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# Sugarcane as Future Bioenergy Crop: Potential Genetic and Genomic Approaches

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## Abstract

*Biofuels* are gaining increased scientific as well as public attention to fulfill future energy demands and can be the only potential candidates to safeguard and strengthen energy security by reducing the world's reliance on exhausting fossil energy sources. Sugarcane is an important C<sub>4</sub> crop with great potential to contribute to global biofuel production as sugarcane juice can be easily fermented to produce ethanol. The success of bioethanol production from sugarcane in Brazil has widened the scope of the technology and has led to increased demand of purpose-grown sugarcane for biofuel production. Scientific interventions have not only helped to improve the cane crop but industrial procedures have also been upgraded resulting in improved production of bioethanol. Likewise, advancements in omics have led to high hopes for the development of energy cane. This chapter highlights the advancements as well as potential and challenges in the production of sugarcane biofuel, focusing on genetic and genomic interventions improving the crop as energy-cane. Further, controversies in the production and usage of biofuel derived from sugarcane have also been discussed.

**Keywords:** Biofuel, Sugarcane, Genetic and genomic approaches, Future energy-cane

## 1. Introduction

Increasing energy demands for erratically increasing population, urbanization, industrialization and environmental concerns related to fossil fuels have inclined researchers to explore alternative resources of energy. Compared with the current global energy consumption of 400 exajoule, an increase of 200 exajoule is expected by 2025 [1]. Biofuels have gained much importance due to the depleting fossil fuel resources and the over-accumulation of CO<sub>2</sub> and other greenhouse gases in the environment. Biofuels can play a part to achieve targets to replace fossil fuels to reduce carbon dioxide released into the atmosphere and to attain environmental and economic sustainability. Though bioenergy is already contributing more than 10% of global energy supplies huge potential is there to uplift its contribution. Lignocelluloses are accounted for more than 20 billion metric tons of biofuels worldwide. Owing to the outstanding features of these biofuels i.e. enhanced octane number: MON (motor octane number) and RON (research octane number), they are the most desired source of biofuel. Further, they would prove an

environmentally friendly fuel source having the ability to generate less black smoke with fewer hydrocarbon emissions and NO<sub>x</sub> [2]. Hence, fuels of the renewable kind (bioethanol and biodiesel) are subjects of increased attention in this context [3]. For the development of efficient and viable alternative fuel having the ability to provide environmental safety and energy gain, the fundamental procedures need to be improved [4]. Normally, biofuels are produced either by biochemical or thermochemical strategies. Production by the biochemical method includes biomass retreatment, biomass handling, fermentation, and hydrolysis. The thermochemical processes can convert non-food and food biomass to fuel through gasification and pyrolysis [5]. A combination of both of these processes (biochemical or thermochemical) has been worked out by various research groups and has proved to be effective for the economic production of biofuel [6].

Sugarcane, being a C<sub>4</sub> plant is photosynthetically more competent to produce higher content of dry mass. Commercial production of sugar from sugarcane was initiated in India and China almost 2500 years back whereas it was domesticated in Western Europe during the 18th century [7]. Various *Saccharum* species prevail all around the world. These include *Saccharum officinarum*, *Saccharum edule*, *Saccharum barberi*, *Saccharum robustum*, *Saccharum spontaneum*, and *Saccharum sinense*. Among these *Saccharum spontaneum* and *Saccharum robustum* are wild species; *Saccharum officinarum*, *Saccharum barberi*, and *Saccharum sinense* are early cultivars whereas *Saccharum edule* is a marginal specialty cultivar. Anyhow, *Saccharum officinarum* is the most widely cultivated species because of its higher sucrose content and wide-spread adaptability. All the *Saccharum* genotypes are polyploid with variable ploidy level (5× to 16×) and chromosome number ranging from 80 to 130. Hence, they have the most complex genome among the plants. Researchers have been striving to develop hybrids having the ability to produce more dry mass with more sugar content. Certain hybrids have been found to have 15–25% chromosomes from *Saccharum spontaneum*, 60–70% from *Saccharum officinarum*, and 5–10% of recombinants hybrids of homologous chromosomes from both species. These hybrids are expected as better performers as compared with existing germplasm [8]. Since sugarcane is an outstanding source of biofuel production as compared with other crop plants and competitors. This manuscript highlights the significance of biofuel usage in the current scenario.

