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Review on Adaptation and Breeding Mechanisms of Salinity Stress for Sugarcane

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Abstract

Salinity stress due to environmental climate change is one of the major abiotic stresses for plants that adversely affects soil health, environmental quality, and agricultural production. Sugarcane is important for economy of many in both tropical and subtropical countries, playing a central role as a primary sugar- producing crop and has major potential for bioethanol and biofuel production. It is moderately sensitive for salinity stress and highly affected under high salt concentration. Soil salinity influences the sugar cane productivity by altering the morphological, physiological and biochemical systems and affecting ionic balance inside the cell. The plant produces new compatible solutes (osmolytes) as a result of salinity stress. There are six types of Saccharum species and breeders are engaged on improving the cane verities through adaptation and breading mechanisms. Now a day's biotechnology plays critical role in the development of abiotic stress tolerant sugarcane cultivar through genotype Based breeding, genetic engineering and genome editing technologies. This paper is aimed to review the current adaptation and breeding mechanisms salinity stress for sugar cane.

Keywords: Salinity Stress, Sugarcane, Osmolytes, Genome Editing, Adaptation

1. Introduction

Sugarcane (Saccharum spp.) is one of important commercial crop grown in tropical and subtropical part of world. It is most commonly produced in Brazil, India, Thailand, China, Australia, Pakistan, Philip- pines, Cuba, Colombia, and the USA. Sugarcane is known for the production of various industrially valuable products, such as sugar, waxes, biofuels, bioethanol and bio-fibers [1,2]. According to (USDA 2020) global market analysis report-2020, Brazil is the leading world country for largest production and commercialization of sugarcane and its products (39.4 mt) of sugar [3]. Sugarcane is a type of glycophyte crop plant which leads to low spout emergence, nutritional imbalance and growth reduction and low productivity when it is cultivated in the high salt-affected soils. The modern sugarcane cultivars are originated from the two wild Saccharum species (*S. spontaneum* and *S. robustusm*), four cultivated species (*S. officinarum, S. sinense, S. barberi* and *S. edule*) [4,5].

Salinity is the presence of dissolved salt in excessive concentration in soil. Salinization is a process in which water-soluble salt ions are gradually accumulated in to the soil and deposited in the soil to a large extent affecting crop productivity, microbial community, and agricultural economics. As a process ultimately converts a fertile land to unfertile state, it causes negative effect on the vegetation and other organisms living in the soil and thus it is totally harmful to the environmental health [6,7]. The salinized area have been increasing at a faster rate annually due low precipitation, excessive evaporation, irrigation with saline water, and poor agricultural practices [3]. Too much amount of salt in the soil will reduce the availability of water by plants, leads to osmotic stress in cells, causes cell dehydration and threatening plant survival [8].

High salinity is caused by the increase in concentration of Na+ and Cl- ions triggering hyperosmotic and infiltration conditions of the soil solution which will hinder the absorption of water and nutrients by plants. Removal of Na+ from photosynthetic organ is therefore crucial for the maintenance of adequate metabolism and efficient carbon fixation and restriction of the amount of Na+ ions in the cytosol by selective uptake and efflux of Na+ ions have been identified as a mechanism for removing toxic Na+. Under salt stress, K+ helps to maintain ion homeostasis and regulate the osmotic balance and a major portion (90%) is localized in

the vacuoles [9]. The adverse effects of salinity stress on plant phenotype are characterized by inhibited and slow growth, reduced leaf area, succulent, and death in severe cases. Beyond this

There are many changes in physiological effects including, low water absorption, reduced transpiration eventually decreases photosynthetic rate due to reduced stomatal opening and increased oxidative stress [10].

One of sugarcane adaptation and protective mechanism for salinity is selection of closely related genotypes and selection of the best one for the better and transformation of sugarcane with gene of interest coding for enzymes important for salinity tolerance [11]. The main aim of this review is to discuss research advances on the adaptation and breeding mechanisms of salinity for sugarcane plant.

1.1 Adaptation Mechanisms under Salinity Stress

1.1.1 Morphological Responses of Sugarcane to Salt Stress

Plant morphology under salt stress results influence on dry or fresh total biomass, the plant height the length of roots and shoots, root architecture and the number of secondary branches on them, diameter of shoot, the tiller numbers and leaves number, generation rate of young leaves and the flowering time on the reproductive stage of plants [12]. According to Gomathi et al., (2014) [13] Propagation of sugarcane under salt stress brought overall reduction in number stalk per unit area, stalk length, number of internode per stalk, internodal length, cane diameter and single cane weight.

1.2 Physiological Responses of Sugarcane to Salt Stress

Beyond to morphological responses to abiotic stresses for salinity plants naturally produce excess amount of stress responsive compounds which assist them in developing stress tolerance. These are types of low molecular weight organic metabolites called the compatible solutes, is one such abundant mechanism in plants during salt stress. These compatible solutes act as crucial osmoprotectants and help the plant system to survive at severe osmotic and ionic stress. Furthermore, Compatible solutes have been depicted for other mechanisms, including prevention of ion entry into the sensitive plant parts, increasing the ion exclusion from them, maintaining the hydration sphere of proteins under desiccating conditions, allow proper folding of proteins thus acting as chaperones, stabilizing macromolecules or molecular assemblies, prevent dissociation of enzymatic complexes and act as the scavengers of reactive oxygen species [14].

Kasirajan et al. (2021) [15] findings demonstrate that the reduction of RWC (%) and nitrate Reductase activity, Increment of proline and lipid peroxidase activity with an increase in the severity of salt stress in all the seven genotypes in comparison to control. Moreover, it hinders the reduction of nitrate reductase activity in the clones leads to a significant decrease in nitrate uptake and total nitrogen content. Nitrate reductase activity is involved in the nitrate assimilation pathway and regulates the protein synthesis.

