

ADVANCED TRANSGENIC STRATEGIES TRIGGERING ENHANCEMENTS OF SUGAR PRODUCTION IN COMMERCIAL SUGARCANE CROP

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Abstract

Despite the water-demanding crop, sugarcane is economically significant crop in Asia for sugar production. In recent decades, due to extreme climate changes, dry and warm weather conditions, sugarcane production is highly affected leading to drought stress which not only directly affects the sugarcane production, but also affects its sugar contents. Several stress-responsive genes were shown to have various gene expressions under drought stress. Specifically, ScDIR5, ScDIR7, ScDIR11 and ScDIR40 were found with differential expression levels. In this review, we have discussed the water deficit impact on sugarcane at the cellular and molecular levels. Low water availability affected the morphology as well as the water content of plants and resulted in the excessive accumulation of reactive oxidative species (ROS). Sugarcane plants respond to drought by activating various genes and signaling pathways. But to survive severe water stress and produce better-yielding sugarcane crops, transgenic sugarcanes with effective drought tolerance are introduced. As well as, to increase the sucrose sugar content, scientists are working for years to enhance its percentage, so that even under the biotic or abiotic stress factors, it continues to produce the maximum sucrose amount. Many countries especially Brazil is using genetically modified sugarcane for sugar production on commercial scales. Modern genetic approaches and gene manipulation strategies including the conventional breeding, genomic selection, gene transformation, gene silencing and CRISPR have been used to raise the yield of sucrose accumulation which are discussed here with future prospects.

Keywords: *Saccharum Officinarum*; RNAi; Transgenic Sugarcane; Sucrose Enhancement.

1. INTRODUCTION

Saccharum officinarum with a common name of sugarcane is a perennial grass belonging to the Poaceae family. Sugarcane is a center of attention for researchers due to the production of biofuels, food products especially sugar, and chemical compounds for the cosmetic and food industry (Wei et al., 2017) as well as due to the recent advancements in the analysis of sugarcane juice which revealed the potential of nutrients and electrolytes on public health (Abbas et al., 2014). Known for the long duration, high water and high nutrient demanding crop, it requires about 12-18 months to mature before harvesting. Despite the given fact, it is an economically highly productive crop with a tremendous capacity for sucrose accumulation. The sugar producing industry is the largest agro-processing industry next to the cotton textile industry and fulfilling about 70% of the sugar need of the world (G S et al., 2017).

The increasing demand for sugarcane for its products due to the rapidly growing population has given rise to two hot topics in its research field. One is the environmental stresses which affect sugarcane production and its yield. Abiotic stresses include drought, flooding, high salinity, cold and high temperatures. In this review, we discussed particularly drought's effects on sugarcane growth, its yield and sucrose content. Researchers are focused to introduce more techniques for transforming sugarcane plants to drought tolerant so that the yield can not only be maintained but increased. The second area of research about which the scientists are concerned is, how the sugar content that is affected by the low water deficit conditions, can be enhanced by genetic transformation and modern genetic approaches. However, a lot of research is being carried on to enhance the sucrose content of sugarcane for economic purposes. With the increasing demand for sugarcane, biotechnologists are now paving the ways to produce cultivars with higher sugar content to compensate the quantity required.

Conventional breeding has provided varieties of sugarcane but it has been proven to be the most time taking method of producing a desired trait and also the results were not much accurate (Yadav et al., 2020) while gene transformation has brought about the remarkable success in acquiring the traits by gene transfer methods. The transgenes of various enzymes involved in the metabolic pathways from other sources have increased sucrose content to some extent. But the complexity and polyploidy nature of sugarcane as well as the biotic and abiotic stress factors have hampered most of the research work. Now-a-days the transfer of sucrose isomerase gene from bacterial strains is known to have promising outcomes by producing the high value sugar i.e. isomaltulose or trehalulose stored in the tissues without decreasing the content of sucrose, thus doubling sugar quantity (Wu & Birch, 2007).

These findings suggest that biotechnological interventions are paving the new pathways to get economic gains from this crop. But enough research is still required regarding the complete understanding of the complex genome of sugarcane.

2. COMPLEX POLYPLOID AND ANEUPLOID GENOME OF SUGARCANE

The extreme complexity of the genome of sugarcane led to less scientific research in this area. The reason for this complexity is a high level of polyploidy and the heterozygous genome. The modern sugarcane is the hybrid of two species i.e. genetically modified female thick-stalked, high sugar *Saccharum officinarum* and wild male thin-stalked, low sugar *Saccharum spontaneum* (Thirugnanasambandam et al., 2018). The size of the sugarcane genome is 10GB, the homologous genes range from 8-12 copies, and the monoploid genome size is 750-930 MB.

The genomic tools and the advancement in the next generation sequencing methods are now unveiling the complexity of the genome. *Saccharum officinarum* is octaploid specie with a basic chromosome number of 10 while *Saccharum spontaneum* is monoploid specie with a basic chromosome number of 8. The hybrid of the sugarcane contains chromosomes that are unevenly distributed from their progenitors. In some studies, it was shown that about 80% of the chromosomes were contributed by *Saccharum officinarum* while 10-20% of chromosomes were contributed by *Saccharum spontaneum*. Furthermore, 5-17% has resulted from the high levels of recombination. Polyploidy nature increases the repeats which leads to more irregularity (Zhu et al., 2020). Phenotypically, wild-type *Saccharum spontaneum* contributes to the fiber content, hardness and complementary characteristics of the hybrid (Thirugnanasambandam et al., 2018).