## 2. Potential candidate crops for biofuel production

Production of different versions of biofuels *i.e.* butanol, methanol, ethanol, isoprene, vegetable oil, hydrogen, biodiesel, jet fuel, and gasoline various types of substrates have been tested [9, 10], using renewable biomass sources including lignocellulosic sources. In the current scenario, ethanol has drawn the most attention owing to its suitability as biodiesel [11]. Various plant sources have been tested to assess their efficiency for bioethanol production and its implication as biofuel. In addition to sugarcane and maize, different plant species have shown the potential to be a valuable source for the production of biofuel (**Table 1**). Quinn et al. [13] reported the use of 49 potential plant species as feedstock for biofuel production and most of them can be used to produce ethanol. Johnsongrass, *Erianthus*, switchgrass, napiergrass, and sorghum are also valuable candidate crops for bioethanol production and can produce high biomass with fewer inputs [14]. Further, corn fiber is also a valuable source for the production of biofuel.

Perennial grasses like *Halopyrum mucronatum*, *Desmostachya bipinnata*, *Phragmites karka*, *Typha domingensis* and *Panicum turgidum* grows in saline coastal areas of Pakistan are good candidate plants for bioethanol production. These

Feedstock	Conditions	Biofuel production (l/ha)	References
Corn	Hydrolysis/fermentation	3,800	[12]
Sugarcane	Fermentation	7,200	
Sugar beet	Hydrolysis/fermentation	7,900	
Wheat	Hydrolysis/fermentation	1700	
Cassava	Hydrolysis/fermentation	137	

**Table 1.**  
*Conditions and comparative efficiency of different feedstocks for biofuel production.*

halophytes contain a good amount of cellulose, hemicellulose, and lignin (26–37%, 24–38%, and < 10% respectively) and have a better growth rate [15]. Miscanthus a potential candidate crop for the production of bioethanol has gathered much attention because of its elemental composition, lignin and polysaccharide content, and final biomass yield. The most important property of the miscanthus is the production of the desired chemical component by thermochemical conversion because of the low ash and moisture content. These properties explored the ability and potential of this plant as a good feedstock for ethanol production in the future. Hence, the production of ethanol from edible sources faces criticism and is not economical which demands exploring non-edible plant species having the ability to grow on the marginal soils thus not interfering with the cultivation of food crops. This necessitates the utilization of saline soils to produce non-food lignocellulosic biomass which is a valuable source of bioethanol without competing for human food production [16, 17].

Another biofuel type is biodiesel that can be mixed with fossil fuels or is used directly in the engines with certain modifications [18]. The maximum oil content was determined in canola crops. Similarly, flax and camelina also appeared to be a promising source of biofuel (biodiesel) in terms of alternate energy crops. In terms of land use and competition with food crops, Camelina sativa proved to be more promising. This crop has fewer problems in comparison with Glycine max and canola and has high land-use efficiency. Further, it can be successfully grown in rotation with wheat or other winter cereals. LCA (life cycle analysis) of *C. sativa* proved that it can reduce CO<sub>2</sub> emission and can provide biofuel resulting in reduced consumption of fossil fuel [19]. Another valuable aspect of bioethanol is that it is an environmentally friendly fuel source compared with fossil fuels. Hence, the emission of greenhouse gases could be minimized by using it as a fuel source in various daily life necessities including cooking, heating, water pumping, and generation of electricity [20]. Biodiesel mixed with fossil fuel can be used in diesel engines and it does not require any kind of change in chemistry. The biodiesel produced from *B. carinata* showed prominent results and proved to be the potential crop for the production of biofuels especially in the areas where other crops are unable to provide good yield due to the adverse and variable climatic conditions [21]. *Jatropha curcas* L. also can be grown on marginal lands and can yield an oil that has been proposed to be good in performance as a biofuel. Biodiesel produced from this crop proved to be eco-friendly, biodegradable, and less toxic properties as compared with fossil fuels. The literature review highlights its contribution to therapeutic and medicinal properties as well, also, to be the source of biofuel [22]. Two wild species of perennial trees grown in the amazon were analyzed and their seed oil was examined i.e. *Carapa guianensis* and *Terminalia catappa*. Oils of the above-mentioned species were used to convert into biofuels, the resultant produced biodiesel proved to be good and comparable with the fossil fuels in terms of chemical properties and the



