REVIEW

GROWTH AND DEVELOPMENT OF SUGARCANE UNDER SALINITY

S. Vasantha* and R. Gomathi

Abstract

Sugarcane is grown in India in about 5.0 million hectares, and one fourth of the acreage is affected by salinity and alkalinity. Long term salinity effects were studied in tolerant and sensitive genotypes of sugarcane in order to understand the mechanism of salinity tolerance. Water and osmotic potentials were distinctly different between tolerant and sensitive genotypes during grand growth phase (150-240 DAP). Failure of osmotic adjustment (with only marginal increase in osmotic potential in saline condition) coupled with reduced photosynthetic rate resulted in poor dry matter production in sensitive genotypes viz., Co 97010, Co 95007 and Co 97009. On the contrary, the tolerant genotypes exhibited better osmotic adjustment, minimal reduction of photosynthesis and less reduction in biomass production. The long term maintenance of water status, osmotic adjustment, maintenance of high photosynthetic rate and biomass production are essential features for a sugarcane genotype to perform as tolerant type. Further, lower lipid peroxidation and higher membrane stability, are related to the levels of activity of antioxidant enzymes particularly POX and APX both through either induction of new isoforms or / and enhanced activities, provide protection under saline conditions in tolerant genotypes. Cane length, girth and number of internodes showed reduction due to salt treatment, which ultimately reduced the cane weight and yield. Cane yield reduction was from 20% in tolerant types viz., Co 99004, Co 85019, Co 94012 and Co 86032 to 45% in sensitive genotypes. Well pronounced differences in jaggery quality as indicated by net rendement value and colour were observed among the tolerant genotypes. Under high soil salinity, the tolerant genotypes Co 85019, Co 94008 and Co 97008 produced jaggery with poor grade, colour and taste since the juice quality characters were affected. The genotypes Co 94012 and Co 99004 produced good quality jaggery even under salinity as sodium and chloride content increased only marginally while cane yield and juice quality were not affected. The studies indicated that salinity tolerance in sugarcane involves improved osmoregulation, ROS enzyme activities as well as less reduction in photosynthesis, biomass production, cane yield and sucrose % juice. However, jaggery characteristics differ widely even among the tolerant types. Wherever juice Na and Cl content is less the jaggery qualities are maintained, while higher Na and Cl content produced poor quality jaggery.

***Key Words**: Salinity, osmoregulation, jaggery quality, sodium, biomass production, ROS enzymes, genes and transcripts*

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Introduction

Soil salinity threatens agricultural productivity globally in 77 M ha of agricultural land, including 45 M ha of irrigated (20% of irrigated area) and 32 M ha of unirrigated (2.1% of dry land) fields all over the world (Munns, 2002). The salts that largely contribute to salinity include the chlorides and sulphates of sodium, calcium, magnesium and potassium while alkalinity is imparted mainly by sodium carbonate. The electrical conductivity of these soils is more than 4dS m^{-1} and in such soils plants fail to absorb water

and nutrients in adequate quantities due to high osmotic pressure of the soil water. The salinity effects are aggravated when irrigation water becomes scanty and EC of irrigation water is high (>3.0). With heavy rainfall, a temporary relief of salt stress could be observed due to leaching of the salts from root zone. Sugarcane is grown in India in about 5.0 million hectares and about one fourth of the acreage is affected by salinity, alkalinity and (saline) irrigation water. The crop is ranked moderately sensitive to salinity with a threshold value of 1.4 dS m^{-1} (Maas, 1986). Soil root zone EC below 2.0 dS m^{-1} shows no effect on growth and yield; at 5.0 - 7.0, the yield decreases by 50 % and at EC of 8.0, stools of some cultivars do not survive and are killed. A yield reduction up to 60% has been recorded due to salinity. The symptoms of salt damage are pale green or yellow leaves, scorched tips and margins, reduced leaf area and stunted canopy.