A high salt concentration for the plant causes two main harmful effects. These are osmotic stress and ionic stress. Osmotic stress is caused primarily due to high concentrations of Na+ and Cl- in the environment that decrease the osmotic potential of water by plant roots. This causes various physiological effects, such as low photosynthetic activity and the production of reactive oxygen species (ROS) [15]. Ionic stress is caused by an increased absorption of ions, primarily Na+ and Cl-, which results in biochemical and physiological damage moreover, high concentrations of intracellular Na+ prevent the uptake of K+, which is an essential element in several cellular processes [16]. ROS including single oxygen (O2), superoxide ions (O2-) and peroxides are widely distributed toxic molecules which have negative effect to DNA protein and lipid. The negative effect of these ROS is due to their ability to perform autoxidative chain reactions on unsaturated fatty acids [18].

Therefore, the regulation of Na+ and Cl– uptake is essential for plants to avoid toxic Na+ accumulation in leaves, and maintain a high K+/Na+ ratio which is important for the activity of K+ dependent enzymes [19]. Plants possess natural adaptive mechanism through accumulation of Organic solutes (compatible osmolytes) as biochemical and physiological mechanisms that can enable plants to tolerate stress effects. The amino acids proline (Pro) and the quaternary amine glycine betaine (GB) are among organic solutes indicating plant stress, and accumulate in plant tissues to protect against toxic accumulation of salt and promote osmotic adjustment of the cytoplasm in response to various stresses including salinity [20].

Leaf net photosynthesis rate, transpiration rate and stomatal conductance are highly affected by salinity stress influences. Salt tolerant clones of some sugar cane cultivars reviled for uniform rates of photosynthesis, conversely the sensitive genotypes show dramatic reduction due to salt treatment. The reasons for reduced photosynthesis include stomatal closure following the osmotic effects of salinity. The rate of photosynthesis is different among salinity stress resistant and susceptible genotype [21]. Researchers has been reported that Salinity has gained a global.

Concern due to its severe environmental stresses that inversely affect the growth and productivity of crop plants with regulation of metabolic changes [16]. Sugarcane cultivation require soils with high water content and nutrients for efficient productivity, due to this cultivation in salinized soils can result in a drastic reduction of growth, with losses of 50% or more in comparison to the productivity in unsalted soils [22].

1.3 Salt Overly Sensitive (SOS) Stress Signaling Pathway

The Salt Overly Sensitive (SOS) signaling pathway is a type of classical signal pathway occurring when plants are exposed to high levels of NaCl and play a great role in regulating osmotic homeostasis in plants in response to salt stress [23]. It is a major regulatory mechanism of plants for ion homeostasis that originally

was considered to regulate Na+ exclusion in their roots [24].

SOS consists of three major proteins: SOS1 protein that encodes a plasma membrane Na+/H+ antiporter, is crucial in controlling Na+ outflow at cellular level and allow long distance transport of Na+ from root to shoot [25]. SOS2 protein that encodes protein kinase and it is activated by the action of both SOS3 protein and salt stress provoked Ca2+ signals. The third SOS3 is a myristoylated Ca2+ binding protein along with a myristoylation site at its N-terminus. This myristoylation site shows a crucial role in salt tolerance. C-terminal regulatory domain of SOS2 protein performs as a site of interaction for Ca2+ binding SOS3 protein resulting in the initiation of the kinase. The activated kinase then phosphorylates SOS1 protein thus mounting its transport activity through Na+/H+ antiporter. This result increase Na+ efflux and maintain cellular osmotic homeostasis [17]. SOS3 perceives the increase of intracellular calcium due to salt stress and then recruits SOS2 to the plasma membrane forms a complex. The SOS3–SOS2 complex further activates the downstream target protein SOS1 to prevent the accumulation of Na+ [26].

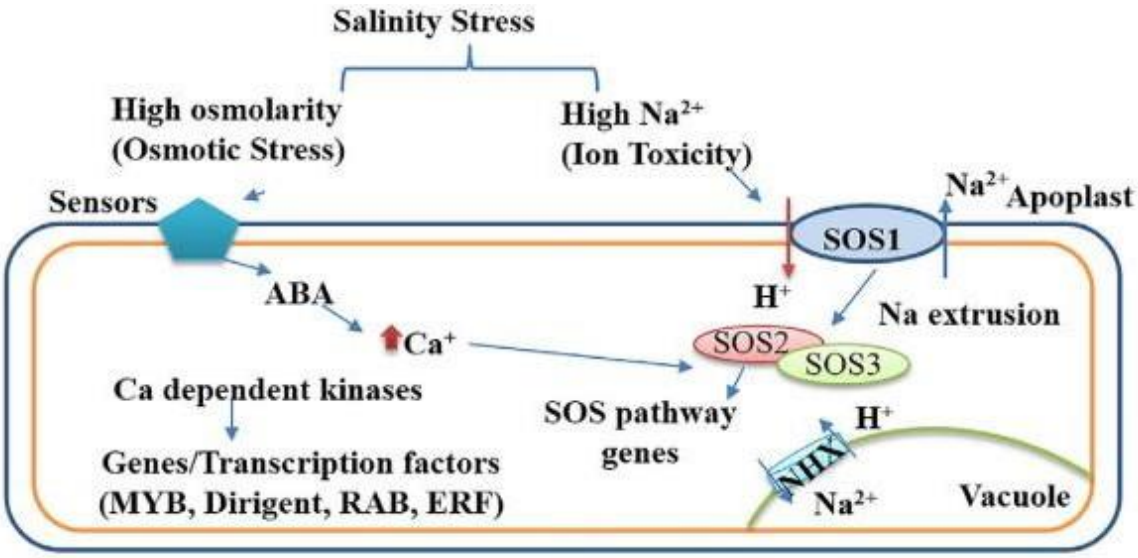

Figure 1. Schematic representation of ABA and SOS signaling pathway in S. spontaneum [15]

1.4 Production of Compatible Compounds

Plants produce compatible compounds as biochemical adaptation mechanism for salt stress. Compatible solutes are a group of chemically diverse organic compounds that are uncharged, polar, and soluble in nature and do not interfere with the cellular metabolism even at high concentration. Compatible solutes are also known as compatible osmolytes produced by plants during salinity stress [17].

