix in evaporators for better fermentation (Santos et al. 2012). Sulfitation is an additional step in bioethanol production to purge the color from the formed sludge. Under anaerobic condition, the most crucial step of bioethanol production is accomplished by yeast (*Saccharomyces cerevisiae*) which metabolizes sugars to bioethanol. Fermentation process at commercial scales involves: (1) Simple Batch: Yeast is added to the fermenter, with the yeast fermentation process lasting till the presence of nutrients. The process is slow and needs to be cleaned and reloaded with each batch. Supplements and inoculums are incorporated at the start of the reaction, with constant agitation that supports the growth and fermentation process. To moderate pH, chemicals and antibiotics are added to the medium (Maxon and Johnson 1953; Zhang 2009). Often fermenters are operated in series at commercial level to sustain the high demand of bioethanols (Gomez-Pastor et al. 2011). The status of the growth of yeast is regularly monitored. (2) Fed Batch: The fermentation involves the addition of supporting nutrients to the fermenter with the products remaining till the end of reaction. The fed-batch system offers an advantage over the batch process: higher productivity level of ethanol along with lower content of residual sugars, thereby self-inhibition by the presence of substrates and products is minimized. The process requires less fermentation period, reduced toxicity levels to the growing yeast cells, and prevalence of optimum growth conditions (Stanbury et al. 2003). Higher inoculum load is inversely correlated to reduced yeast cell viability (Laluce et al. 2009). (3) Multistage Continuous Process: These fermenter systems are designed to operate continuously and are fed by sugarcane juice to maintain continuous flow towards the distillation units. Often four or more reactors are operated in series. The major advantage this system offers is very high levels of ethanol production coupled with lower operational running costs (Deindoerfer and Humphrey 1959). The drawbacks include higher chances of contamination, therefore requires large amounts of sulfuric acids and antibiotics (Domingues et al. 2000).

11.3 Second-Generation Bioethanol Production

The second-generation biofuels involve the use of lignocellulosic materials. Lignocellulose comprises of cellulose (homo-polymer of glucose units), hemicelluloses (hetero-polymers of D-mannose, D-glucose, D-xylose, L-arabinose, D-galactose, mannuronic acid, and glucuronic acid units), and lignin (phenylpropane units). These three components are responsible for the rigidity of plant cell (Brodeur et al. 2011; Hendriks and Zeeman 2009; Ogeda and Petri 2010; Sarkar et al. 2012). The idea of employing sugarcane straw from crop residues while not competing with food production is building up the buzz. Bioethanol yield through this method can be augmented as much as 100% with a yield of ~300 L of bioethanol from one ton of bagasse. After harvest, the sugarcane straw (comprising 40% cellulose, 30% hemicelluloses, and 25% lignin) is shredded and processed by hydrolysis. The plant cell wall is degraded into monosaccharides to be used as a feeder for fermentation process (Piacente et al. 2015). The hydrolysis of cellulose is catalyzed by cellulase enzymes to produce mono- and disaccharides followed by fermentation to bioethanols. Since the process is slow, a pretreatment is often undertaken (Fig. 11.5).

Pretreatment helps disrupt the cellulose structure, breaking down hemicelluloses and modification/removal of lignin (Mosier et al. 2005). The methods include physical, chemical, and biological pretreatments (Alvira et al. 2010). Physical processes include steam explosion, mechanical reduction in size, and hot water application, often added in combination with catalysts to improve efficiency (Agbor et al. 2011). Physicochemical methods include CO_2/SO_2-steam explosion, acid-steam explosion, and ammonia fiber explosion (Agbor et al. 2011). Chemical pretreatments involve the use of dilute acids like H_2SO_4 and HCl; dilute alkalis like