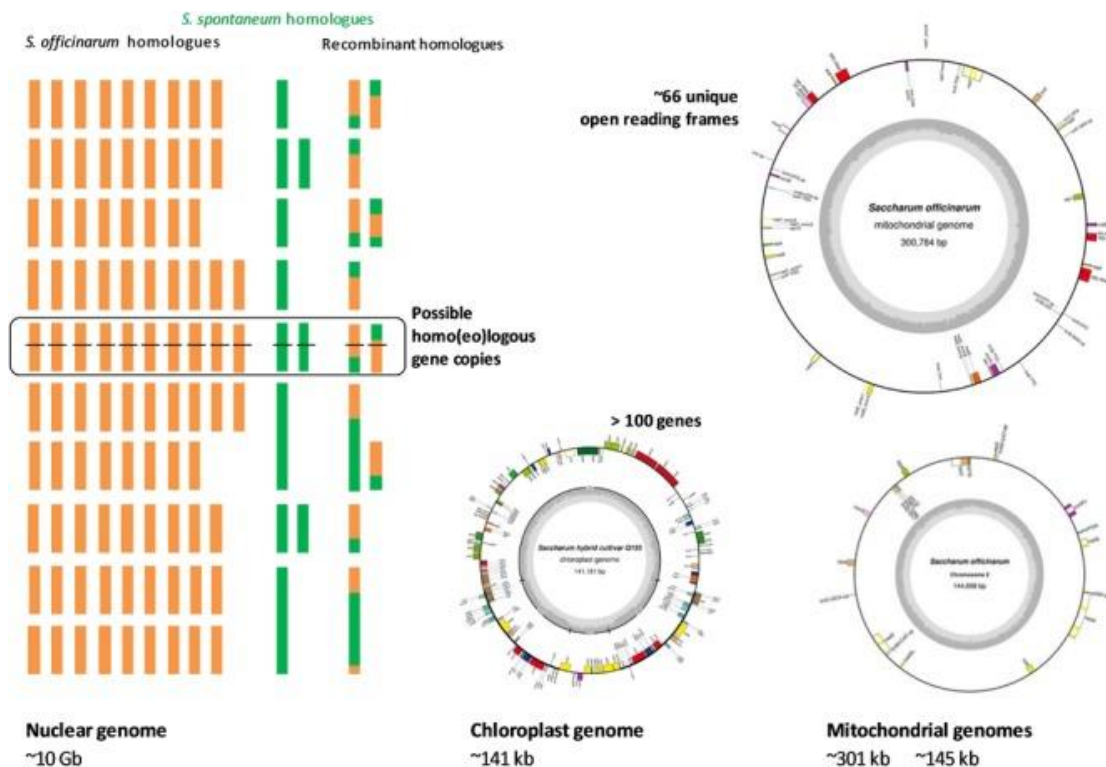


Figure 1: Sub-genome of sugarcane hybrid (Thirugnanasambandam et al., 2018)

The modern sugarcane hybrids are produced by the crossing of different species which fall under the *Saccharum* genus and it comprises six species including *S.spontaneum*, *S.robustum*, *S.barberi*, *S.sinense*, *S.officinatum* and *S.edule*. *Saccharum* belongs to the PACMAD clade in which other different other genera are included such as *Miscanthus*, *Erianthus*, *Zea*, *Sorghum* and *Setaria* (Thirugnanasambandam et al., 2018).

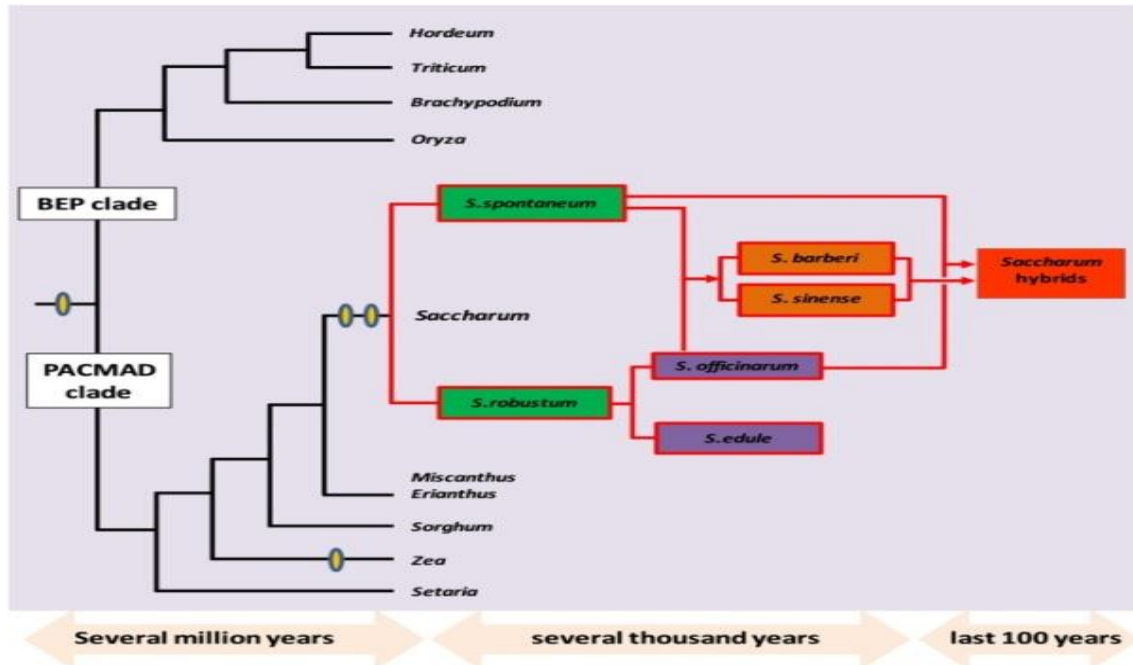


Figure 2: Phylogenetic tree of *Saccharum officinarum* (Thirugnanasambandam et al., 2018)

