Pak. j. life soc. sci. (2003), 1(2): 114-121

Pakistan Journal of
Life and Social Sciences

Soil Salinity Effects on Sugarcane Productivity, Biochemical Characteristics, and Invertase Activity

Altaf Hussain, Zafar Iqbal Khan , Muhammad Hamid Rashid[1], Muhammad Ashraf and Muhammad Saeed Akhtar.
Department of Botany, University of Agriculture, Faisalabad – Pakistan.
[1]National Institute for Biotechnology & Genetic Engineering (NIBGE) Faisalabad – Pakistan

Abstract

An effort was made to draw relationship among acid invertases, biochemical characteristics, growth of sugarcane under salinity stress. COJ-84 was found to be more productive as compared to CP-77-400 under saline conditions. Low productivity of CP-77-400 might have been due to the inhibition of its invertases by high concentration of absorbed metal ions. Specific activity values of invertases under saline conditions suggest that accumulation of high concentration of metal ions in sink cells might have affected the expression of invertase genes.

Key words: Sugarcane, Bio-chemical characteristics, Productivity, Invertase, Gene expression

Introduction

Modern sugarcane cultivars are multispecies hybrids, primarily of *Saccharum officinarum L.*, *Saccharum spontaneum L.*, and *Saccharum robustum* Brandes et Jeswiet ex Grassl (Zhu *et al.*, 1997; Ming *et al.*, 2002), with high sucrose content, low fiber content, thick stalks, little pubescence, rare flowering, and limited tillering (Ming *et al.*, 2001).

The ripening of sugarcane is associated with an increase in sucrose concentration in mature stalk tissue (Botha and Black, 2000). In fully mature internodes, the sucrose content can be more than 50% of the total dry weight. Despite numerous studies, biochemical basis of sucrose accumulation in sugarcane is still poorly understood (Moore, 1995). Invertases, a family of enzymes that hydrolyse sucrose into hexoses, have been proposed to carry out critical functions during sucrose accumulation by sugarcane (Ma *et al.*, 2000). Sucrose stored in sugarcane internodes is re-synthesised from the breakdown products of translocated sucrose that is primarily cleaved by invertase (Hatch, 1963).

Sucrose plays a central role in plant growth and development. It is a major end product of photosynthesis and functions as a primary transport sugar, and in some cases as a direct or indirect regulator of gene expression (Winter and Huber, 2000).

Corresponding author: Altaf Hussain
Department of Botany, University of Agriculture, Faisalabad – Pakistan
E.Mail: ashrafm@fsd.paknet.com.pk.

Research during the last two decades has identified the pathways involved and which enzymes contribute to the control of flux. Availability of metabolites for sucrose synthesis and demand for products of sucrose degradation are important factors. In terms of sucrose degradation, invertase catalyzed hydrolysis generally has been associated with cell expansion. Metabolism of sucrose, a mobile source of energy and carbon, is an absolute requirement for the survival of heterotrophic plant organs (Sturm *et al.*, 1999).

Carbohydrates are synthesized in photosynthetically active source tissues and exported, in most species in the form of sucrose, to photosynthetically less active or inactive sink tissues. Sucrose hydrolysis at the site of utilization contributes to phloem unloading. This phenomenon links sink metabolism with phloem transport to, and partitioning between sinks. Invertases catalyse the irreversible hydrolysis of sucrose and thus are expected to contribute to carbohydrate partitioning. Different invertase isoenzymes may be distinguished based on their intracellular location, their isoelectric points and pH optima (Roitsch *et al.*, 2000). Extracellular, cell wall bound invertase is uniquely positioned to supply carbohydrates to sink tissues via an apoplastic pathway, and links the transport sugar to hexose transporters. A number of studies demonstrate an essential function of this invertase isoenzyme for phloem unloading, carbohydrate partitioning and growth of sink tissue. Extracellualr invertases were shown to be specifically expressed under conditions that require a high carbohydrate supply to sink tissues. Substrate and reaction products of invertases are not only nutrients, but also signal molecules like hormones and in combination with hormones and other stimuli, they can regulate many aspects of plant development from gene expression to long distance nutrient allocation.

The level and timing of sucrose accumulation in the whole stalk and within individual internodes is correlated with the down- regulation of soluble acid invertase (SAI) activity above which high concentration of sucrose do not accumulate (Zhu *et al.*, 1997). This low level of SAI activity always exceeds in the internodes of the lower sucrose storing genotypes.

Since sugarcane is the prime source of sugar production in Pakistan, it is imperative to investigate the physiological and biochemical basis of yield reduction under saline conditions.

Materials and Methods

Sugarcane varieties

Two local varieties of sugarcane (*Saccharum officinarum* L.) CP-77-400 and COJ-84 were obtained from the Directorate of Sugarcane, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan.

Cultivation of canes

The canes were grown in field plots (2 x 1.5 x 0.5m). Plots were dug in the field and lined with double layer of good quality polythene sheet before refilling with 760 kg of loam soil. The control and each salinity level contained 20 one-eyed sets of each variety. The sets were placed in soil with eye position upward. After proper germination, 15 plants were maintained for each of the salinity levels till the grand growth stage (180 days after sowing) was reached.