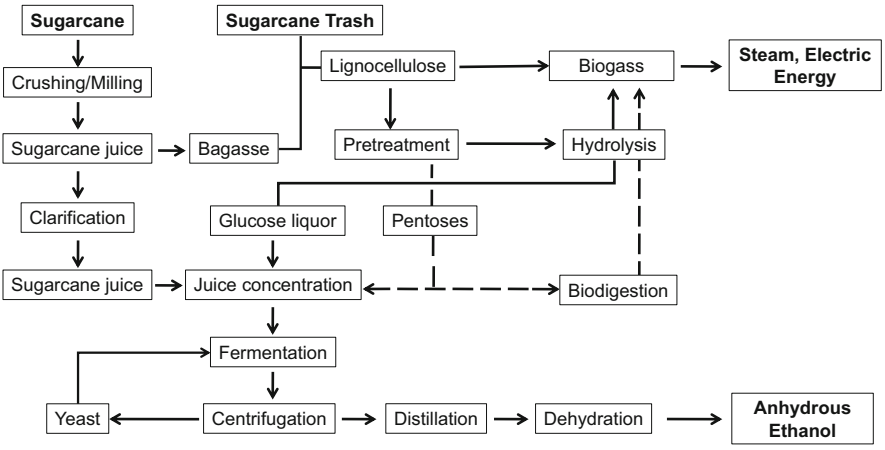

Fig. 11.5 Block flow diagram of a sugarcane-based second-generation bioethanol production (Adapted from Dias et al. 2013)

NaOH, ammonia; oxidizing agents like hydrogen peroxide and peroxyacetic acid; organic acids like formic acid and acetates; and inorganic salts like $FeCl_3$ and $CaCl_2$ (Ngyen et al. 2010; Brandt et al. 2013; Zhang et al. 2012, 2013). To improve reaction efficiency one or more methods are used in combinations.

11.4 Yeasts in Bioethanol Fermentation

Saccharomyces cerevisiae, the most commonly employed ethanol producing yeast, offers distinct advantages in terms of owing to its high ethanol production from hexoses, low cost and easy availability, high tolerance to ethanol, and other inhibitory compounds and ability of fermenting wide range of sugars. Studies conducted for ethanol production by *S. cerevisiae* from different substrates at varying treatment and optimization conditions are compiled in Table 11.1.

The commonly used *Saccharomyces cerevisiae* yeast in industrial fermentation processes lack the ability to metabolize pentoses such as xylose and arabinose. These pentoses are present in large quantity in hemicelluloses, which forms a major component of plant biomass (De Souza et al. 2013, 2015). Bio-prospecting for new strains of pentose-fermenting microbes has gained prominence as a source for the development of recombinant yeast strains with improved fermentation abilities (Zhang and Geng 2012; Harner et al. 2015). Some of the new yeast species identified for fermentation of pentose from diverse sources are listed in Table 11.2. The whole genome sequencing of these newly identified strains of pentose metabolizing strains will divulge the new genes of biotechnological importance for the development of recombinant strains of *S. cerevisiae.*

11.5 Biotechnological Approaches

The second-generation biofuels are produced from the lignocellulosic material of the plants. In the current scenario, uses of sugarcane bagasse for second-generation biofuels emerged with great potential. The bottleneck in uses of lignocellulosic material is the production cost, preventing this technology from the commercialization on a large scale (Halling and Simms-Borre 2008) due to use of expensive microbial enzymes for pretreatment of the bagasse fibers to remove the recalcitrant components (Yuan et al. 2008). For accelerating higher biofuel production from sugarcane, requires a strategic shift to incorporate both first- and second-generation biofuels production. This strategic shift can be achieved by the implications of biotechnological practices such as improving the sugarcane yield, increasing the sugar content, developing the faster-growing cultivars, modified bagasse lignocellulosic fiber quality which requires less or cheap pretreatments and faster biodegradable property of these fibers (Hoang et al. 2015). Transgenic approaches to engineer any organism have the unique ability that it can be applied independent of closeness or relativeness of the source of the genes, i.e., a gene from any organism can be transferred to any organism from other kingdoms.

Table 11.1 Bioethanol production by *Saccharomyces cerevisiae* from different feedstock at varying pretreatment and optimization conditions (Mohd Azhar et al. 2017; Tesfaw and Assefa 2014)