3. DROUGHT EFFECTS ON THE SUGARCANE PRODUCTION

This review is majorly concerned with the effects of drought on sugarcane which causes the gradient difference between the ions and water, therefore reduces the water flow to plants (Mahajan & Tuteja, 2005). According to (Ferreira et al., 2017), the total loss caused by low water availability can reduce the production of sugarcane up to 60%. Currently, Brazil is a lot more focused on the production of sugarcane with modified traits of tolerating abiotic stresses, specifically water deficit for the dry mid-east Brazilian regions (Ramiro et al., 2016). Under low water availability, plant cells generate reactive oxygen species (ROS) (Cruz de Carvalho, 2008). These reactive oxygen species (ROS) initiate the defense mechanism to adapt the plant to a fluctuating environment. However, under severe water deficit, elevated levels of reactive oxygen species (ROS) disrupt the biochemistry of DNA, proteins, lipids and other bio-molecules of plants (Dat et al., 2000; Ramiro et al., 2016).

The structural losses during drought are more severe than during the waterlogging stress. For instance, the cane height, stalk diameter, tillering, root weight, number of internodes

were decreased in drought condition (Misra et al., 2020). The appearance of plants also changed in terms of impaired growth, wilting and yellowing of plants. Furthermore, the affected growth rate leads to the reduction of the sucrose content of sugarcane during the early periods (Reyes et al., 2021). (Million et al., 2018) observed that the plant height plays important role in determining the yield of sugarcane. The recorded plant height under water deficit was about 18.25% lesser than the plants grown under normal conditions (Misra et al., 2020). It was analyzed through carrying out experimentation that the cane yield is correlated with plant height, and sucrose content is directly related to cane yield (Q. Ali et al., 2014; Million et al., 2018). (Inman-Bamber, 2004) investigated that sucrose content was actually enhanced under average water stress at maturation stage as the more assimilated carbon dioxide is converted to sugars. However, severe water stress has negative effects on both cane and sucrose yield (Ferreira et al., 2017). Tillers are known to serve as plant's sugar sink (Ramesh & Mahadevaswamy, 2000). Drought negatively affects the number of tillers and ultimately the yield and sucrose content. It is reported that the reduction in tillering can cause up to 49% loss of cane yield (C. Mahadevaiah et al., 2021). In a study, it was reported that the levels of glucose and fructose under drought stress were almost similar as under normal conditions, whereas the levels of sucrose levels in leaves were greatly reduced on dry weight basis under water deficit (Iskandar et al., 2011).

Moreover, under water deficit stress, there will be limited nutrients available to plants due to disrupted transportation of ions (Reyes et al., 2021). Potassium is one essential element required for tolerance against drought. Potassium is considered to be involved in the higher cane yield and thus higher sucrose accumulation (Samuels & Landrau, 1954). Also phosphorous level is considered to be an important factor in maintaining plant's turgor pressure and the diffusion of gases, known as stomatal conductance, low levels of phosphorous retard the growth and photosynthesis of plants (Reyes et al., 2021).

4. Drought Stress Response Mechanisms At Molecular Level

Plants have mechanism to adapt themselves for some degrees under abiotic stresses . It was analyzed that in case of sugarcane under drought condition, the plant has two mechanisms. One is to avoid the dehydration by maintaining maximum water content through reduced transpiration and stomatal conductance rate (Blum, 2005; Ferreira et al., 2017). The other is to create tolerance against stresses to adapt according to the environmental changes. The exact mechanism of sugarcane stress response to water deficit is complex and still not fully understood because of the different gene expression pattern of various genotypes studied (Andrade et al., 2015; Ferreira et al., 2017). Abscisic acid (ABA), however, as the main component involved in the stress perception and signal transduction pathways, has role in the stomatal closure under extreme water stress as well as in the gene expression of proteins and enzymes to counter act the oxidative stress (Lim et al., 2015). Figure 3 represents different mechanisms by which sugarcane respond to abiotic stress, water deficit in this case.

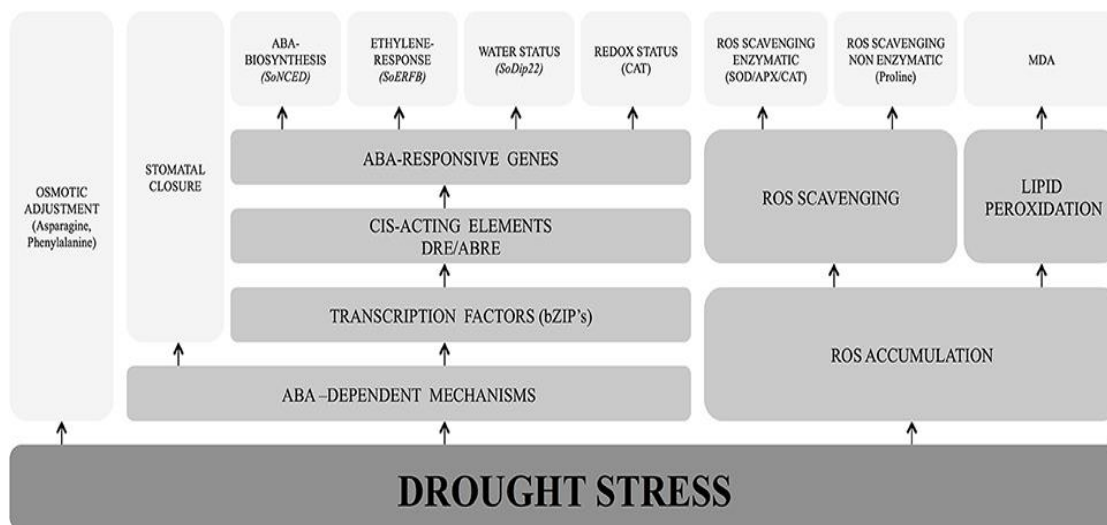


Figure 3: Sugarcane response to water stress at various levels (Ferreira et al., 2017)

Expressions of 51 stress-related genes of sugarcane linked with sucrose accumulation were investigated, which showed a different expression pattern under drought condition as compared to those associated with sucrose accumulation alone (Iskandar et al., 2011). A research conducted by Li X. and his coworkers reported 64 ScDIR genes involved, and their gene expression was measured. But the four genes (ScDIR5, ScDIR7, ScDIR11, and ScDIR40) showed notable drought tolerance response. ScDIR7 from the subfamily ScDIR-c exhibited highest transcriptional levels (Li et al., 2022).