biodiesels were acceptable for use. This showed that these species have the potential to provide oil for biodiesel production [23]. *Calophyllum inophyllum* can be the best alternative crop for the provision of oil feedstock for the production of biodiesel. Another advantage of this plant was that it can be grown in coastal regions so does not demand any sort of sacrifice in the form of food crops. The physical and chemical properties concluded that this tree has the potential to be a sustainable source of feedstock for biodiesel production. Hence, Calophyllum oil can replace palm oil to produce biodiesel. Moreover, owing to the status of a non-conventional crop it requires further research to maximize benefits from it [24]. Switchgrass is another potential crop for the production of biofuel and has gathered much interest due to its better adaptability and good performance in the field. Anyhow it needs proper attention for the economic production of biofuel from this grass [25].

3. Different generations of biofuel production (biofuels: from ethanol to biodiesel)

Biofuels are classified into different generations or groups based on their method of production and raw material used (Figure 1). In first-generation biofuels, cultivated crops providing polysaccharides and starch are the main raw material used in the production [26] but this generation is not much appreciated due to various reasons such as the increased amount of inputs use, cultivated crops used for biofuels are decreasing the availability of the food and cultivable land for food production. The first-generation biofuel is produced from sugars or starch. Globally, sugarcane contributes 21 million m<sup>3</sup> of ethanol whereas 60 million m<sup>3</sup> comes from corn and grains. The important step is liquefaction of sugar residues followed by hydrolysis that release sugar-monomers which are converted into CO<sub>2</sub> and ethanol by yeast fermentation. Ethanol yield can be increased by augmenting the sugar contents of sugarcane. It is very difficult to enhance sugar production as sugarcane owns one of

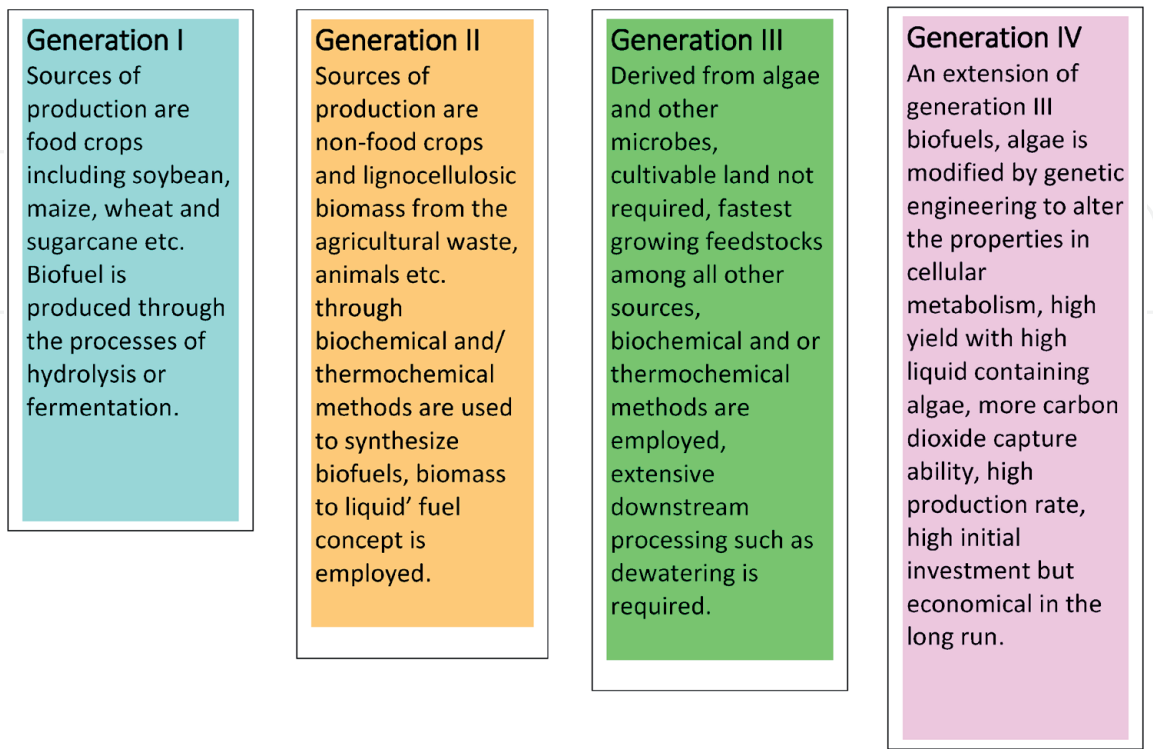


Figure 1. Comparison among different generations of biofuel production based on their method of production and raw material used.