1.4.1 Production of Proline

Proline accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell. Many previous studies shows that an exponential increase in proline content in the salt stressed plants was observed [27]. There are two major enzymes for proline production through biosynthetic pathways during salt stress in plants. These are pyrroline carboxylic acid synthetase (5PCS) and pyrroline carboxylic acid reductase (P5CR), which are responsible for overproduction of Proline in plants under stress [28]. Salt stress resulted in growth reduction, increase in the Na+/K+ ratio, increase in Proline level and up-regulation of proline synthesis gene as well as accumulation of H2O2, the production of proline increases activity of antioxidative enzymes SOD, POX, APX, CAT during the significant inhibition of salt [29]. Intracellular Proline provides tolerance toward stress and also acts as an organic nitrogen reserve during stress recovery. Proline helps in stimulating the expression of salt-stress-

Responsive proteins acts as an antioxidant feature, signifying ROS scavenging activity. It protects the photosynthetic apparatus through plant adaptation against salt stress [25, 26].

More over high-level expression of proline enhances the photosynthetic activity, and thus preserve well plant growth and water influx, mitigate the reduction of photosynthesis (Pn), fluorescence (Fv/Fm), and chlorophyll (Chl) content under saline conditions. According to [29] exogenous supplementation of Proline suppressed the uptake of Na+ in rice plant. Other researcher also demonstrated that application of proline to the salt stress environment repressed Na-induced trisodium-8-hydroxy-1,3,6-pyrenetrisulphonic acid uptake and Na+ accumulation, whereas the K+ content was fairly increased, leading to a high K+/Na+ ratio under salt stress [32].

Proline content of sugarcane under salinity stress can be estimated by the following procedures. Taking and weigh Five hundred mg of fresh leaf sample and transfer to a mortar. Macerate the sample with 10 mL of 3% sulphosalicylic acid and transfer to a centrifuge tube. Centrifuge the solution at 4,000 rpm for 10 minutes. After

centrifugation, collect 2 mL of the supernatant in a test tube. Add 2 ml of acid ninhydrin and 2 mL of glacial acetic acid. Keep the test tubes in water bath for one hour and then cool under tap water. Transfer the solution to a separating funnel and then added 4 ml of toluene. Shak the solution uniformly for 30 seconds until it forms two different layers. Discard the colorless bottom layer and collect the upper pink color solution in a fresh test tube and measure the optical density at 520 nm. Prepare standard curves with every set of plant samples. Finally analyze the standard in duplicates, unknown samples, in triplicate [33].

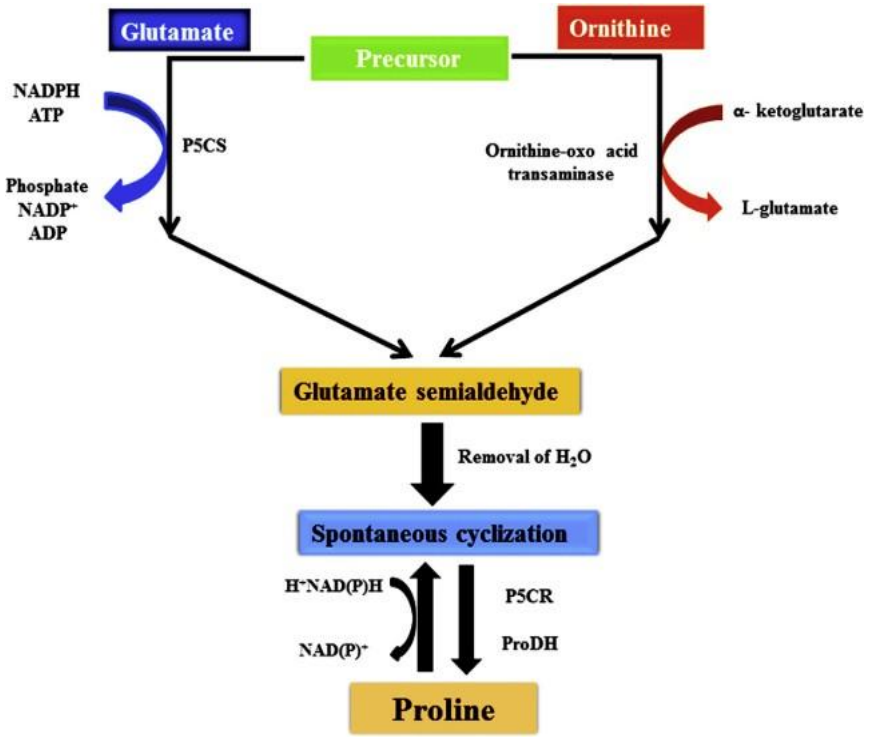

Figure 2. Biosynthesis of Pro from glutamate during salinity [34]

1.4.2 Production of Glycine Betaine

Glycine betaine is a type of compatible solute which play critical role in mitigation of salinity, drought and extreme temperature stress. It is dipolar at physiological pH, but electrically neutral in nature. The essential role of GB in plants exposed to salinity stress is protecting the cell by osmotic adjustment, stabilizing proteins and protecting the photosynthetic apparatus from stress damages and reduction of ROS [17], [35]. GB is a non-toxic even at higher concentrations in cell which plays a role to salt stress tolerance. GB is synthesized by two step reaction pathways from choline, first cholin is oxidized to betaine aldehyde by choline monooxygenase (CMO) which is further undergoes oxidation to form glycine betaine by BADH. Another pathway of synthesis involves three successive N-methylation which are catalyzed by GSMT and SDMT. The exogenous application of GB helps to the reduction of Na+ accumulation alone with the maintenance of higher K+ concentration within all parts of salt-stressed plants. This effect might be due to the creation of numerous vacuoles in the root cells in which Na+ is stored and prevent its accumulation in the shoots. More over the studies are revealed that GB treated plants exhibited higher water use efficiency (WUE) and pigment stabilization, leading to high CO2 assimilation, photosynthetic performance as well as plant height under salinized environment [32].