Table 1: Meta-analysis of Drought Responsive Genes of Sugarcane

Gene	Protein	Function	Reference
ScCAT1	Catalase	Protect against oxidative stress	(Ferreira et al., 2017)
SoNCED	9-cis-epoxycarotenoid dioxygenase	Involved in ABA biosynthesis	(Ferreira et al., 2017)
SoDip22	Sucrose phosphate synthase	Regulation of water	(Ferreira et al., 2017)
ScDIR5, ScDIR7, ScDIR11	Dirigent protein	Lignin biogenesis, stress resistance (ScDIR7 with highest tolerance)	(Li et al., 2022)
ScDIR40	Dirigent protein	Lignin biogenesis, stress resistance	(Li et al., 2022)
G6PDH	Glucose-6-phosphate dehydrogenase	Oxidative pentose pathway, tolerance to abiotic stresses and pathogenesis	(Yang et al., 2014)
ScDREB2B-1	Dehydration responsive element binding protein	Drought tolerance by activating ABA pathway, response against higher oxidative stress, activation of enzymes (superoxide dimutase, peroxidase, catalase)	(Chen et al., 2022)

SoSnRK2.1	Sucrose non-fermenting1-related protein kinase 2 (SnRK2)	Role in ABA transduction pathway, response to drought and ionic stress	(Phan et al., 2016)
SoP5CS	Pyroline-5-carboxylate synthase	High expression under stress, tolerance response to drought, salinity and cold temperatures.	(Li et al., 2018)
ScMT2-1-3	Metallothioneins (metal binding proteins)	Role in detoxification and metal ion homeostasis	(Guo et al., 2013)

5. BIOTECHNOLOGICAL INTERVENTIONS FOR GROWING DROUGHT TOLERANT SUGARCANE

In order to maintain high sucrose levels of sugarcane under water deficit conditions, the plant cells must be able to tolerate high solute concentrations (Iskandar et al., 2011). For that purpose, transgenes from various other plant species have been identified by researchers to induce abiotic stress tolerance in sugarcane. This allows investigating more thoroughly the functions of targeted genes and related proteins (Ferreira et al., 2017). Overexpression of DREB2A CA transcription factor of *Arabidopsis thaliana* induced drought resistance in sugarcane and the relative water content along with sucrose concentration was increased as reported by (Reis et al., 2014). Also, Trehalose synthase enzyme is known to provide protection to the protein structure as well as to abiotic stress – drought/water deficit. Reported by (Zhang et al., 2006), expression of trehalose synthase gene from *Grifola frondosa* exhibited increased tolerance to drought by accumulating relatively high trehalose levels of about 8.805-12.863 mg/g as compared to normal non-transgenic sugarcane in water stress. Sugarcane gene SoP5CS is responsible for the synthesis of bifunctional pyroline-5-carboxylate synthase enzyme, involved in the biosynthesis of pyroline as well as in response against pathogen infections and drought stress (Qamar et al., 2015). (Li et al., 2018) in the research, isolated SoP5CS gene and overexpressed it in the sugarcane lines which showed higher RWC, ABA and pyroline synthesis. These transgenic sugarcane plants expressed enhanced resistance to infection and drought. (Mall et al., 2022) identified tomato ethylene responsive-factor 1, AVP1 genes which induced protective response to environmental abiotic stresses. Many other genes are known to create tolerance against drought and other stresses. Currently, researchers are paving even new methods and techniques to further identify the genes and factors involved in response to abiotic stresses (Ramzan et al., 2016) including drought so that the sugar content could also be enhanced and not affected by low water availability.

6. ENHANCING DROUGHT AFFECTED SUCROSE CONTENT VIA GENETIC APPROACHES

6.1.1 Plateauing Genetic Gain by Conventional Breeding for Sucrose Enhancement

Increasing demand as well as the abiotic/biotic stresses has challenged the new research for the producing high yield crops with better tolerance against diseases and environmental stresses. (A. Ali et al., 2014; Muzaffar et al., 2015). Sugarcane breeding began in the late 1800s as a prevention response to the sereh disease. (Snyman, 2004). Breeding process begins with the selection of parent plants and allows them to produce flowers for crossing under proper conditions. Parent variety selection is based on the high productivity, high sucrose content and resistance to different viral, bacterial and fungal diseases. More than 60 varieties are gained from the breeding programs (Zhou, 2013). Superior genotypes are produced by the crossing over of a large number of different specie individuals from segregation populations. The resulting seeds produce a large number of progeny (seedlings) from which the improved cultivars are obtained from superior combinations. (Awan et al., 2019)

Saccharum officinarum is domesticated from the *Saccharum robustum* specie and it was originated from New Guinea. *Saccharum officinarum* (noble cane) has a very high importance commercially in the views of high sugar content, low impurity levels and low fiber (Yadav et al., 2020).

But with beneficial characteristics, there are some major drawbacks (Jackson, 2005) in this specie which includes the lacked vigour, rationing performance and the susceptibility to various fungal, viral and bacterial diseases. These drawbacks led to draw the attention of the scientists to *Saccharum spontaneum* which is wild but thin stalked specie with high rationing performance but low sugar content. Furthermore, this specie has more tolerance to harsh environment like drought, moisture stress, water logging, high salinity, low and high temperature and micro nutrients (Yadav et al., 2020).