the most complex genomes with extremely complicated genetic networks and pathways. Moreover, modification in one process can decline the expression profiles of the other desired traits resulting in an unsolicited tradeoff. First-generation biofuels are criticized for food security as there are increasing concerns that the diversion of sugarcane towards ethanol production will reduce sugar availability, which could cause a consequent rise in prices. So, second-generation biofuels are now being used to generate energy so that co-generation would help to minimize competition with sucrose supplies. Second-generation biofuels are produced from different lignocellulose (substrate). Sugarcane bagasse and leaves are used as a by-product to produce ethanol. Various studies showed that assimilating either biochemical or thermochemical routes significantly enhances ethanol production, as compared with first-generation ethanol production. A Norwegian company (Borregaard) is the largest producer of second-generation bioethanol all over the World, with an annual production of 20,000 m<sup>3</sup>. Milling, pretreatment (thermophysical), fermentation, distillation, and processing are the fundamental steps to produce bioethanol from lignocelluloses hence, needs due consideration to improve its production [27]. In comparison to the first generation, second-generation biofuels are cost-effective as they utilize mostly those parts of the plants that are not used as food or are considered waste. Such as crop waste or municipal wastes [28] but still there is a need to produce these biofuels at a consumer's acceptable price. Many researchers are aiming to increase the production efficiency of this type of biofuels produced but chemical or enzymatic methods are found to be more efficient. In third-generation biofuels algae is the main substrate while in fourth-generation biofuels microorganisms and genetically engineered crops and algae are used as raw material. Biofuels produced from algae are less stable and less cost-efficient. While fourth-generation biofuels are under development [29].

#### **4. Sugarcane a bioenergy source: an overview**

Energy cane, unlike conventional sugarcane, contains more fiber content than sucrose. Alexander [30] coined the term “Energy Cane Management” and proposed that sugarcane being a high biomass producer is a potential candidate for the production of bioethanol. The high biomass production is a valuable biological factor of sugarcane that contributes to the high positive LCEB (life cycle energy balance) of bioethanol produced from it with a positive balance of GHGE (greenhouse gas emission). Sugarcane biofuel generates low net greenhouse gases (GHG) and hence reduced adverse environmental impact in terms of pollution indicators. In Brazil, net greenhouse gases emission was estimated to be reduced by 25.8 million tons CO<sub>2</sub> equivalent in 2007. This was only because of the replacement of non-renewable energy sources with renewable energy sources [31].

Sugarcane is the most economical source of bioethanol with 9.8% fermentable sugars in its juice whereas sweet sorghum has 11.8% fermentable sugars. Sugarcane bagasse contains 22% lignin, 24% hemicellulose, and 43% cellulose whereas sweet sorghum has 21% lignin, 27% hemicellulose, and 45% cellulose with theoretical ethanol production of 12,938 and 5,804 kg per ha respectively. Though sugarcane has higher productivity as compared with its counterparts (sweet sorghum, sugar beet) yet further improvement in its fiber and sugar contents is desired to transform it into energy cane [32].

The sucrose contents present in sugarcane is about 50% of the culm dry mass whereas fiber contents are about 14%. Besides, the following features make sugarcane an ideal choice as an energy crop: drought tolerance, cold tolerance, pest and disease resistance, less flower production, erect growth habit, ratooning ability,

and fast early growth [33]. Brazil is a pioneer country in launching sugarcane as an energy crop and produced about 23.4 billion liters of bioethanol in 2014 and fulfilled 15% of the country's electricity needs from sugarcane. Brazil is expecting to meet 30% (equivalent to hydropower) of energy needs from sugarcane during the current year. Thus biofuels are outcompeting the fuel market owing to their potential to fulfill future energy needs with the ability to contribute towards a safer climate. It is mandatory now in European Union and the United States to use biofuel as a fuel source. It was obligatory in the EU, to use at least 10% of the transport fuel from renewable sources by 2020. They have planned to produce 36 billion gallons of biofuel by 2022 compared with 4.7 billion gallons in 2007. Though the dominant source of production will be non-corn starch feedstock [34]. Considering this scenario, bioenergy, and energy cane seem to be the most desirable source of bioethanol all over the world.