The accumulation of Glycine betaine in salt stressed sugarcane can be analyzed according to Grieve et al., (1983) [36]. Accumulation of GB increased in response to salinity and the accumulation increases with increasing the concentration of NaCl. Recent reports revealed that Exogenous GB treatments significantly enhance the growth in both stressed and non-stressed plants they suggest the most effective dose of GB to be 25 mmol l−1 for the mitigating of salinity effect on the growth of lettuce. They also conclude from their results on average 25 mmol l−1 GB treatment increased fresh and dry shoot and root weight by 54.9%, 80.5%, 40.0% and 64.3%, respectively [37]

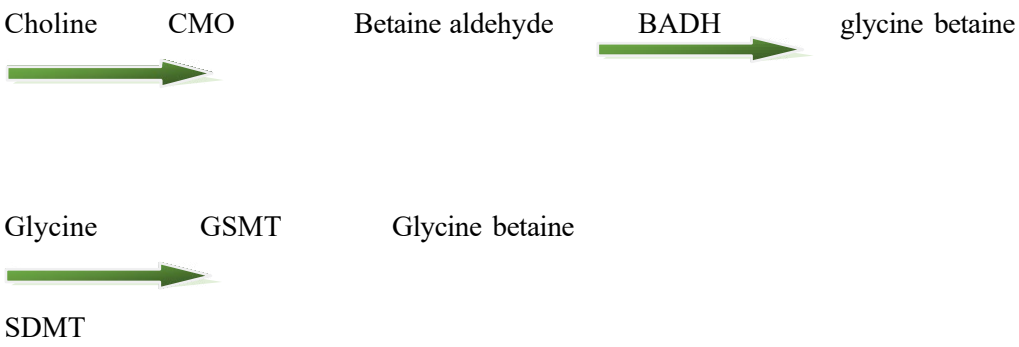

Figure 3. Biosynthesis of GB from choline and glycine during salinity [32]

1.5 Antioxidant Enzyme

Reactive oxygen species (ROS) are produced under normal conditions in plants; but under stress conditions, their level is highly increased. Plants have devised antioxidative defense system to scavenge harmful ROS, and protect plant cells from oxidative injury [38]. The peroxidation of lipids is the most obvious symptom of oxidative stress in plants, caused by ROS [39]. The most common lipid peroxidation pathway includes O2 molecules originated from photosystem II (PSII). These molecules are incorporated into plastid membranes and catalyzed by lipoxygenases (LOX) into LOOH (lipid hydroperoxide), which makes the membrane vulnerable to fragmentation and leads to a cascade of damaging events [40].

Salt tolerant plants besides being able to regulate the ion and water movements also have an efficient antioxidative system for effective removal of the reactive oxygen species (ROS). Plants protect cells and sub-cellular systems from the effects of ROS by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutatione reductase,

Polyphenol oxidase and non-enzymic antioxidants such as ascorbate and glutathione [41]. SOD (superoxide dismutase) has the ability to eliminate the large amount of superoxide anions from the cells and therefore acts as a defense system for salinity stress. CAT is an enzyme which is present in peroxisomes and it deplete the amount of H2O2. CAT eliminates the hydrogen peroxide (H2O2) which is formed by light reaction. APX is an important enzyme which helps in the elimination of hydrogen peroxide by using ascorbate for reduction [38].

APX is one of the most important antioxidant enzymes of plants that detoxify H2O2 using ascorbate for reduction. In different plant species, APX activity increased in response to a variety of biotic and abiotic stresses. APX enzyme is involved in protecting the oxidative damage, such as hydrogen peroxide generated in the chloroplast. CAT enzyme is involved in protecting the oxidative damage, such as hydrogen peroxide generated in the chloroplast. CAT is important for the elimination of H2O2. Several studies have reported that salinity may induce increase in CAT activity [42]. Enzymatic activities for the enzymes APX, CAT and SOD were estimated from leaves of sugarcane stressed with salinity for both tolerant and sensitive cultivars. The hydrogen peroxide dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 290 nm for three min at the interval of 30 sec. The hydrogen peroxide dependent oxidation was estimated by the decrease in absorbance at 240 nm. Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium [43].