S. cerevisiae strain	Feedstock	Pretreatment	Enzymatic hydrolysis	Ethanol produced (g/l)	References
MTCC 173	Sorghum stover	NaOH	Cellulase	68.0	Sathesh-Prabu and Murugesan (2011)
MTCC 174	Rice husk	NaOH	Crude unprocessed enzyme	14	Singh et al. (2014)
RL-11	Spent coffee grounds	H_2SO_4	Cellulase	11.7	Mussatto et al. (2012)
ATCC 26602	Wheat straw	H_2O_2	Cellulase	10	Karagoz and Ozkan (2014)
L2524a	Empty palm fruit bunch fibers	NaOH	Cellulase	64.2	Park et al. (2013)
KL17	Galactose and glucose	–	–	96.9	Kim et al. (2014)
Y5	Corn stover	Steam explosion	Cellulase and glucosidase	50	Tian et al. (2013)
ATCC 6508	Sweet potato chips		α-Amylase and glucoamylase	104.3	Shen et al. (2012)
DQ1	Corn stover	H_2SO_4	Cellulase	48	Chu et al. (2012)
CHY1011	Cassava starch	–	α-Amylase and glucoamylase	89.1	Choi et al. (2010b)
TISTR 5596	Sugarcane leaves	H_2SO_4 or $Ca(OH)_2$	Cellulase	4.71	Jutakanoke et al. (2012)
Y5	Corn stover	Steam explosion	Cellulase	40	Li et al. (2011)
TISTR 5596	Starch cassava pulp	–	α-Amylase and glucoamylase	9.9	Akaracharanya et al. (2011)
CHFY0321	Cassava starch	–	α-Amylase and glucoamylase	89.8	Choi et al. (2010a)
DQ1	Corn stover	Steam explosion	Cellulase	55	Bi et al. (2011)
Var. ellipsoideus	Corn meal	–	Heat stable α-amylase and glucoamylase	79.6	Nikolić et al. (2010)
ZU-10	Corn stover	H_2SO_4	Cellulase	41.2	Zhao and Xia (2010)

Table 11.2 Novel yeast species isolated and identified for xylose and arabinose fermentation

Yeast species	Isolation	Pentose substrate	References
Scheffersomycesshehatae and *S. stipitis*	Gut of Guatemalan passalid beetles	Xylose, arabinose	Kurtzman et al. (2011)
Meyerozyma guilliermondii	Termites (*Nasutitermes* sp.) in the Amazonian habitat	Xylose	Matos et al. (2014)
Scheffersomyces shehatae	Natural habitats in Brazilian forest	Xylose	Martiniano et al. (2013)
Sugiyamaella xylanicola, Scheffersomyces queiroziae, and *Scheffersomyces stipitis*	Rotting wood of Atlantic rainforest	Xylose	Morais et al. (2013)
Spathaspora brasiliensis, Spathaspora roraimanensis, Spathaspora suhii, Spathaspora xylofermentans	Rotting wood of the Brazil forest ecosystem	Xylose	Prompt (2012)
Spathaspora passalidarum, Scheffersomyces stipitis	Rotting wood samples of the Amazonian forest ecosystem	Xylose	Cadete et al. (2009)
Scheffersomyces insectosa, Scheffersomyces lignosus	Baotianman Nature Reserve, China	Xylose	Ren et al. (2014)
Zygoascushellenicus, *Candida blankii, Candida saraburiensis*	Agricultural residues	Xylose	Nitiyon et al. (2011)
Spathaspora passalidarum and *Candida jeffriesii*	Gut of passalid beetles in the USA	Xylose	Nguyen et al. (2006)
Candida tropicalis, *Candida parapsilosis*, *Candida mengyuniae*, *Sporopachydermia lactativora*, *Trichosporon asahii*	Rectum of Murrah buffalo and Swamp buffalo in Thailand	Xylose	Lorliam et al. (2013)

Among the several monocots which are being used for biofuel, sugarcane was extensively studied through the genetic transformations to improve its potential (Hoang et al. 2015 and the references therein). The genetic modification of sugarcane plants which have a desired ratio of cellulose to noncellulose content; transgenically expressing some of the cellulolytic or hemicellulolytic enzymes prior to which are being used for pretreatment before its conversion to ethanol; improving the pest and disease resistance by expressing disease resistant genes; improving the abiotic stress tolerance; or improving the agronomic performance by incorporating some of the regulatory genes enhancing the growth parameters (Khan et al. 2019; Hoang et al. 2015; Sticklen 2006; Yuan et al. 2008; Matsuoka et al. 2009; Arruda 2012). In line with changing the carbohydrate composition, changing the cell wall carbohydrate would facilitate in achieving the easier processing of the biomass in the form of the end products for biofuel generation (Harris and DeBolt 2010).