Selected hybrid was backcrossed to noble types for several times to dilute the negative effect of the wild germplasm in a process known as nobilization. As a result, the modern sugarcane now shows the high degree of co-ancestry. So the commercial sugarcane is derived from twenty *Saccharum offiicinarum* clones and less than ten *Saccharum spontaneum* species (Cheavegatti-Gianotto et al., 2011). During a cross, *S. officinarum* transmits two haploid chromosome set while *S. spontaneum* transmits one haploid chromosome set. The outcome of crosses in breeding programs is unpredictable due to the high polyploidy and interspecific origin (Snyman, 2004).

In the Australian sugarcane breeding program, the objectives were the increased productivity, sugar content upto >300 kg sugar/ha/year, profitability, disease resistant varieties and the cultivars with the improved fiber content and the maintenance of germplasm collection for the cross pollination. The main purpose of breeding program is the collection and improvement of parents which are used further in the crossing for the production of improved and better genotypes. Suitable parental clones are retained to use them in crossing while the others are discarded. The progeny generated is evaluated

and characterization of parents is done by the progeny performances and the breeding value is determined for the desirable traits. Computer algorithms based on the best linear unbiased predictors (BLUP's) uses data to predict the appropriate crossing i.e. parental disease ranting, whether the cross has been made before from this parent and whether there is inbreeding (Park et al., 2007).

Breeding programs brought significant changes in attaining the desirable traits in the sugarcane but the rate of genetic gains is plateauing. The reasons include the breeding cycle lengths and the low narrow-sense heritability for major commercial traits.

6.1.2 Sucrose Enhancement by Genomic Selection

Sugarcane with desirable traits is mostly the outcome of the inter-crossing of improved hybrids and then the selection of desirable genotypes. But due to the high complexity of the sugarcane genome for which non additive gene action play a significant role, the polyploidy nature of the genome and the long breeding and selection cycles, (Channappa Mahadevaiah et al., 2021) it became a challenge for the scientific investigators to bring about the efficient changes in the sugarcane contents until in the late 1980's when molecular tools were available for the analysis of sugarcane genome.

A modern breeding tool i.e. Genomic Selection (GS) has been successfully used in the plant breeding procedures. High-density SNP arrays and genotyping sequencing approaches have been implemented in animal and plant breeding (Majid et al., 2017). Genome selection accelerates the genetic gain by increasing the accuracy of selection and by reducing the length of breeding cycle (Aitken et al., 2008). It is crucial to generate large reference populations to determine accurately the effects of DNA markers associated with mutations which affect the traits. Traditionally, molecular markers have been used for the mono- or oligogenic traits to increase the efficiency of breeding program (Yadav et al., 2020).

In a study, different population clones were used to predict the accuracy of cane yield and sugar content. Genomic prediction tools like GBLUP, BayseA, BayesB and RKHS were used and prediction accuracy for the sugar content was highest in the advanced stage trials and low for cane yield (Channappa Mahadevaiah et al., 2021). The accuracy found was 0.25-0.45 which was showing the effectiveness of Genome Selection for sugarcane breeding. Some studies showed that RGS-based program could produce more genetic gain than GS strategy within breeding cycle. RGS breeding schemes improve additive genetic effects in each generation cycle. It increases the long term selection gain in hybrid sugarcane (Yadav et al., 2020).

The measurement and estimation of activities of enzymes i.e. invertase, sucrose phosphate synthase and sucrose synthase in four high and four low CCS clones from cross between *Saccharum officinarum* and commercial cultivar Q165 as biochemical markers is the accurate way to confirm the high yield of sucrose in the hybrids. The results showed that sucrose phosphate synthase has a significant role in the sucrose sugar content accumulation (G S et al., 2017).

6.2 Sucrose Accumulation By Sucrose Phosphate Synthase And Trehalulose Gene Transformation

Molecular breeding and genetic engineering has proved to modernize the scientific field and has brought about the gigantic improvements in the natural species for the welfare of humankind. In sugarcane, transgene methods have been developed to increase resistance to biotic and abiotic stresses, productivity and the high yield sugar content (Mudassar Fareed Awan et al., 2022; Zhang et al., 2018). Sucrose phosphate synthase is one of the importance regulatory enzyme in the sucrose metabolism of *Saccharum officinarum* (Anur et al., 2020) but the over expression of this gene alone has not brought about the significant improvements in the accumulation of sucrose content. Overexpression of invertase and sucrose phosphate synthase led to high sucrose accumulation in sugarcane but to a certain threshold. The increased activity of invertase in the cell wall leads to the high yield of sugar content (G S et al., 2017).

The sugar content, metabolism for the sugar production, growth and development can be greatly affected by the different biotic and abiotic stresses which include high or low temperature, drought conditions and salinity. Trehalose, a non-reducing disaccharide acts as a protectant against abiotic stress in bacteria, yeast, invertebrates and in highly resurrection plants. It stabilizes the dehydrated proteins, enzymes and membranes in drought condition. Moreover, it can lead to the higher yield and sugar content in the drought conditions. Trehalose synthase gene II showed maximum sugar recovery of 14.9% in transgenic sugarcane lines (M. F. Awan et al., 2022).