## **5. Challenges in sugarcane biofuel production**

Since the last two decades, biofuels have proved their worth by reducing greenhouse gases and contributing to energy self-sufficiency along with increased agricultural yield. Developed countries have enthusiastically introduced subsidies for rapid adoption of this technology but the net gains have been unsatisfactory because of rising prices of fossil fuels, high agricultural inputs, processing, transport, etc., and food crisis lead to controversy about biofuels. Researchers are of the view that competition for the production of biofuel will not only result in competition with food crops but will also lead to additional pressure for land, water, fertilizer, and other natural resources to produce feedstock. This demands reclamation of the salt-affected/waterlogged soils for the cultivation of energy crops. This will make possible the cultivation of uncultivable land resulting in enhanced production of feedstock. Further, the cultivation of neglected non-food plant species will result in their conservation thus securing biodiversity, etc. This also demands the development of developing new accession of different plant species, suitable for energy production having wide-spread adaptability to produce more biomass with least nutrients [35].

Fermentation is the most established technology for the production of biofuel from sugarcane and other feed/food crops, all over the world. In our country too, ethanol is produced from sugarcane molasses, etc. The average ethanol production is one-liter ethanol from 12 to 14 kg of sugarcane [36]. Also, bagasse is a prominent source of heat and energy. Sugarcane production requires significant investment in the form of energy and inputs including farm machinery, labor, fertilizers, and pesticides. The development of input efficient varieties with better ability to produce energy and sucrose are core challenges for the transformation of conventional sugarcane into energy cane.

## **6. Genome engineering in sugarcane for improvement in biofuel production**

Since the advent of molecular biology, researchers are striving hard to help mankind by fulfilling their needs and uplifting living standards. Engineering plant genome for tailoring targeted cellular mechanisms and to express desired recombinant protein has proved its worth and more than 190 million hectares of agricultural land is occupied by transgenic crops. The concept of producing industrial

Sr. #	Name of the gene	Putative role of the gene	Possible outcome/ role	References
1.	<i>Sucrose isomerase</i>	Production of sucrose isomer isomaltulose	Increased sugar accumulation	[32]
2.	<i>CslF</i>	catalyze MLG biosynthesis	Cell wall biosynthesis	[49]
3.	<i>COMT</i>	Lignin reduction	Biofuel production	[50]
4.	<i>Phenoloxidase laccase</i>	Laccase enzyme activation	Detoxification of sugarcane bagasse increased second-generation biofuel	[51]
5.	<i>PvCCR1</i>	Lignin biosynthesis	Increases lignocellulosic material	[52]
6.	<i>COMT</i>	Methylation of 5- hydroxconiferyldehyde	Increases lignocellulosic material	[52]
7.	<i>OMT</i>	Pathway fluxes indicate the manipulation of the expression of a gene	Lignin modification	[53]
8.	<i>CAD, COMT</i>	LIGNIN S/G ratio	Increased biofuel production	[53]
9.	<i>C4H</i>	encode monolignol biosynthesis enzymes	Lignin biosynthesis	[54]
10.	<i>PAL</i>	Encode phenylalanine ammonia-lyase	Lignin biosynthesis	[55]
11.	<i>HCT</i>	Encodes shikimate hydroxycinnamoyl transferase	Lignin biosynthesis	[56]
12.	<i>Sh4CL1</i>	lignin biosynthesis	Lignin reduction upto 16.5%	[57]
13.	<i>CesA, CSL</i>	Lignin biosynthetic pathways	Second-generation biofuel production	[57]
14.	<i>CCoAOMT</i>	Synthesis of G and S monomer of lignin	Lignin biosynthesis	[58]
15.	<i>F5H</i>	production of the lignin S monomer	Lignin biosynthesis	[59]
16.	<i>CAD</i>	monolignol biosynthetic pathway control	Lignin biosynthesis	[59]
17.	<i>nptII</i>	Control transcriptional control of ubiquitin promoter	Increases Biomass production	[60]
18.	<i>CesA</i>	cellulose biosynthesis	Increases cellulose synthesis	[61]
19.	<i>Csl</i>	Biomass synthesis	Increase biomass synthesis	[61]
20.	<i>SuSy</i>	Cellulose synthesis	Increased cellulosic contents by 2–6%	[61]
21.	<i>CslH</i>	catalyze MLG biosynthesis	Cell wall biosynthesis	[62]
22.	<i>Sh4CL1</i>	Lignin production	Increases lignin biosynthesis	[63]