11.5.1 Biomass Improvement

Increasing biomass yield of sugarcane would also enhance the quantities of ethanol produced from the same area of cane cultivation. It was showed that the *ScGAI* gene regulates the growth and development of the sugarcane culm by modulating the ethylene signaling pathway (Garcia Tavares et al. 2018). They showed that silencing the *ScGAI* gene increases the internode length, bigger height, and increased carbon allocation to the stem (Garcia Tavares et al. 2018). For second-generation biofuel the sugarcane bagasse fibers composed of lignocellulosic materials are being used. The lignocellulosic biomass yield is about 22.9 tons dry weight per hectare per year and thus the total available estimated dry weight of sugarcane lignocellulosic material worldwide is approximately 600 million tons (Van der Weijde et al. 2013) and combined bioethanol yield of 9950 L per hectare can be achieved (Khan et al. 2019 and the references therein). Hence, increasing the biomass potential is another promising strategy for producing higher amounts of biofuels from sugarcane.

11.5.2 Abiotic and Biotic Stress Tolerance

Drought is one of the most devastating abiotic stresses causing severe damage to crop productivity. Similar to several other crops, scarcity of water can negatively affect the growth of the sugarcane and could result in decrease of the biomass yield by 50% (Inman-Bamber 2004). Many sugar molecules in plants serve as an osmolyte to increase the solute concentration intracellular and thus promoting the efficient water uptake during the mild drought stress. Trehalose is one of the good examples which functions as an osmolyte and has been reported to protect the cellular structure from dehydration induced damages (de Jesus Pereira et al. 2003). Developing genetically modified sugarcane which expresses the genes of trehalose biosynthetic pathway showed better growth, improved drought tolerance, and produced higher sugar content than the WT plants (Zhang et al. 2006). Similarly, overexpression of a drought responsive transcription factor cloned from *Arabidopsis At*DREB2A CA in sugarcane upregulates the expression of stress responsive genes, maintains better relative water content and photosynthetic efficiency, and performs better vegetative sprouting (Reis et al. 2014). Moreover, transgenic sugarcane overexpressing another transcription factor BcZAT12 cloned from *Brassica carinata* enhanced both salinity and drought stress tolerance (Saravanan et al. 2018). To improve the salinity stress tolerance in the sugarcane, transgenic sugarcane overexpressing *Arabidopsis* vacuolar pyrophosphatase (*AVP1*) or Δ1-pyrroline-5-carboxylate synthetase (*P5CS*) gene has been developed which showed the improved endurance against the salinity stress (Kumar et al. 2014; Guerzoni et al. 2014).

On the other side, genetically engineered sugarcane to mitigate the diseases caused by the biotic factors or fighting against the pests were also developed and tested. Transgenic sugarcane resistant to the yellow leaf virus has been developed very early as in 1997 (Khan et al. 2019; Arencibia et al. 1997, 1998, 1999). Glufosinate resistant sugarcane was developed by expressing the phosphinothricin

acetyltransferase (*bar*) gene and by spraying the glufosinate, the weeds are selectively killed without having negative effect on the transgenic sugarcane (Manickavasagam et al. 2004). To fight against several pest and insects, Monsanto has already developed the transgenic sugarcane using the Bt technology and it is being used commercially (Maldonado et al. 2010).

As above discussed, approaches are useful for the improvement of the yield potential for both first- and second-generation biofuel from the sugarcane, in the following sections we would emphasize the specific genetic engineering approaches used for either first- or second-generation biofuels.

11.5.3 Increasing Cellulose Content

Obviously, it is clear that modifying the cell wall composition of the sugarcane by increasing the cellulose and hemicellulose content will increase the fermentable sugars produced from the same amount of the materials. Transgenic sugarcane plants expressing the cellulose synthase gene *CsCesA* from a marine invertebrate *Ciona savignyi* increased the cellulose synthase activity and also the cellulose content in the transgenic plants (Ndimande 2014). Additionally, the hemicellulosic glucose content and the uronic acid content of the transgenic sugarcane have also been increased with the decline of lignin content (Ndimande 2014).