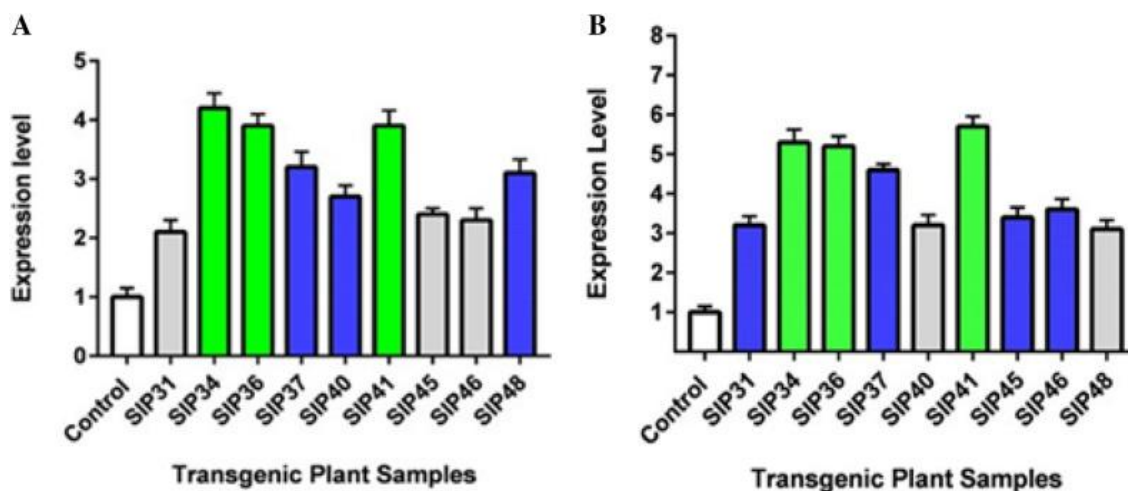


Figure 4: A represents expression levels of ThSyGII in leaves of transgenic lines, while B shows the expression of gene extracted from stem tissues of transgenic sugarcane. Green; maximum expression, blue; average expression, white; non-transgenic controls (M. F. Awan et al., 2022)

Trehalose synthase (TSase) gene were isolated from *Grifola frondosa* for improving the drought tolerance and the sugar content in *Saccharum officinarum*. The expression of this

transgene was controlled by two tandem copies of CaMV35S promoter. The transformation into the sugarcane was *Agrobacterium tumefaciens* EHA105. In a study, it was shown that up to 8.805-12.863 mg/g of trehalose was accumulated in transgenic sugarcane. Furthermore, the stalk lengths and weights were also different from non-transgenic plants (Zhang et al., 2006).

6.3 Application of Genome Editing Tools in Complex Sugarcane Genome

Scientists are trying to implement different genome editing tools to gain the desired traits, but due to the sugarcane genome highest levels of complexities, there are always some hindrances that come forward. Out of which transgene silencing is the major one. Genome editing or gene editing is the modification of genetic material of the organism in which DNA is inserted, deleted or replaced using nucleases (Georges & Ray, 2017). The nucleases work by creating site specific double stranded DNA breaks at specified desired locations of genome. Now these induced double strand breaks are repaired by the process of homologous recombination or the non-homologous recombination which result in the targeted mutations. Four families of engineered nucleases are being used in creating the desired traits in different organisms as well as for the treatments of diseases i.e. meganucleases, ZFNs, TALENs and the CRISPR/Cas9 (Liang et al., 2014). Among these technologies, CRISPR/Cas9 has surpassed the others.

6.4 Post Transcriptional Silencing Of Cas9 Genes

CRISPR technology has been successfully applied in the model plants like *Arabidopsis thaliana* as well as in the monocot species like rice, sorghum, wheat and maize. It has helped the scientists to edit the genome from single to multiple genes by knocking-in and knocking-out mechanism (Belhaj et al., 2015). This technique depends on the RNA/DNA hybrids for the determination of sequence rather than the protein sequence. In the gRNA, 20 nucleotide sequences determine the specificity by PAM (Protospacer Adjacent Motif) and then the Cas9 enzymes cleave the strand. Furthermore, it has the ability of multiplexing which makes it less time consuming (Bortesi & Fischer, 2015). CRISPR/Cas can improve crop yield and can make the crops herbicide and disease tolerant (Zhu et al, 2020). Sugarcane has a complex genome which makes it extremely time consuming to create a desired variety. Conventional breeding takes about 12-15 years to produce an improved and desired variety.

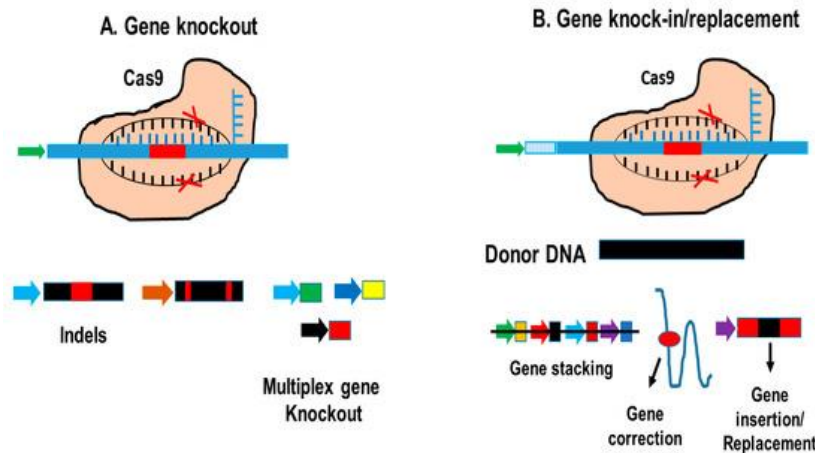


Figure 5: CRISPR Cas9 applications in (A) indels and gene deletions (B) gene correction and gene insertion (Hussin et al., 2022)