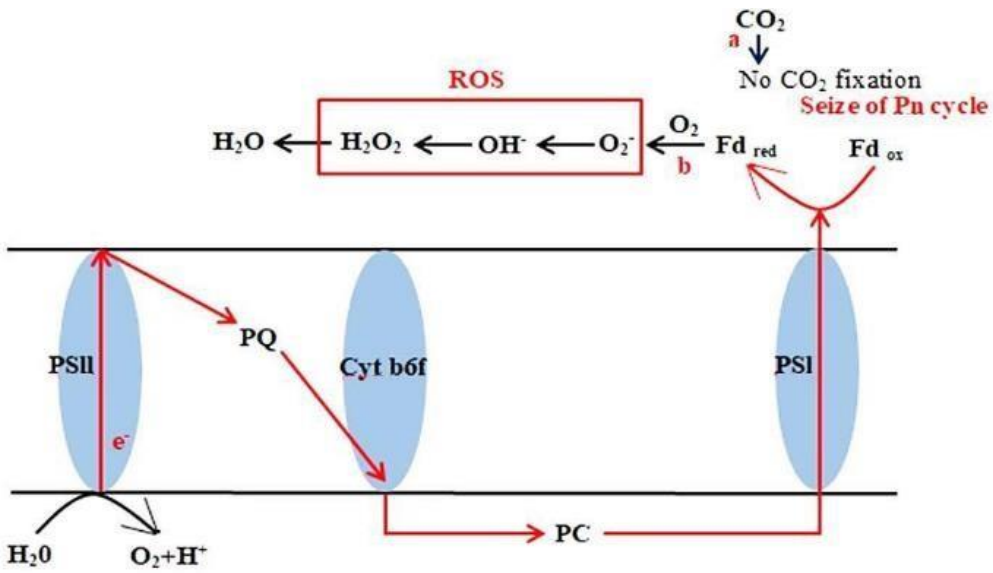

Figure 4. An overview of oxidative stress during salinity stress. Where, a- No CO2 fixation due to stomatal closure; b- Initiation of ROS generation via. mehlar reaction [32]

2. Evaluation of Salinity Stress at Gene Level

Recent studies are showing that salinity tolerance has been studied at a molecular approach. Regulation of gene expression for salinity stress tolerance may manifested either by upregulation or downregulation of a gene for the production of specific gene products (protein or RNA) [17].

2.1 Upregulated Genes

2.1.1 Heat Shock Proteins (HSPs)

Heat shock proteins (HSPs) are dispersed extensively in plants naturally and also expressed during stress conditions. HSPs are molecular chaperons which have a significant role in gathering and folding of proteins and eradication and destruction of non-useful proteins. These include small family Hsp, Hsp 100 family, Hsp 90 family, Hsp 70 family also known as DnaK family, and chaperonins such as Hsp60 and GroEL [44]. Salinity as well as drought stress is instigated by heat shock proteins (HSPs). Under stress conditions, several heat shock proteins are found to be upregulated such as HSP70-9-12 and -33 in poplar and HSP70 in wheat and rice seedlings. Additionally, other heat shock proteins were upregulated in salinity stress such as HSP100-75 and -21; HSP90-12 and -9; HSP40-117 and -113; HSP60-49, -38, -33, and -31; and HSP21 in poplar and HSP40 in rice. The overexpression of Hsp70 genes correlates positively with enhanced tolerance to salt [38].

2.1.2 Salt Overly Sensitive Gene

A plant cell under salinity stress will experienced with increased Na+ ion in the cytoplasm, it is compartmentalized into vacuole due to the expression of Na+/H+ antiporter (SOS1) gene. SOS1 is a plasma membrane bound Na+/H+ antiporter gene, which mediates Na+ efflux, and its activity is regulated by the SOS3/ SOS2 kinase complex during salt stress [45]. The expression of SOS and Ras-associated binding (RAB) protein become higher for maintenance of ion homeostasis for salinity tolerance. The higher concentration of Na ions is received through sensors and activates the abscisic acid (ABA) dependent signaling pathway, which leads to the accumulation of ROS. Consequently, ABA and ROS regulate ionic and osmotic homeostasis to control stress induced cell damage. Hence based on the increased evidence of interlinkage between ABA-induced SOS pathway and the increased expression of SOS and RAB genes, which play a major role in ion homeostasis and salt tolerance in many crops [15].

2.1.3 G-Protein Coupled Receptor Gene

G-protein-coupled receptors (GPCRs) are a conserved family of membrane-bound proteins present in most eukaryotes [46]. GPCRs mediate responses to several physiological processes, such as growth, development, and extracellular stimuli. Members of the GPCR protein family share a common central core domain composed of 7–9 transmembrane (TM) helices connected by three N-terminal extracellular loops and three C-terminal intracellular loops, a distinct characteristic not seen in other classes of cell membrane receptors [47].

One way to enhance sugarcane stress tolerance to abiotic stresses is by manipulating GPCR activity. In this study we report the identification, isolation, and functional characterization of a GPCR gene from Saccharum spp. hybrids (ShGPCR1) and show that genetically modified sugarcane plants overexpressing ShGPCR1 are more resistant to drought, salinity, and cold stresses. Ramasamy and the team [48] analyzed transformation and expression of GPCR gene in the following procedures. They took DNA sequence of GPCR from rice for performing a BLAST

search against the sugarcane expressed sequence tag and prepared cDNA libraries. After they sequenced the samples, they got coding sequence matching with rice GPCR by using bioinformatics tool and the resulting shGPCR was cloned into a vector for transferred to embryonic callus of obtained from sugarcane. Finally, explants were allowed to grow and analyzed for the expression of GPCR by using selectable marker and southern hybridization techniques. Generally, the team concluded over expression of GPCR gene is important for abiotic stress tolerance including salinity.

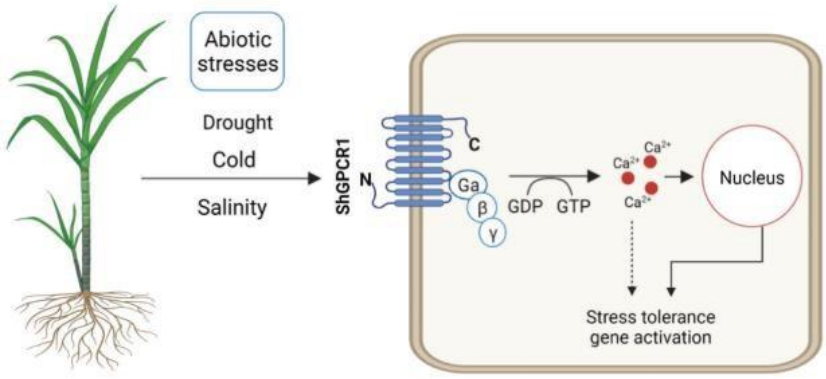

Figure 5. Hypothetical model of ShGPCR1 function in sugarcane to confer tolerance to multiple abiotic stresses.