11.5.4 Enhanced Sucrose Accumulation

Sugar is the first product of photosynthesis which is further modified in different structural, nutritional, protective, or storage metabolites in the plants. Enhancing the sugar synthesis either by increasing the photosynthesis efficiency or by manipulating the sugar synthesis or sugar degradation pathway has not been successful so far. Because an increase in any of these components sends feedback signals to the photosynthesis and thus the photosynthesis is inhibited. To overcome the feedback inhibition of sugar synthesis, the pathway has been modified, where the natural sugar product of photosynthesis is modified in a different form of sugar. The modified form does not send any feedback signal and is relatively more stable. These modified sugars were designed in such a way that it can be used for food as well as for the biofuel sector. Isomaltulose (IM) is a stable sugar which shows slower digestion property than the sucrose and non-hygroscopic (Khan et al. 2019; Lina et al. 2002). Expression of bacterial sucrose isomerase (*SI*) in vacuole of sugarcane accumulated the IM in the vacuole without affecting the cellular sucrose concentration and thus doubled the total sugar concentration of the sugarcane juice (Wu and Birch 2007). Interestingly, the transgenic lines also showed increased photosynthesis, sucrose transport, and increased sink strength (Wu and Birch 2007). Targeted expression of the *Saccharomyces cerevisiae* invertase gene (*SUC2*), which has been expressed in the apoplast of the sugarcane callus/liquid culture cells, showed the rapid conversion of sucrose to hexose and increased hexose concentration in the medium (Ma et al.

2000). Alternatively, several other strategies like improving the photosynthetic capacity by expressing cyanobacterial genes, metabolic engineering for modifying the photorespiratory pathways, Calvin–Benson cycle, or modifying the sugar forms in the sink tissue will increase the photosynthetic efficiency of the sugarcane and would also result in higher sugar yield (Lin et al. 2014; Shih et al. 2016).

Second-generation biofuel generation was adopted to avoid the competition between the crops for feeding the growing population or for the fuel. The second-generation biofuel is being produced from the lignocellulosic biomass of several grasses with a higher growth rate and rich potential of yield and can be grown in the marginalized lands. Traditionally, the lignocellulosic fiber of the sugarcane bagasse obtained after extracting the juice is being used in the fertilizer industries or in sugar mills for producing heat, steam, and electricity (Pandey et al. 2000). Including the sugarcane lignocellulosic materials along with the sugar for bioethanol production would make the breakthrough by enhancing the total yield of bioethanol of 9950 L per hectare (Hoang et al. 2015; Somerville et al. 2010). Producing ethanol from the bagasse lignocellulosic material is not as convenient and cost-effective as from the sugar derived from the sugarcane. Enzymatic degradation of lignocellulosic biomass to fermentable sugar requires several enzymes in huge quantities. For example, 15–25 kg cellulase is required for the processing of a ton of biomass (Carroll and Somerville 2009; Fan and Yuan 2010). These degrading enzymes are derived from microbial sources and thus the requirement of these huge quantities of enzymes making the whole process expensive. The presence of recalcitrant material in the cell wall arises additional bottleneck preventing the enzymatic access to the cellulose or hemicellulose for their degradation. A new approach adopted to tackle these issues was to express these enzymes required for pretreatment of the lignocellulosic materials stably in the leaf of the sugarcane or metabolic engineering of the cell wall content to reduce the recalcitrant material. Transgenic sugarcane lines with reduced lignin content, higher cellulose to noncellulose ratio, and expressing the lignocellulosic processing enzymes inplanta has been successfully reported (Khan et al. 2019 and the references therein).

11.5.5 Modifying the Cell Wall Content of the Sugarcane

Removal of recalcitrant compounds in the bagasse lignocellulosic fibers is required before they can be used for bioethanol production. Sugarcane bagasse constitutes of cellulose, hemicellulose, and lignin at the ratio of 50, 25, and 25% of dry weight, respectively (Khan et al. 2019; Hoang et al. 2015; Loureiro et al. 2011; Mutwil et al. 2008; Pauly et al. 2013). Lignin of the cell wall is one of the large barriers which prevent the access of the cellulase to the cell wall. The biosynthetic pathway of the lignin is complex which involves 10 enzymes (Whetten and Sederoff 1995), and monolignol, the starting material for the lignin biosynthesis pathway whose biosynthesis in plants is linked with 28 unigenes (Bottcher et al. 2013). A wise strategy can be applied to suppress these genes or a candidate gene regulating these pathways to reduce the lignin content in the sugarcane bagasse. It is important to be noted that the