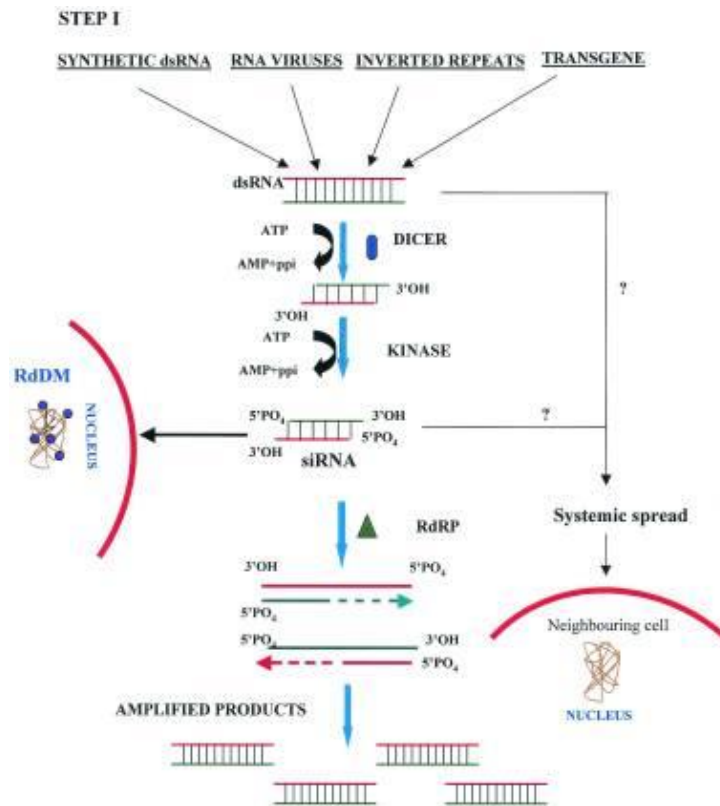
Transgene silencing hinders the molecular improvement in sugarcane. In a study, transcriptional and post transcriptional transgene silencing effects have been reported. Post transcriptional silencing in sugarcane is promoter-cassette sequence specific which emphasise the use of promoters to lower the effects of silencing (Jiang et al., 2013). The maize ubiquitin promoters as well as sugarcane derived promoters have been used. Therefore, there is a fundamental need of specific Cas9 genes with highly efficient promoters to bring about the significant crop improvement. CRISPR/Cas9 may also result in random unnecessary and unwanted mutations. It can therefore cleave the DNA sequence at other genomic sites due to mismatches in the guide sequence (Mohan, 2016).

CRISPR/Cas9 uses transformation methods including protoplast transfection, ago-infiltration and generation of stable transgenic (Ma et al., 2015). Argo-infiltration and protoplast fusion methods are not successful in sugarcane, so *Agrobacterium*-mediated transformation was highly used but it is time consuming and less efficient. Another drawback is that there is a lack of mutant studies in sugarcane for the functional studies of multiple allelic gene forms. Most of the traits in sugarcane are polygenic so there is a lack of available targets (Mohan, 2016).

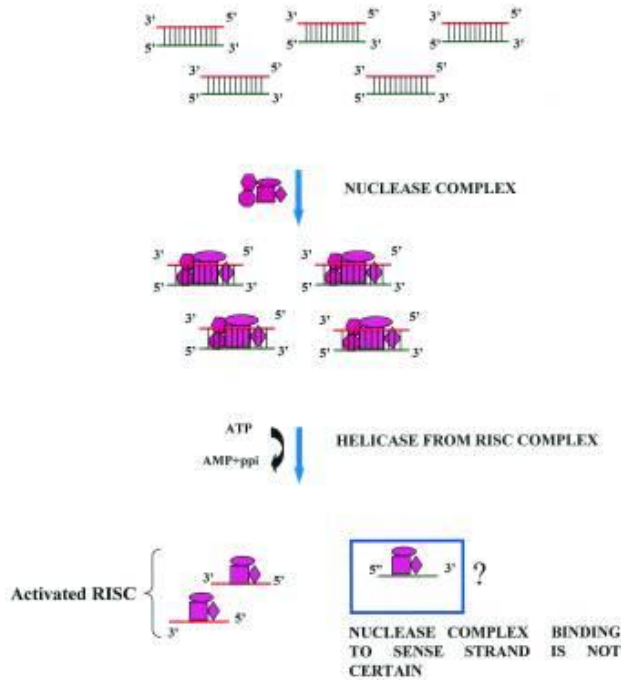
6.5 Improved Sugar Production by Rnai Mechanism

Every year, world's majority sugarcane yield is produced in subtropical areas. By silencing the specific genes, we can enhance growth and ultimately traits of agriculturally important crops (Sharif et al., 2022). Sugarcane is crucial for producing sugar and other sugarcane products such as ethanol and biofuels (Murugan et al., 2021). Cell wall of sugarcane contains lignin components which have become a hurdle for processing polysaccharide and ultimately hinders the sucrose production. It was studied that by reducing or silencing the lignin components from sugarcane we can increase the sugar production (Bewg et al., 2016).

The expression of lignin gene was silenced by RNAi. This resulted in reducing the lignin components in the sugarcane which ultimately increased the production of sugar content (Murugan et al., 2021). By the help of RNAi reduction in gene expression of caffeoyl-CoA O-methyltransferase (CCoAOMT), ferulate 5-hydroxylase (F5H) and caffeic acid O-methyltransferase (COMT) led to reduced lignin production. The analysis was done on the bases of qRT-PCR which showed that the suppression of three genes of lignin improved glucose production (Bewg et al., 2016). A complete mechanism of gene knockout by RNA interference has shown in figure 7 below.