3. Evaluation of Salinity Stress at Protein Level

Now a days, the research and knowledge of the mechanisms for the tolerance of sugarcane to salt stress has increased. These recent studies have identified genes and metabolic pathways that might be important in the response to salt stress; particularly, proteins associated with carbohydrate metabolism and energy might be involved in the response of sugarcane to salt stress [49].

In recent studies scientists reveled that differential expression of proteins investigated for many types sugar cane cultivars from young leaves for both tolerant and sensitive cultivars. Eight upregulated and ten down regulated proteins were investigated through NaCl treatment of cultivars. GDSL-motif Lipase/ Acylhydrolase were among up regulated proteins showed greatest abundance. Similarly, lipoxygenase enzyme 9-LOX which is involved in lipid metabolism was showing increment in concentration. In other ways there have been down regulated Proteins that are mainly concerned with photosynthetic carbon assimilation and metabolism. For salt sensitive cultivars ten proteins were identified as upregulated and twenty-three of the others were down regulated. Triosephosphate isomerase (TPI) showed the greatest abondance. Type III chlorophyll a/b-binding protein was downregulated in response to stress in the sensitive cultivar, and lipoxygenase was not expressed in the salt-treated sensitive cultivar The other four proteins that were upregulated in the tolerant plants, the H+-transporting ATPase, 50S ribosomal protein, Histone 2A and peroxyredoxin Q were similarly upregulated in the sensitive plants [50]. The differentially regulated proteins identified in response to salt stress were all related to photosynthesis and energy generation, antioxidant activity, and lipid metabolism. Additional energy is needed by plants in saline soil to regulate the uptake and transport of Na+ and Cl– and to provide osmotic adjustment by compartmentation of Na+ and Cl– within vacuoles of cells, along with the synthesis of organic solutes to avoid toxic accumulation of Na+ or Cl– [51].

Proteins hexokinase and GST, have been identified to be associated with responses to different abiotic stresses. Since these proteins were observed in cultivars only under salt stress conditions. They have been reported as important proteins involved in the response to salt stress and useful as bio- markers associated with the response of sugarcane to salt stress. More over proteins such as photosystem I sub- unit I which exhibited a higher abundance in the stress-tolerant cultivar might be directly associated with response to salt stress conditions. However the photosynthesis

Rate in susceptible cultivar was reduced [49]. This decrease in photosynthetic activity under stress conditions might affect energy metabolism and increase the production of ROS. Peroxidase and GST proteins are acting as ROS scavengers preventing high accumulation of ROS [16], [52]. GAPC is an enzyme is also overexpressed in salt tolerant cultivar and plays a direct role in photosystem repair and consequently increases the photosynthetic rate. The acceleration of photosystem repair is achieved primarily by decreasing ROS accumulation and increasing CO2 fixation. It is also reported that GAPC overexpression in Oryza sativa under salt stress effectively decreases the intracellular H2O2 [49].

Moreover, upregulated proteins are also known to protect plants from lipid peroxidation. Lipoxygenases (LOX) catalyze the oxygenation of long chain polyunsaturated fatty acids into short chain and volatile compounds. Lipoxygenase enzyme is involved in many physiological processes during plant development and under stress, acting to modulate ROS accumulation, lipid peroxidation and gene expression, and conferring tolerance to biotic and abiotic stresses including pathogen attack, drought, salinity and oxidative stress [53], [54]. Peroxiredoxins (Prx) are a widespread family of one of the major ROS-scavenging enzymes that act as antioxidants

in ROS elimination, specifically on hydroperoxidases, protecting the plant from lipid peroxidation. Peroxiredoxin Q is a member of this family and overexpression of this gene from the halophyte Suaeda salsa L. conferred salt tolerance to a non- halophytic species Eustoma grandiflorum Shinn. These enzymes are responsive to salinity and may be an essential response to the increase in ROS. [55].

Other upregulated proteins in the tolerant cultivar are involved in energy production. Type III chlorophyll a/b-binding proteins, although they were present in the sensitive cultivar under salinity, only showed increased activity in the tolerant one. These proteins are involved in the photosynthetic light reactions of plants. Their upregulation suggests that photosynthetic mechanism is not impaired and able to respond to increased demand for energy production under salt stress. The chloroplastic 50S ribosomal protein L12 is a component of the chloroplast translation machinery for the synthesis of chloroplast-encoded proteins for the photosynthetic apparatus. Upregulation of 50S ribosomal protein L12-in both sugarcane cultivars indicates that protein synthesis had increased in response to the salt stress [50].

Triosephosphate isomerase (TPI) is identified from salt sensitive strain which is an important enzyme in primary metabolism, which catalyzes the reaction between dihydroxyacetone phosphate and 3-P glyceraldehyde which feeds into the glycolysis pathway.

Other proteins concerned with energy production were upregulated under salinity. Among them, ATP synthase b subunit and ATP synthase g chain, both related to glucose metabolism in the production of ATP and consequently to the TPI mentioned above. Increase in ATPase subunits another important upregulated stress protein in the sensitive cultivar was 2-Cys peroxiredoxin BAS1. It plays an important role in the elimination of hydroperoxides, both alkyl hydroperoxides formed from lipids by lipoxygenase and of hydrogen peroxide. These are reactive oxygen species and show increased production when the plant is undergoing salinity stress [56].