Sr. #	Name of the gene	Putative role of the gene	Possible outcome/ role	References
23.	COMT	Lignin biosynthesis	Second generation biofuel production	[64]
24.	CYSOLE1, WRI1	Supresses spd1	Increased biofuel	[64]
25.	JU A10 T	Lc biomass	Cellulase production	[65]
26.	E2 ADHE, EUTE	Liginin biosynthetic pathways	Ethanol production	[66]

**Table 2.**  
*Potential candidate genes that can be engineered to enhance biofuel production.*

enzymes, therapeutics, and nutraceuticals in plants further validated the worth of plants being transformed into bio-factories. Likewise, sugarcane is an ideal candidate for the expression of desired recombinant proteins and to engineer endogenous cellular mechanisms for enhanced production of sucrose and bioethanol [8].

After addressing recalcitrance in this complex grass [37–39], efforts have been made to engineer its nuclear [40] as well as plastid genome [41] for the expression of valuable proteins in this C<sub>4</sub> plant. The first transgenic sugarcane was developed by Bower and Birch in 1992 [42]. Thereafter, efforts were made for herbicide tolerance [43] flowering inhibition [44], disease or pest resistance [45], drought tolerance [46], and for the expression of cellulosic enzymes in its leaves [47]. Some new genes for cold and drought tolerance are being identified in other genus and species like *S. spontaneum*, *Miscanthus*, and sorghum. Integration of these genes in sugarcane will help to generate more biomass in temperate areas or under dry conditions [48, 49]. These engineered sugarcane genotypes will provide better germplasm for the development of future energy cane for biofuel production.

Manipulating growth hormones and biomass synthesis pathways (Table 2) may play a vital role in plant cellulose content and total biomass leading to the development of energy cane [67]. Initial hydrolysis of cell wall polysaccharides may be increased up to 46% by reducing the cross-links of the cell wall in maize [68]. Lignin contents are the main hurdle in saccharification during conversion to ethanol and it accounts for 25% of sugarcane total lignocellulosic biomass [69]. For the saccharification process, lignin contents are needed to be removed as they prevent cellulase from accessing the cellulose molecules [67]. Almost 10 different enzymes are involved in the lignin biosynthesis pathway in sugarcane making it more complicated to engineer [70]. Some genes targeting enzymes (involved in the lignin biosynthesis pathway) like COMT (caffeic acid O-methyltransferase) and CAD (cinnamyl alcohol dehydrogenase) can be downregulated to alter its composition for biofuel production [71]. Biomass recalcitrance can be increased not only by lignin contents but also by lignin S/G ratio [72]. RNAi (RNA interference) suppression was used to downregulate the COMT gene by 67–97% to reduce lignin content and lignin S/G ratio by 3.9–13.7% and 1.47 to 1.27–0.79, respectively [50]. These findings suggested that RNAi-mediated gene suppression is a promising tool for the suppression of target genes not only involved in the lignin pathway but also the cell wall biosynthesis [73].

Biofuel production cost can be decreased by *in planta* enzyme production, as it reduces the expense of enzymes and enzyme treatment. Cellulase has been successfully produced in plants (i.e. *Arabidopsis*, rice, and maize) without affecting their growth and other developmental pathways [67]. However, in sugarcane *in planta* expression of enzymes is at infancy owing to its complex genome and recalcitrance. Compartmentalization of the recombinant enzymes (expression in the vacuole, chloroplast, and endoplasmic reticulum may further promote this concept, as

endogenous cellular mechanisms will be least affected [47]. *Agrobacterium* and biolistic mediated transformations are well-established in sugarcane so the expression of above said enzymes in leaves and other tissues is doable [74]. Three cellulolytic enzymes (cellobiohydrolase I, cellobiohydrolase II and bacterial endoglucanase) were expressed in the leaves under maize PepC promoter and with various subcellular targeting signals to assess the feasibility of accumulation of these enzymes in the vacuole, endoplasmic reticulum, and chloroplast. Expression of cellobiohydrolase (CBHs) was maximum in the vacuoles whereas expression of endoglucanase was maximum in the chloroplasts. Hence, these studies proved that the sugarcane genome may be targeted for the expression of cellulolytic enzymes leading to the economical production of bioethanol [47].