Relative salt tolerance of sugarcane at various growth stages

Many experiments conducted over several decades showed sett germination (bud sprouting) to be the most resistant phase whereas shoot growth following germination is the most sensitive phase to salinity in the life of a sugarcane plant (Liu, 1967; Chowdhury et al. 2001; Kumar and Naidu, 1993; Kumar et al. 1994). Plants with two or more fully expanded leaves are more resistant than those with one fully expanded leaf. Shoot growth inhibition in sand culture starts even at a level of 30 meq salts/l whereas root growth inhibition starts by 100 meq salts/l during settling growth indicating root growth to be more resistant than shoot growth. Salinity reduced tillering and other growth parameters, leaf/ shoot elongation being the most sensitive and leaf/ internode number being the least sensitive parameter (Vasantha, 2007). Sugar accumulation in the cane, even though invariably reduced, may not show its effect upon juice analysis of the harvested canes in terms of sucrose % juice because reduced internode growth at moderate levels of salinity may compensate for reduced accumulation of sugars (Vasantha et al. 2008b).

Sett germination

Germination is delayed progressively with increasing salinity and reduction in final germination percent was observed at higher salinity levels (EC >5 dSm^{-1}). Varietal response to salinity varied with the temperature and temperatures below 25°C were more damaging under saline conditions. In sand culture, levels up to 200 meq salts/l did not affect emergence but further growth of the shoot was inhibited at levels 30 meq salts/l and above and the settlings did not survive at levels of 200 meq salts/l beyond two leaves stage (Kumar and Naidu 1993). Growth of leaf blades showed maximum reduction compared to stem and sheath whereas growth of sett roots was the least affected during germination phase (Kumar et al. 1994). Reduced germination with biomass variation for root and shoot has been well documented in several works (Liu, 1967; Chowdhury et al. 2001; Kumar and Naidu, 1993).

Soil salinity has a profound impact on the crop growth especially on germination. Germination was delayed under salt treatment and reduction in final germination percent was observed at higher salinity level (EC >5 dS m^{-1}). Higher concentration of NaCl, i.e. 0.5 N completely inhibited germination while at moderate levels the germination was reduced (Rizk and Normond, 1969). Higher reduction in germination of setts with increasing salinity levels has been reported in sugarcane. Kumar and Naidu (1993) observed that soil salinity was more damaging for germination of setts at low temperature (below 25°C). Varietal response is a critical factor in determining the final germinant. Genotypes like Co 97010 and Co 95007 recorded a reduction of over 50% in germination indicating their sensitivity. During germination and early growth of the crop, nitrogen requirements are more, as sink production is at its peak. Nitrate reductase, an important enzyme in N-metabolism, showed significant reduction in salinity treatment (Jasmine Rani et al. 2004). Sett treatment with GA_3 (150 ppm) showed improvement in germination percent in saline soil (Gomathi and Thandapani 2005a).

Tillering and early growth

Tiller production per main shoot decreases under saline as well as sodic conditions. In a study with 10 popular varieties, the reduction in tiller production due to salt treatment ranged from 16.3% in Co 6304 to 49.8% in Co 86010 (Anon, 1996a). Shoot population was reduced resulting in poor and patchy

field stand. Shoot height, number of internodes, number of leaves and leaf area per plant were significantly less in saline soil. Decreased or nil expansion growth of leaves and young internodes results in stunted canopy and poor tillering leading to poor crop. Varieties exhibiting better tolerance (Co 7717, Co 86011 and Co 6806) had more tillers/ shoots (Muniasamy, 1998). Reduction in tillering due to soil salinity as well as high salt concentration in irrigation water has been reported in sugarcane (Robinson and Worker, 1965; Syed and El-Swaify, 1972). Restricted growth in terms of reduced shoot height and less green leaf production for photosynthesis was reported (Joshi and Naik 1977 and 1980; Naik and Joshi 1981). A reduction in the elongation and expansion of sugarcane leaves under salinity has been attributed to a lowered efficiency of growing tissues to utilize sugars for growth (Kumar et al., 1994). Shoot growth rate was reduced even under mild salinity (EC of 2dSm^{-1}) in different cultivars of sugarcane (Meinzer et al.1994).

Ion-toxicity was the main determinant of salt tolerance at the grand growth stage while the osmotic component of NaCl mainly appeared to affect the transport of sucrose to stalks, followed by stimulated sucrolytic activity in the internodes, resulting in reduced final cane yield (Wahid, 2004).