STEP II



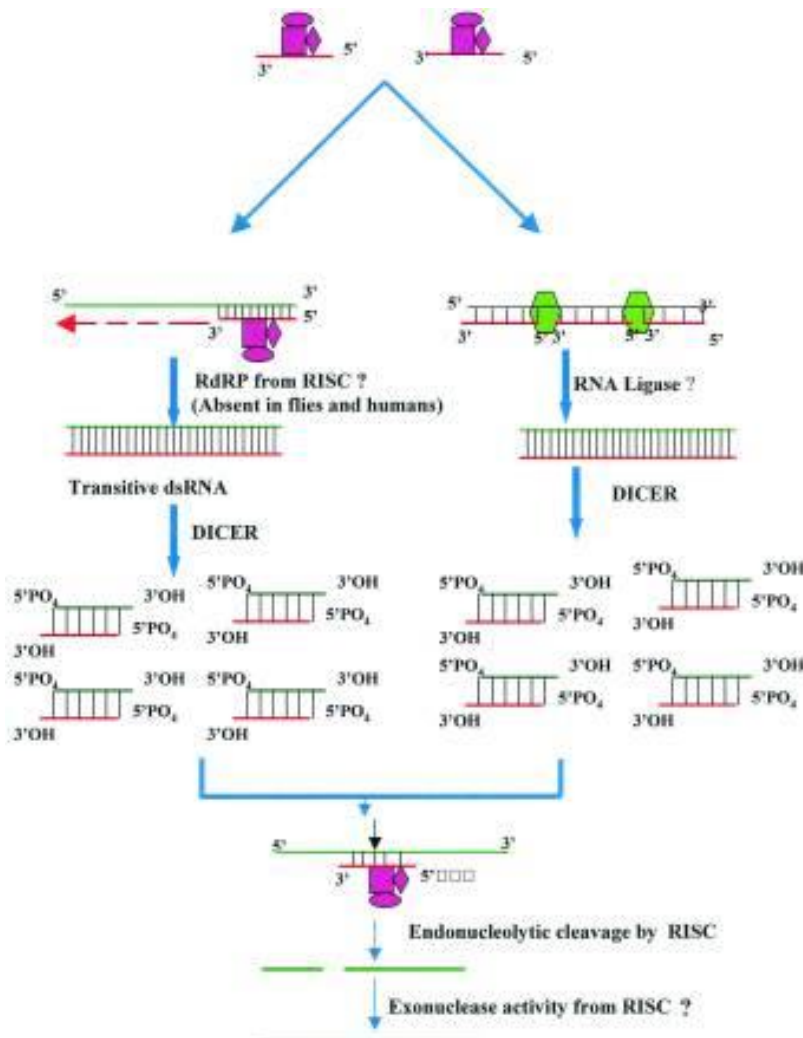


Figure 6: (Step 1 & 2) represents the whole mechanism of gene silencing by RNA interference (Agrawal et al., 2003)

6.6 Sucrose Isomerase Gene as a Revolutionary Advancement for Higher Sucrose Content

Sucrose isomerase (SI) is an enzyme which is used to convert sucrose into its isomers; more beneficial than sucrose which are further used in food. There are many different bacterial strains which are used to produce sucrose isomerase enzyme which can be extracted and further used in our industries for beneficial purposes (Goulter et al., 2012).

Bacterial strains from which sucrose isomerase genes can be extracted are *Pantoea dispersa* UQ68J, *Klebsiella planticola* UQ14S, and *Erwinia rhapontici* WAC2928, they are cloned and expressed in *E.coli* which was further introduced in sugarcane, which convert sucrose into its isomers such as isomaltose or trehalulose which are more beneficial than sucrose (Wu & Birch, 2005).

The major source of sucrose is sugarcane and beet. Scientists have been working on improving sugar contents present in the sugarcane by conventional and molecular breeding but they failed to do so. After that they cloned some bacterial genes which contain isomerase enzyme for the conversion of sucrose into different isomers of sugar (Wu & Birch, 2007).

By introducing sucrose isomerase gene in sugarcane we can improve sugar production as well we can achieve double quantity of sugar than it was before in the sugarcane, when SI gene was introduced in the sugarcane the sugar value in the sugarcane boosted up (Liu et al., 2021), as when sucrose isomerase was introduced in the sugarcane the sucrose was converted into isomaltulose whose storage was observed in tissues of sugarcane without even decreasing the previous value of sucrose present in the sugarcane. In fact, the quantity of sugar was doubled than before along with that the photosynthetic efficiency was increased due to increase in sugar storage (Wu & Birch, 2007). Therefore sucrose isomerase has enlightened our way to increase the sugar production and other byproducts of the sugarcane.

7. CONCLUSION

Saccharum officinarum is a large-stature perennial crop which is majorly cultivated in the tropical and the sub-tropical regions. It has been a great source for sucrose as well as for the production of various sugarcane products. Out of which, sugar production is of gigantic importance. The rapidly changing environmental conditions and global warming are causing critical water stress on its crop production. Drought affects sugarcane production drastically and thus affecting its yield and sucrose content as tillering majorly depend on the water available. Apart from that, to enhance the economical value of sugarcane, conventional breeding, molecular breeding, transformation and various gene editing tools have been applied to increase the sucrose content of this crop, but due to the highly complex genome, biotic and abiotic stresses, the polyploidy of this crop and the post transcriptional silencing factors research has been impeded in this area. Recent advancement is the isolation of sucrose isomerase gene from the different bacterial strains and incorporating in the *Saccharum officinarum* has proved to be an effective tool for enhancing the sugar content of this crop.

8. FUTURE PROSPECTS

For future research, the area of bioinformatics is wide open as the complex polyploid and aneuploid genome of sugarcane hinders most of the research for inducing drought tolerance in sugarcane as functions of many genes are yet to investigate. It is crucial to have the complete understanding of its genome including the functions of genes as well as the promoters. Last but not least, as we will know the complete genomics of sugarcane, we may be able to increase the sucrose yield by using genome editing tools rather than transformation methods.

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