(MG) is a highly cytotoxic metabolite formed as a result of growth under abiotic stresses in higher plants (Kaur et al., 2014). However, plants have developed an efficient tolerance mechanism to remove an increased accumulation of toxic metabolites, namely ROS scavenging enzymes of the glyoxalase pathway [glyoxalase I (Gly I), glyoxalase II (Gly II) and glyoxalase III (Gly III)]. the EaGly III gene in sugarcane obtaining transgenic lines that exhibited significantly higher water status, gas exchange parameters, chlorophyll, carotenoid, and proline content, total soluble sugars, superoxide dismutase and peroxidase activity compared to non-transformed parental plants under salinity stress [57].

4. Breeding Mechanisms Used For Adaptation

Plant breeding has been used for the development of high productive and stress tolerant crops. The breeders had been relied at intraspecific, interspecific and intergeneric levels to produce lots of salt tolerant crop cultivars. Current sugarcane cultivars are generated as a result of interspecific hybridization between Saccharum officinarum a species with high sugar content and Saccharum spontaneum a wild species tolerant to various abiotic and biotic stresses [58].

4.1 Conventional Breeding

Hybridization techniques are mainly used to generate new recombinants in the sugarcane breeding program, followed by a selection of superior clones with high stress tolerance and cane productivity. The main criteria for the selection of good parental lines should depend upon the response of clones to various biotic and abiotic stresses in addition to the cane yield and sucrose content. By following these approaches, it can be easy to identify the early maturity, high vigorous, and better performing entries ideal

To particular climates. Sugarcane improvement through conventional breeding approaches has been successfully implemented throughout the world. Sugarcane breeding involves mainly two approaches for selection: (i) Individual selection, and (ii) Family selection [59].

Sugarcane being a glycophyte plant exhibits moderate tolerance to salinity and high sensitivity to high salt concentration at various growth stages leads to significant product losses. Salinity tolerant clones accumulate fewer Na+ and higher K+ in plant tissues compared to susceptible clones, and therefore a tolerant clone shows a higher K+: Na+ ratio [60]. Similarly, tolerant sugarcane clones show a higher level of flavonoids accumulation, which is an important antioxidant that protects the sugarcane against ion-induced oxidative stress caused due to salinity. Moreover, seedling priming which is an important practice adopted for sugarcane to improve germination and tillering, is now gaining importance. Priming of seedling with NaCl treatment improves seedlings tolerance against salt stress [61]. The early stages of the crop, such as germination, tillering and cane formation, are more sensitive to salinity than the later stages. It has also been noticed that the ratoon crop is more sensitive than the plant crop of sugarcane, and characteristics such as good germination, high tillering, stem color, pink and waxy stem, number of green leaves and leaf area index (LAI), root-and-shoot ratio, and ratooning potential have a positive correlation with salinity tolerance. The use of salt-tolerant cultivars represents an innovative and cost-effective strategy to sustain crop production in saline environments. Identification and development of crops and their cultivars with improved salt tolerance have been the key to improve productivity. Sugarcane genotypes, due to their divergent genetic background, significantly differ with respect to salinity response [59].

Generally, the continuing conventional breeding efforts have brought a significant change in cane yield and stress resistance, but a lesser gain on sucrose content due to the limited levels of

genetic variations in sugarcane. The limited introgression in sugarcane has resulted in a narrow genetic base of the current commercial varieties. Therefore, in sugarcane breeding, it is still a very important task to broaden the genetic base of sugarcane crops and improve stress resistance and sucrose content by using the gene pool of wild relatives [62].

4.2 Molecular Based Breeding

Molecular markers can be used to track genetic loci related to salt tolerance by using molecular biology techniques without phenotypic measurements. Indeed, several PCR-based markers have been employed to assess the genetic diversity of sugarcane cultivars regarding their relative salt tolerance [63].

Nowadays scientists had been developing breeding technology based on the molecular characteristics of sugar cane to improve quantitative traits. One of the technologies deriving the modern breeding technology are the emergence of next generation sequencing and the finding of single nucleotide polymorphism among sugarcane individuals. The large-scale deployment of NGS and SNP genotyping and the development of SNP analysis algorithms opens the door for the use of markers in sugarcane breeding. In the selection process, the use of a large number of markers with small effects simultaneously can be applied in commercial breeding programs for quantitative traits. This new approach is termed genomic selection (GS). Most papers on genomic selection refer to the first publication of this approach the revolutionary advances in SNP marker discovery and their utilization in commercial breeding enhances the rates of genetic gain and could shorten the breeding cycle in sugarcane. GBS derived SNP markers were used for the first time in marker-trait associations for sucrose content and cane yield. After validation, these markers can be successfully applied in marker-assisted breeding programs [64].

The revolutionary advances in SNP marker discovery and their utilization in commercial breeding enhances the rates of genetic gain and could shorten the breeding cycle in sugarcane. Reports show the successful application of SNP markers in sugarcane breeding. The groundwork for the employment of SNP markers for sugarcane breeding was put forth. High-quality markers are recently discovered based on the reference and were further utilized for QTL mapping for important traits and the identified QTLs are promising for future use in molecular breeding with sugarcane. More over Different molecular markers, such as RAPDs (random amplified polymorphic DNA), RFLPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms), TRAPs (target region amplification polymorphisms), SSRs (simple sequence repeats), and ISSRs (inter simple sequence repeats) have been widely used in modern sugarcane breeding technology [65], [2].

5. Developing Transgenic Sugarcane Resistant to Salinity

Beyond different types of conventional and molecular breeding technologies Genetic engineering is becoming the most significant technique for salt stress tolerant sugarcane genotypes. The technique involves combination of recombinant DNA technology, gene transfer method, and tissue culture techniques [33]. Halophytes such as Aeluropus, Mesembryanthemum, Suaeda, Atriplex, Thellungiella, Cakile, and Salicornia serve as a potential candidate for the salt- responsive genes and promoters. Several known genes like antiporters (NHX, SOS, HKT,

VTPase), ion channels (Cl−, Ca2+, aquaporins), antioxidant encoding genes (APX, CAT, GST, BADH, SOD) and some novel genes such as USP, SDR1, SRP etc were isolated from halophytes and explored for developing stress tolerance in the crop plants (glycophytes) [7].