Yield and quality characters as influenced by salinity

Salinity reduced the number of millable canes up to 37% in popular genotypes whereas tolerant genotypes recorded less reduction. Cane length, girth and number of internodes showed reduction due to salt treatment which ultimately reduced the cane weight and yield**.** Cane yield recorded significant reduction of up to 64% in sensitive genotypes while it was marginal (27%) in tolerant types (Anon, 2003). Decrease in cane yields of the order of 5.45 t/ha for every 1 dSm^{-1}ha^{-1} was experienced due to soil salinity. Experiments conducted at Sugarcane Breeding Institute, Coimbatore, over the years have shown that soil salinity had reduced single cane weight, cane length and cane diameter (Anon, 1994 and 1996a; Vasantha and Gururaja Rao, 2005). Further, Thomas et al. (1981) found that under mild salt stress condition (4 dsm^{-1}), the individual cane weight was not affected in resistant variety NCO 310. However, Syed and El-Swaify (1972) observed a significant reduction in single cane weight when the EC of irrigation water increased from 2.0 to 8.0 dsm^{-1}. Several studies have also shown that salt stress reduced the number of internodes, cane length and internodal length which depends on genotypes (Rozeff 1995; Dang et al. 1998) and the detrimental effects of excess salt on sugarcane are greater on the cane and sugar yields than sugar recovery.

Juice and jaggery quality characters as affected by salinity

Sucrose % juice, Brix and purity are reduced by salinity. Increased non-sugar solids and salts reduce the purity. The salt content of cane juice ranges from 900 -1 900 ppm in non-saline soils whereas it ranges from 4000-4500 ppm in saline soils. The electrical conductivity of the juice at harvest increased in all the genotypes under saline conditions due to irrigation with saline water; increased accumulation of Na, K and Cl ions caused a reduction in sucrose per cent juice due to salinity. In varieties Co 94012, Co 99004 and Co 97001, the increase in Na and EC was marginal and consequently there was not much reduction in sucrose% juice due to salinity (Vasantha et al. 2009).

In order to study the effect of salinization on sugar accumulation, Co 7717 plants growing under normal conditions were exposed to saline water irrigation. Brix value after 25 days of salinization decreased only in the upper young immature internodes and not in the rest of the mature internodes. Juice analysis after two months indicated that sucrose decreased only in the upper young immature portion of the cane and not in the mature middle or basal portion. An analysis of 34 genotypes (grown under saline conditions from the time of planting) showed reduced sucrose % juice in the upper and/or basal portions of canes of all but four genotypes tested. These studies indicated that (i) sucrose accumulation in canes is reduced by salinity, (ii) the capacity of canes to accumulate sugars in the mature portions is not appreciably affected by moderate level of salinity and (iii) salinity delayed maturity in sugarcane. So the juice quality may not be appreciably affected in some clones by moderate salinity level even though sugar yields would decrease because of reductions in cane yields. The

cane maturity is delayed by salinity. In some genotypes the juice quality is severely affected so also the sugar yield (Sharma et al. 1997).

Sugarcane clones vary significantly with respect to their Na, K and Cl concentrations in the juice. Concentration of Na is generally below 10 mM whereas that of K and Cl may go up to a maximum of 150 mM under saline conditions (EC 7.5 dSm^{-1}). K and Cl concentrations were negatively correlated with sucrose and purity % of the juice and stalk diameter but were positively correlated with number of millable stalks in inter-specific hybrid (ISH) clones tested. In another study, increasing salinity led to increased Na, K, Mg, Ca and Cl in the juice. Sucrose increased from top to bottom but potassium decreased. Sodium concentration was higher in the lower section (Vasantha, 2007).

About 26% of the sugarcane produced is diverted for the production of jaggery which provides an alternative market to sugarcane growers. The quality of jaggery is dependent on the cane juice which in turn is determined by the variety and the environment in which the cane is grown. Adverse conditions, such as salinity, drought, etc. affect cane yield and quality. Among the tolerant genotypes, variations for quality parameters have been recorded which determine their relative suitability for jaggery. The electrical conductivity of the juice at harvest increased in all the genotypes due to increased accumulation of Na and K ions causing a reduction in sucrose per cent juice. In genotypes Co 94012, Co 99004 and Co 97001, the increase in Na and EC was marginal and consequently there was not much reduction in juice sucrose content under salinity. Increase in EC of the cane juice was observed in crop irrigated with saline water. However, well pronounced differences in jaggery quality as indicated by net rendement value and colour were observed among the tolerant genotypes among which Co 85019, Co 94008 and Co 97008 produced jaggery with poor grade, colour and taste. The genotypes Co 94012 and Co 99004 produced good quality jaggery even under salinity as sodium and chloride content increased only marginally while cane yield and juice quality were not affected. There was no setting of jaggery in the genotype Co 95003 under salinity while good quality jaggery was produced under normal conditions. The colour and taste of the jaggery are the choice of the consumer which ultimately decide its market value. In the context of sizeable area of sugarcane being grown under saline soils, there is a need for identification of genotypes like Co 94012 and Co 99004 capable of producing good quality jaggery under saline conditions. Content of Na in juice should be given greater priority than salinity tolerance *per se* in identifying the suitability of genotypes exclusively for jaggery making purpose (Vasantha et al. 2009).