lignocellulosic fibers serve as the skeleton of the sugarcane (Khan et al. 2019) and precaution must be taken that the modification of the lignin content should not affect the plant growth and development. Some examples of modifying the lignin biosynthesis pathway for the purpose to reduce recalcitrance of lignocellulosic fibers come from the studies where enzyme like caffeic acid O-methyltransferase (COMT) expression of the lignin biosynthesis and cinnamyl alcohol dehydrogenase (CAD) enzyme expression of the monolignol biosynthesis were suppressed (Jung et al. 2012; Sticklen, 2006). In these studies, it was found that the growth and development of the plants were not affected in the controlled growth conditions, while the reduction of the lignin content resulted in a significant increase in the fermentable sugar content without any pretreatment (Khan et al. 2019; Jung et al. 2012; Sticklen 2006). Field trial study of these COMT-suppressed transgenic lines in the USA revealed that the lignin content of the transgenic was reduced by 12% as compared to the WT plants and reduction of lignin content has reduced the hydrolysis time by one-third and enzyme consumption decreased by 3- to 4-fold (Khan et al. 2019; Jung et al. 2012). Using the similar strategy to engineer another biofuel grass, switchgrass has shown better efficiency of cellulase treatment and increased production of glucose and bioethanol (Fu et al. 2011; Saathoff et al. 2011).

Alternative to reducing the lignin content of the cell wall, approach where changing the composition of the lignin polymer composition can also be employed. It has been reported that the lignin in angiosperm is composed of guaiacyl, syringyl, and p-hydroxyphenyl units derived from the monolignols (Vanholme et al. 2010), where syringyl units are better-degrading type than that of recalcitrant guaiacyl-rich lignin (Papes et al. 2015). Changing the syringyl and guaiacyl levels by manipulating the gene expression has a minor effect on the plant development (Vanholme et al. 2010) and the genetically modified sugarcane having altered cell wall lignin composition can be easily processed, adding advantage in terms of cost-effectiveness of the second-generation ethanol production (Maldonado et al. 2010).

11.5.6 *In-Planta* Processing

The idea of expressing cellulolytic and hemicellulolytic enzymes in sugarcane using genetic engineering is to degrade or digest the cell wall cellulose and hemicellulose within the sugarcane plants after harvesting, so that the highly cost consuming pretreatment process can be mitigated. Maize *PepC* promoter-controlled expression of the cellulolytic fungal cellobiohydrolase I (CBH I), CBH II, and bacterial endoglucanase (EG) shows stable expression in different cellular compartment of the leaf in transgenic sugarcane (Harrison et al. 2011). It was shown that the accumulation of exo- or endoglucanase in the transgenic plants had no any negative impact on the growth of the transgenic sugarcane plants (Harrison et al. 2011). But this strategy also comes with the challenges and the detailed knowledge to overcome these challenges are still limited. To achieve the full purpose of this strategy, extensive knowledge of several inducible promoters are required, so that these enzymes are expressed only after harvesting of the biomass. Use of constitutive

promoter was limited due to occurrences of transgene silencing in the sugarcane caused by its complex genome structure (Harrison et al. 2011). Expression of these enzymes at the early developmental or growth stages could also be devastating and may negatively impact the growth and development of the transgenic sugarcane plants (Dale 2007; Harris and DeBolt 2010; Maldonado et al. 2010).