The high ploidy and complex sugarcane genome structure creates challenges for developing elite cultivars with higher productivity and stress tolerance by conventional breeding. Consequently, the development of transgenic plants through insertion of genes that confer salt tolerance is a feasible strategy for the cultivation of sugarcane. Transgenic sugarcane plants expressing the heterologous Vigna aconitifolia P5CS gene controlled by the stress inducible promoter AIPC (ABA-inducible promoter complex) gave tolerance to salt stress conditions. The transformed sugar cane line had expressed P5CS gene and conferred to have the following physiological traits under salinity stress 25% of more proline was produced,VaP5CS mRNA content became highest, lipid peroxidation increased, photosynthesis efficiency remained constant (PII level remained constant), Na+ accumulation reduced [11].

The first transgenic sugarcane plant was obtained by Bower and Birch in 1992 who described a simple and efficient system of micro projectile bombardment of embryogenic callus, obtaining transgenic plants with selectable genes. This procedure was optimized during the following years and transgenic plants with a commercial trait (herbicide resistance) were obtained [57].

DREB2 is one of stress tolerant genes play a crucial role in providing tolerance to multiple stresses and display overlapping responses to different biotic and abiotic stress conditions. These genes control the expression of stress-responsive genes through the ABA-independent pathway in abiotic stress. Recent reports are indicating that DREB2a are over expressed by twofold increase of underwater stress in E. arundinaceus compared to the moderately drought-tolerant sugarcane cultivar. Similarly, Reis et al. (2014) [66] have recently reported the overexpression of AtDREB2A in sugarcane imparting drought tolerance, salinity tolerance of DREB2 in sugarcane. DREB2 is the major transcription factor that binds to the cis-acting elements of most of the osmotic-stress- inducible genes responsible for osmotolerance to the plants under stress conditions [67]. Augstin et al 2015a [68] has done transformation experiment by using DREB2 gene. The DREB2 gene was isolated from E. arundinaceus using gene-specific

primers designed from Sorghum bicolor and Zea mays through polymerase chain reaction. The pCAMBIA1305.1

Vector was restricted with BamHI and NcoI restriction enzymes to release the CaMV 35S promoter driving the GUS gene and to insert Port Ubi 2.3 promoter.

After sequencing and confirmation, the full-length sequence of EaDREB2 was amplified with two specific primers: forward, 50-GATTACTAGTATGGAGCTGGGA GACGC-30 (SpeI site underlined) and reverse, 50-GAT TGCTAGCCTAATAT-GCAAAAAGGCTAAACCCA-30 (NheI site underlined), and cloned into pPORT-UBI2.3- GUS in place of the GUS gene. The resultant construct pSBI-DREB2 was transferred to Agrobacterium tumefaciens LBA4404 by the freeze–thaw method (Sam- brook and Russel 1989). For Agrobacterium-mediated transformation in sugarcane variety, the method described by Arvinth et al. (2010) was followed, and transgenic plants were selected on hygromycin (50 mg/l) selection medium. Finally Standard PCR technique was used to detect the presence of the transgene in regenerated putative transgenic sugarcane plantlets.

Augustine et al (2015b) [69] isolated the gene coding for HSP70 from E. arundinaceus and incorporated it into the sugarcane genome. They demonstrated that overexpression of EaHSP70 enhances tolerance to drought and salt stresses in hybrid sugarcane as the first report on the overexpression of HSP70 conveying salinity and drought tolerance in sugarcane crop pant. Among different transformation methods particle bombardment and Agrobacterium- mediated gene transformation are the most common methods applied in sugarcane due to a higher transformation efficiency.

6. Genome Editing

Now adays biotechnologists have developed specific and precise genome editing approaches for plants replacing random mutagenesis. Genome editing is the most recent technique and it is based on the activity of sequence- specific engineered nucleases and takes advantage of the DNA repair system that exists inside each cell [70]. These designed nucleases target specific DNA sequences and provoke double-stranded breaks (DSB) which are repaired either by non- homologous end joining (NHEJ) or homology directed repair (HDR), resulting in diverse outcomes, such as site- directed mutagenesis, gene replacement, nucleotides insertions or deletions.[40]

Genetic modification is insertion/deletion of DNA sequence in an organism mediated by four nuclease families namely, meganucleases, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease (CRISPR/Cas9 technologies). CRISPR/Cas9 is the adaptive immunity of prokaryotes against invading genomes or viral predators, established by memorizing the previous infections and integrating the spacers (short sequences of the invading genomes) within the CRISPR locus. Integrated complexes, termed crRNAs (CRISPR RNAs), are then used by Cas nucleases for targeting invader sequences in case of reinfection. This ability of CRISPR/CAS9 to target the DNA sequences through programmed RNAs opened new approaches in genome editing [71].

Although there are many types of genome editing techniques are developed the success is limited due to the complex genome, large genome size, highly polyploid nature of the plant. Generally genome editing with genomic-assisted breeding could contribute significantly to reduce the impact of abiotic stresses on future scenarios of sugarcane cropping [3].

7. Conclusion

Sugarcane is economically important crop plant in the world for the production of sugar, ethanol and biofuel. However, its productivity is highly affected by abiotic environmental stresses including salinity. Salinity of the soil through different reasons affect the overall productivity of sugarcane. Salinity stress for sugarcane triggers different morphological, biochemical and physiological changes as adaptation mechanisms. Furthermore, scientists have been developing modern biotechnological techniques including breeding, genetic transformation and genome editing to overcome salinity stress and aimed to increase the overall yield of sugar cane around the world.

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