Root mass under salinity

Tolerant genotypes had better root penetration, mass and density than sensitive genotypes (Plate 1 & 2) in pot culture experiments. Live and rope types roots were noticed in tolerant types under salinity while the roots were less dense and fresh in sensitive genotypes. The improved root mass apparently supported more number of canes in tolerant types under salinity (S. Vasantha, unpubl. data).

Physiological and metabolic behaviour under salinity

In an attempt to equilibrate with the osmotic potential of the soil solution under saline conditions and to increase water absorption, the crop not only accumulates salts but also stress specific osmolytes such as proline, betaine, etc. Osmotic potential of the leaf tissues increased by 50 to 200 mmole kg^{-1} in salt treatment as compared to normal plants. Proline is one of the widely studied osmolytes that is accumulated in response to stress conditions. In popular varieties several fold increase in proline content was observed (Vasantha, 2003).

Salinization leads to decrease in rates of transpiration, stomatal conductance and CO_2-assimilation of all the leaves and the extent of damage increases with time. Gas-exchange measurements suggested that variation in carbon isotope discrimination (delta) was attributable largely to variation in bundle sheath leakiness to CO_2 (phi). Salinity-induced increases in (phi) appeared to be caused by a reduction in C_3 pathway activity relative to C_4 pathway activity rather than by physical changes in the permeability of the bundle sheath to CO_2 (Meinzer et al., 1994). The rates of transpiration continued to decrease

probably due to its effect on stomatal conductance whereas it was not the case with rates of assimilation. The effect appears to be due to their effects on its efficiency to fix CO_2 present in the leaf rather than its deficiency. Accumulation of sugars in the leaves upon salinization appeared to result from reduced rates of their translocation, which in turn appeared to be related with their reduced utilization in the sink tissues. Thus, reduced rates of photosynthesis were not directly responsible for reduced growth under saline conditions. Results of another experiment at grand growth phase revealed that the tolerant clones maintained more or less uniform rates of photosynthesis while the sensitive types showed sharp decline due to salt treatment. Net photosynthetic rates were reduced when the leaf water potential was < -0.9 MPa on diurnal basis, suggesting the sensitivity of the photosynthetic process to water potential gradients. During grand growth phase, the tolerant clones maintained more or less uniform rates of photosynthesis while the sensitive types showed sharp decline due to salt treatment. The reasons for reduced photosynthesis include stomatal closure, and feedback inhibition due to reduced sink activity. A reduction in stomatal conductance may result from the osmotic effects of salinity. Fluctuations in photosynthetic rate during hours of day in stress free environment and stressful environment would account for the variation in net photosynthetic rate and photosynthate production. Long term salinity effects were studied in tolerant and sensitive genotypes of sugarcane in order to understand the mechanism of salinity tolerance. Water and osmotic potentials were distinctly different between tolerant and sensitive genotypes during grand growth phase (150-240 DAP). Failure of osmotic adjustment (with only marginal increase in osmotic potential in saline condition) coupled with reduced photosynthetic rate resulted in poor dry matter production in sensitive genotypes, *viz*. Co 97010, Co 95007 and Co 97009. On the contrary, the tolerant genotypes exhibited better osmotic adjustment, minimal reduction of photosynthesis and less reduction in biomass production. The long term maintenance of water status, osmotic adjustment, maintenance of high photosynthetic rate and biomass production are essential features for a sugarcane genotype to perform as tolerant type (Vasantha et al. 2010).