11.6 Genetic Engineering of Sugarcane for Biodiesel

The lipid in plants is stored in the form of triacylglycerols (TAGs) which have the relatively higher energy content than that of the carbohydrates (Durrett et al. 2008). The TAGs are converted to biodiesel by modifying the acyl chains of TAGs to fatty acid methyl esters (Ohlrogge and Chapman 2011). Oil-seed crops tend to have relatively higher content of the TAGs but the use of oil seeds or fruits for the biodiesel product negatively impacts the food produced from those crops and thus focus has been diverted towards use of the vegetative biomass of the crops without affecting the food productivity (Chapman et al. 2013). Being a C4 grass, sugarcane has efficient photosynthetic capability and extensive production of the vegetative biomass drew attention of the scientific communities to explore the possibility of biodiesel production from the sugarcane. Genetic engineering approaches are focused to upregulate the lipid biosynthesis pathway in the sugarcane by rerouting the carbon flux (Vanhercke et al. 2014; Zale et al. 2016). TAGs accumulation up to 19% dry weight of the total biomass production in the tobacco has been achieved by expressing three genes, namely WRINKLED1, DGAT, and Oleosins (Vanhercke et al. 2014; Zale et al. 2016). Similar strategy was adopted in sugarcane which resulted in accumulation of 5% TAGs and 10% total fatty acids (Huang et al. 2015; Zale et al. 2016). As most of the biomass in sugarcane is contributed by the stem, the metabolic engineering using the stem-specific promoters could have large impact on the TAGs production in sugarcane (Khan et al. 2019 and the references therein). It will be an additional breakthrough in the biofuel industry if the metabolic engineering for TAGs synthesis in sugarcane would be successful which has a great potential for biodiesel production due to its huge biomass production rate.

Disadvantages of Sugarcane-Based Biofuel Production The main drawback that questions the sustainability of sugarcane-based biofuel production is the competition between the land usage for food production and biofuel production. The possibility of horizontal land expansion is not possible. This would lead to deforestation and loss of soil diversity. The forest is a great carbon sink, so loss of forest would lead to global warming. Sugarcane also requires substantial inputs of fertilizers and water that lead to eutrophication. The use of pesticides and machine leads to soil pollution and erosion. The other disadvantages are the GHG emissions from agricultural inputs and farming operations. Therefore, the alternatives to sugarcane-based biofuel which would be more sustainable like third and fourth generation biofuel should be discussed.

Alternatives to Sugarcane-Based Biofuel: 3rd- and 4th- Generation Recently, the idea of algal biomass-based biofuels also called third-generation biofuel is getting more acceptances. The algae have higher energy conversion efficiency and surface area-to-volume ratio as compared to sugarcane. Hence the amount of lipid is more in the algae, and biofuels from algae usually relies on the lipid content of the microorganisms, for example, Chlorella has high lipid content (around 60 to 70%; Liang et al. 2009) and high productivity (7.4 g/L/d for *Chlorella protothecoides*; Chen et al. 2011). However there are geographical and technical challenges associated with algal biomass production. First, algae production requires a large amount of water with specific nutrient and temperature condition. Second, the harvesting of algae, removal of water from them, and lipid extraction need technical skills. The idea of using 3rd generation biofuels is setback by the cold countries and countries lacking enough fresh water. At present, extensive research to improve both the metabolic production and separation of fuels from non-fuels is underway.

To meet up such challenges and in order to develop biofuel that can be used universally, the use of nonarable lands and solar energy towards the sustainable development of biofuels is proposed. Such biofuels are also called fourth-generation biofuels and can effectively reduce greenhouse gas emissions and mitigate climate change. They include photobiological solar fuels and electrofuels. It is also based on redesigning the genome of algae and cyanobacteria in such a way that their energy conversion efficiency increases (also called photon-to-fuel conversion efficiency (PFCE)) (Berla et al. 2013; Hays and Ducat 2015; Scaife et al. 2015). Photosynthetic microorganism can be used as biocatalyst for the production of hydrogen by photosynthetic water splitting (water oxidation). This can become a large contributor to fuel production on a global scale, both by artificial photosynthesis (Inganäs and Sundström 2016) and by direct solar biofuel production technologies. However, the production of photobiological fuel and electrofuel requires synthetic biology approach which is still in its beginning stage and requires a lot of optimization.

11.7 Conclusions

Sugarcane is characterized by narrow genetic base with a complex genome and low levels of fertility. To realize the full potential of sugarcane as a bioenergy crop, more efforts need to be directed towards improvements in biomass addition coupled with sucrose accumulation, imparting tolerance to biotic and abiotic stresses. The emerging biotechnological tools of genetic transformation primarily through *Agrobacterium*-mediated genetic transformation are likely to emerge as major force to supplement the classical breeding approaches towards sugarcane crop improvement which is hampered by laborious and long development period. With the availability of whole genome sequence information of sugarcane coupled with ever evolving bioinformatics tools, the enigmatic goal of achieving the plant type with most desirable traits will be within reach. Recent technique of genome editing

and successes in the other crops offers new scope and dimension to sugarcane crop improvement.

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