## **7. Role of omics in the development of future energy cane**

Omics is genuinely an innovative area of research in the field of genomics, transcriptomics, proteomics, metabolomics, and their applications for the improvement of sugarcane to energy cane. Understanding the genetic regulation and mechanisms involved in photosynthesis, nutrient assimilation, disease resistance, sucrose transportation. Advancement in genome mapping, DNA microarray, expression profiling, RNAi (RNA interference), and data mining tools can play a central role in the development of future energy cane. Interventions in next-generation sequencing (NGS) has not only reduced the cost of whole-genome sequencing but have made it possible to sequence complex genomes like sugarcane [75]. It is of particular importance for the aneuploid, polyploid genome of sugarcane for the identification of various alleles of the same gene. This has also helped to devise molecular markers paving the way to tackle bottlenecks in sugarcane breeding. Numerous transcript sequence clusters of sugarcane do not contain information of full-length coding sequences, NGS is expected to resolve the issue by making it possible to get information of complete gene sequences [76].

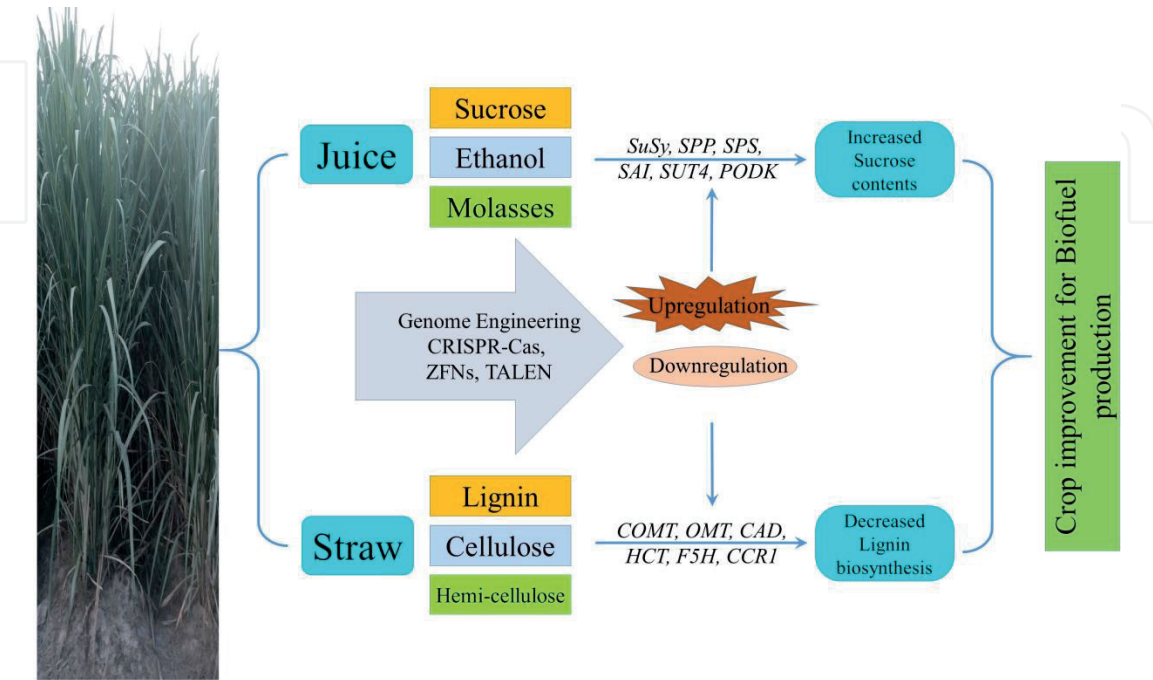
With the advent of recent techniques, conventional breeding can now be integrated with genomic tools to harvest maximum advantage of these innovations. The earlier genomic research led to the development of molecular markers, elucidated genome structure of modern genotypes, and phylogenetic relationship among the complex *Saccharum* species. EST-SSRs have been successfully utilized to understand genetic relationships and genetic diversity. Genome mapping research has helped to determine marker-trait associations and to validate chromosomal localization of valuable genes [77]. The development of new markers and their incorporation in genetic maps will accelerate breeding programs leading to the development of an approved version of sugarcane. Understanding complex connections among genetics, genes, proteome, and metabolome requires integrated research on omics, bioinformatics, and computational biology. A great many sugarcane genes involved in molecular mechanisms of stress (cold, drought, and salinity stress), plant growth, and development have been explored [78]. During the recent decade, transcriptomic research has led to the identification of more than 33000 genes, involved in critical biological functions in this energy crop [79].