In shoot tip raised plantlets, increasing levels of NaCl reduced total chlorophyll (Chl a, Chl b and carotenoids) which possibly resulted in reduced dry weight/shoot. At soil salinity of 8 dSm^{-1}, photosynthetic rates did not vary significantly among commercial genotypes during formative phase; however, the differences became significant at grand growth phase (Gomathi and Thandapani 2005b; Merlin 2008).

Total biomass on an average was reduced by 41% under salt treatment with tolerant clones showing only a moderate reduction of 28 and 17% during formative and grand growth phases, respectively whereas the sensitive clones showed reductions of 60 and 71% at formative and grand growth phases, respectively. In sugarcane, the major share of biomass is diverted towards stem after the completion of grand growth phase. Under saline conditions, the response of genotypes varied with respect to allocation of dry mass towards the stem. In tolerant types, the percent stem dry mass remained more or less uniform both in control and salt treatment whereas in sensitive types, the dry matter allocation to stem was reduced sharply. Biomass accumulation varied in accordance with the potential of a genotype during grand growth phase. The photosynthetic and transpiration rates and leaf water potential showed little difference during the early stage of crop but the tolerance of the clones to salinity was determined by their performance later during the grand growth stage (Vasantha et al. 2010).

Nitrate reductase activity decreased under salinity in all the genotypes with more than 30% reduction in sensitive clones as compared to <20% reduction in tolerant ones. Chlorophyll content was much lower in the sensitive clones due to yellowing of the leaves whereas it was more or less similar in control and salt treated ones indicating an effective pigment protective mechanism. Polyamines accumulated to a greater extent in salinity tolerant genotypes than in the sensitive clones (Gomathi and Thandapani, 2005c; Dhivya 2011; Jennifer Sathiya 2012).

Lipid peroxidation and cell membrane injury

Malondialdehyde (MDA), a lipid peroxidation product varied from 0.85 µg g^{-1} to 1.667 µg g^{-1}in control while it varied from 1.28 to 2.51µg g^{-1} under

saline conditions. Tolerant genotypes recorded an average increase of ~28% in MDA while sensitive genotypes recorded nearly double the increase of ~57% thereby indicating greater damage to the membrane system. Cell membrane injury test conducted with popular varieties showed significant variation in their tolerance capacity. Cell membrane stability is a measure to test the membrane's biophysical / biochemical properties. Under stress situations, the cell membrane loses the selectivity towards ions and macromolecules resulting in heavy influx / efflux of essential ions in and out of cells. A resistant genotype maintains the cell membranes selectivity and thereby supports the maintenance of growth and metabolism. Cell membrane injury test conducted with popular varieties showed significant variation indicating their tolerance capacity. In tolerant genotypes, the MDA content increased by 36% while in sensitive genotypes the increase was 57% (Vasantha et al. 2008a).

Oxidative enzymes under salinity stress

Peroxidase activity: Peroxidase activity increased by 1.6 - 5.1 folds in response to salinity. The increase was highest in tolerant genotype (Co 85019) and less in sensitive genotype (Co 95016). Pox isoforms (cytosolic) were recorded in leaf sample. It is interesting to note that two low molecular forms with faster mobility were induced under higher salinity level only in tolerant genotypes, suggestive of its role in tolerance behaviour. Isolated chloroplast lysate also showed induced isoforms under high salt condition (Vasantha et al. 2008a).

Super Oxide Dismutase activity : SOD activity increased marginally in response to high salt condition in varieties Co 85019 and Co 95003 and in other varieties the activity was on par with control plants. SOD isoforms (five in all) were similar in both control and salt treatment. A single isoform each was located in chloroplast and mitochondrial lysate. Neither treatment nor genotypic influence could be detected in isoforms of SOD or in its activity (Vasantha et al. 2008a). Ascorbate peroxidase activity increased by two folds in tolerant varieties while in sensitive types the increase was only marginal. However, APX isoforms failed to show any variation due to high salt treatment (Vasantha et al. 2008a).

Enzymes of sucrose metabolism : The enzymes of sucrose metabolism, *viz*. sucrose synthase, and sucrose P synthetase activity declined due to salinity. The tolerant genotypes showed relatively less reduction (Gomathi and Thandapani, 2004).

Movement of ions within sugarcane plant

Analysis of the germinating buds and sett roots indicated that uptake of both Na^+ and Cl^- was checked in the roots but not in the case of buds which may be responsible for greater sensitivity of the shoot growth than the root growth during sett germination phase. Potassium levels did not appear to be affected. An analysis of the shoot roots later showed concentration of Na^+ to gradually increase only up to 6 cm away from the root tip. Na was least in the tip region whereas K^+ being not affected by saline conditions, was the highest in the tip region; K^+ decreased only slightly as we go away from the tip region. As the tip region is actively dividing with least development of vacuoles, high K^+/Na^+ ratios favor growth and indicate the successful operation of an efficient K^+-uptake system (HKT genes) even in the presence of high NaCl in the media. Storage of Na^+ in the vacuoles of mature cells is an option to keep Na^+ away from the cytosol and helping the cells to take up water under saline conditions. Long term studies indicated that these patterns of K^+ and Na^+ are maintained with time. This indicates that once the roots fill their vacuoles up to a desired level, the rest of the Na^+ and Cl^- ions are sent to the shoot. A Na^+ exclusion system as operative in other members of the Gramineae such as wheat, rice and barley may also be present in sugarcane since it was observed that sodium entering the shoot was specifically retained in the basal internodes, mainly in exchange for K^+, restricting its concentrations further in the upper leaves and internodes where it was quite low as compared to Cl^- and K^+. Young emerging tillers had lower Na^+ and higher K^+ than the shoot tissue from which they are emerging. This seems to be an adaptive response of the shoots to saline conditions (Anon 1996b). Ion-toxicity was the main determinant of salt tolerance at the grand growth stage while the osmotic component of NaCl mainly appeared to affect the transport of sucrose to stalks, followed by stimulated sucrolytic activity in the internodes, resulting in

reduced final cane yield (Wahid 2004). Micro nutrient availability was restricted under salinity conditions resulting in deficiency of nutrients (Gomathi and Thandapani, 2005d).

Progressive stress responses enlighten us about the metabolic changes during stress adaptation in tolerant types and possible flaws that reflect on the metabolic failures resulting in sensitive behaviour. The salinity (NaCl) effect was noticed in the sensitive variety Co 95007 on day two as poor growth. The visual symptoms, i.e. yellowing of leaves and salt injury in leaves were noticed on day seven in the sensitive variety. Progressive stress responses were studied in contrasting sugarcane genotypes to elucidate the stress adaptive features with regard to physiological and biochemical characters. Varieties showed differences with respect to parameters studied from day four. The tolerant variety Co 85019 maintained stability of plastid pigments (chlorophyll and carotenoids), higher proline level and increased activity of the oxidative enzymes POX and SOD. The sensitive genotype suffered heavy loss with regard to these characters. Lipid peroxidation, a measure of damage to the membrane system, was high in the sensitive variety and difference between genotypes became significant from day four, indicating the progressive nature of adaptation in tolerant variety and its failure in sensitive variety (Vasantha and Rajalakshmi 2009).

Molecular studies on salinity tolerance

The direct introduction of small number of genes by genetic engineering seems to be a more attractive and rapid approach for improving stress tolerance (Cushman and Bohnert, 2000). Present day biotechnological strategies rely on the transfer of one or several genes that encode either biochemical pathways or endpoints of signaling pathways. These genes/products protect either directly or indirectly against environmental stresses. Engineered over expression of biosynthetic enzymes for osmoprotectants, scavengers of reactive oxygen species and stress induced proteins, late embryogenesis abundant proteins are among the attempts made (Srinivasulu et al. 2007). Amino acid proline (Kavi Kishore et al. 1995), polyamines and sugars such as trehalose were the most frequently used genes. Annexin possesses a property of scavenging hydrogen peroxide (Gidrol et al. 1996). The role of annexin in countering the stress by H_2O_2 is attributed to its N-terminal similarity with plant peroxidases. Ascorbate peroxidase is a scavenger of reactive oxygen species during stress conditions.