Plant genomic databases are valuable resources to mine candidate genes for the improvement of crop plants through molecular breeding. Many databases have been developed for various plant species. These include Gramene [80] TropGENE [81], Plant GDB [82], GRASSIUS [83], Phytozome [84], MOROKOSHI [85], Plant TF database [86], PLAZA [87] and KBase [88]. SUCESTFUN is a specific platform for sugarcane/energy cane breeders (<http://sucest-fun.org/>) [89] and was developed keeping in view the five major objectives: gene annotation, expression profiling,

genome sequencing, functional genomics, and integration of public resources. The database was initially based on 43,141 Sugarcane Assembled Sequences (SAS) from the SUCEST Project [90] followed by the generation of 17,500 ORFeome genes using RNA-seq from the hybrid and ancestral varieties of sugarcane [91]. The retrieved data was of great value for single nucleotide polymorphism analysis, protein characterization, identification of splicing variants, and evolutionary studies.

The first transcriptomic analysis was performed to investigate differences between immature and mature leaves and internodes, [92] followed by the genes involved in sucrose transportation, source-sink interactions, tissue profiling of transcriptionally active transposable elements, transcription elements, stress-responsive factors, and resistance gene analogs [93]. Numerous differentially expressed transcripts involved in photosynthesis, assimilate partitioning, cell wall synthesis, phosphate metabolism, and stress were identified through an oligonucleotide array [94]. The advent of micro-arrays appeared a great milestone in expression profiling where GeneChip<sup>®</sup> Sugar Cane Genome Array produced by Affymetrix assaying up to 4715 non-redundant random ESTs.

The available sugarcane genomic resources can be employed to identify genes involved in sugarcane cell wall (SCW) biosynthesis. NAC and MYB transcription factors and gene regulatory network (GRN) involved in SCW biosynthesis. The genes identified through genomic and transcriptomic approaches could either be used as DNA markers or develop value-added transgenic sugarcane [95]. Proteomics approaches have explored the role of DEPs (differentially expressed proteins) in signal transduction pathways for stress tolerance by proteomic approaches i.e. 2D-DIGE (two-dimensional difference gel electrophoresis) [96] and iTRAQ (isobaric tags for relative and absolute quantitation) [97]. More recently, metabolite analysis provides a deeper understanding of the complex regulatory processes of potential metabolites including saccharides and other derivatives helping out to predict resistance mechanisms through the use of high-throughput technologies that can determine metabolic phenotypes [98, 36]. Hence, these advancements can play a crucial role in the development of future energy cane (**Figure 2**).



**Figure 2.**  
Schematic sketch showing different research strategies to uplift biofuel production from sugarcane.

## 8. Conclusions

Fuel energy is an inevitable necessity of life and is anticipated to gain key standing in the world's economy. Exhausting reserves and the ruthless burning of fossil fuels have forced researchers to explore alternative energy resources for the biosecurity of living beings. Biofuels are potential candidates that provide an eco-friendly and sustainable energy source to meet the energy demand of the whole world. The major contributors to global biofuel are maize and sugarcane in addition to sorghum and other grasses. Research efforts are direly needed, not only to increase biomass production but also the betterment of industrial processes involved in the production of biofuel including biodiesel. Advancements in omics and other innovative disciplines have opened new horizons paving the way to develop future energy crops resulting in the replacement of fossil energy with renewable energy.

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