The transciption factor DREB1A specifically interacts with the DRE and induces expression of stress tolerance genes. Yamaguchi-Shinozaki and Shinozaki (1994) showed that overexpression of the cDNA encoding DREB1A in transgenic *Arabidopsis* plants activated the expression of many of the stress responsive genes under normal growing conditions and resulted in improved tolerance to drought, salt loading and freezing. Expression of DREB1A from the stress-inducible rd29A promoter gave rise to minimal effects on plant growth while providing an even greater tolerance to stress conditions than did expression of the gene from the CaMV promoter. As the DRE-related regulatory element is not limited to *Arabidopsis*, the DREB1A cDNA and the rd29A promoter may be useful for improving the stress tolerance of agriculturally important crops by gene transfer.

Changes in gene expression induced by salinity in a suspension culture showed specific proteins and the mRNAs altered by salinity were identified. Multitude of mechanisms at the transcriptional, post transcriptional and post translational levels may contribute to the control of gene expression in the salt-adapted sugarcane cells (Ramagopal and Carr, 1991). Transcript expression of mature *miR159* was studied in sugarcane leaves stressed for long-term (15 days) with NaCl (150 mM) or iso-osmotic PEG 8000 (20% w/v) and for short period (up to 24 h) with NaCl (200 mM) or PEG (20% w/v). The results revealed no significant changes in transcript levels of the miRNA in response to the long-term stress. However, short-term salt or PEG stress led to significant up-regulation over the control. The transcript expression of the *MYB*-related gene indicated up-regulation at 1 h of salt stress with concomitant slight down-regulation of the miRNA. In addition, under short-term PEG stress, the transcript levels of *MYB* and *miR159* were the opposite of each other, suggesting *MYB* as a potential target of *miR159 (*Patade and Suprasanna, 2010).

Semi-quantitative RT-PCR based transcript expression of stress responsive genes was studied in leaves of sugarcane plants exposed to short-term (up to 24 h) salt (NaCl, 200 mM) or polyethylene glycol-PEG 8000 (20% w/v) stress. Transient increase in expression of NHX (sodium proton antiporter), SUT1 (sucrose transporter1), PDH (proline dhydrogenase) and CAT2 (catalase2) was observed in response to 2-4 h PEG stress. However, salt stress imposed repression of NHX, PDH and CAT2 at these time points. The transcript level of the delta (1)-pyrolline-5-carboxylate synthetase (P5CS) increased slightly in salt treatment while in response to the PEG stress, the gene expression increased at 4 h treatment but then decreased considerably by 80% at 24 h. Thus, differential regulation of these stress responsive genes in response to salt or PEG stress indicates existence of different strategies of stress tolerance in sugarcane. Understanding transcript gene expression patterns of the stress responsive genes may provide insights into complex regulatory network of stress tolerance (Patade et al. 2012). The comparative transcript responses to salt stress were monitored by ribotyping of both treated and untreated sugarcane plants at early growth stage. Among 335 differentially expressed transcript-derived fragments, 156 up- and 85 down-regulated were functionally categorized as metabolism, DNA/RNA/ cellular processes, signal transduction/cell rescue/ defense, cell wall modifications, transcriptional regulation, transport/trafficking, retro elements and unknown/hypothetical proteins (Pagaria et al. 2011). As a singular work, SodERF3 gene from sugarcane induced in response to high salinity has been introduced in tobacco and the transformant has shown improved tolerance to salinity without any adverse morphological changes (Luis E Trujillo et al. 2009).

Strategies adopted by plants to become salt tolerant

Exclusion of selected salt ions rather than internal tolerance appears to be the preferred mechanism. Obtaining tolerance to accumulation of sufficient solutes/salts within cells means cells filled with salt have no space for sugar, which is a biophysical fact. To duplicate the mechanisms of drought tolerance would be a futile effort as deeper root system/ reallocation of carbohydrates is practically not possible to obtain. However, scope exists in sugarcane for improving the performance in terms of cane yield under saline conditions, as evidenced by the wide varietal variation, through incorporation of tolerance factors.

The studies reviewed indicated that salinity tolerance in sugarcane involves improved expansion growth of leaves and shoot, better internode and stalk length at harvest, osmoregulation, ROS enzyme activities as well as less reduction in photosynthesis, biomass production, cane yield and sucrose % juice. However, jaggery characteristics differ widely even among the tolerant types. Wherever juice Na and Cl contents are less, the jaggery qualities are maintained while higher Na and Cl contents produced poor quality jaggery. Obtaining improved tolerance through biotechnological means is no more an unattainable task; however, focus should be on identifying genes expressed in tolerant genotypes.

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