Salinity application

At grand growth stage, four salinity levels 50, 100, 150, and 200 mM were developed based upon the full saturation percentage of the soil. The natural level of salinity in the soil was 25 mM (control). The original electrical conductivity of the soil extract (ECe) was accounted for, while developing the salinity levels. Salt solutions were prepared by dissolving NaCl in tap water. Salinity (NaCl) levels were accomplished by a daily increment of 20 mM until the final required levels were attained. The plots were flooded with water overnight to ensure thorough and uniform distribution of salt. The plots were irrigated with underground tube-well water (salt conc. 8 mM) as and when needed. ECe was monitored weekly to maintain the desired levels. Recommended doses of N,P, and K, i.e., 150, 100 and 100 kg per hectare, respectively, were applied to the plants in order to avoid possible effects of nutrient deficiency. The canes were grown for a period of three months under saline conditions.

Harvesting

After three months of growth under salinity, the plants were harvested. After removing trash (dead leaves) from the standing crop, the plants were cut from the soil, washed thoroughly with tap water and immediately stored at 4^{o}C in a cold room.

Effect of salinity on biochemical characteristics of sugarcane

The sugarcane were thoroughly washed with water and then juice was extracted. The juice was filtered through a muslin cloth and then centrifuged at 15000 x *g* at 4^{o}C to remove the solid particles. Following biochemical characteristics were studied.

Total sugars

Total sugar content in sugarcane juice was determined by Dinitrosalicylic acid (DNS) method (Miller, 1959) using glucose as standard.

Glucose assay

Total glucose in sugarcane juice was determined using glucose oxidase based kit (Biocons, Germany)..

Determination of Na^+ and K^+

The concentrations of Na^+ and K^+ in the juice of sugarcane were determined by Flame photometry.

Protein assay

Total soluble proteins in the juice were estimated by Bradford method (1976) using bovine serum albumin (BSA) as standard.

Effect of salinity on invertases

Isolation of invertases

The sugarcane juice was dialyzed against distilled water at 4^{o}C to remove the soluble sugars and salts.

Invertase assay

The activity of invertases was determined using appropriate amount of enzyme in 50 mM sodium acetate (pH 5.5) at 50^{o} C, and sucrose (50 mM) was used as substrate. After 40 minutes of incubation, the reaction was quenched by placing the reaction mixture in boiling water for 5 minutes and then cooled. The amount of product, i.e., glucose released was determined by adding 100 µL of reaction mixture to one ml of glucose oxidase and was incubated at 37^{o}C for 10 minutes. Absorbance was read at 500 nm. To check the amount of free glucose in the substrate and in the enzyme preparation, the assay for the substrate blank and the enzyme blank was also performed.

"One unit of invertase activity was equal to µmoles of glucose equivalents liberated/ml/min at pH 5.5 and 50^{o}C".

Determination of units for invertase activity

Glucose concentration in assay was determined using the following equation:

$$\textbf{Glucose concentration} = \frac{\triangle \text{A of sample}}{\triangle \text{ A of standard}} \times \text{Conc. of standard (100 mg/dl)}$$

Statistical analysis

Data for different biochemical parameters were analysed using MSTAT-C computer program.

Results and Discussion

Biochemical properties of sugarcanes grown under salinity:

Data for different biochemical parameters for both cultivars are presented in Table-1, while, analysis of variance of the data in Table-2. Since, both the cultivars differed markedly under control conditions, the data

were transformed to percent of control (relative salt tolerance) to determine the actual degree of salt tolerance of two cultivars. The yield of total reducing sugars in COJ-84 grown under non-saline conditions (control) was higher than that in CP-77-400. In COJ-84, it was equal to 38.7 g $cane^{-1}$ as compared to 24 g $cane^{-1}$ in CP-77-400. COJ-84 under non-saline conditions absorbed low amount of Na^+ and K^+ from the soil as compared to CP-77-400. The concentration of Na^+ in juice of COJ-84 and CP-77-400 was 0.52 and 0.84 mg ml^{-1}, respectively, while, K^+ concentration in juice was 3.36 and 7.5 mg ml^{-1}, in COJ-84 and CP-77-400, respectively. The specific activity of apo-invertases of CP-77-400 was very high (about 10-fold) as compared to that of COJ-84. On the other hand, *in vivo* activity of invertases of COJ-84 was very high than that of CP-77-400, because the total amount of glucose produced by COJ-84 was 24 g $cane^{-1}$ as compared to that of CP-77-400 having only 10.7 g $cane^{-1}$. Moreover, concentration of glucose in juice in terms of mg ml^{-1} also supported this trend (Table-1 and 2).

The effect of salinity on biochemical properties was significant and like growth parameters both varieties differed significantly. Volume of juice of sugarcanes grown under salinity decreased significantly as compared to control plants. Therefore, the effect of salinity has been attributed in terms of sugars and glucose content per cane juice rather than mg ml^{-1}. The COJ-84 canes, grown at 50 mM NaCl salinity level showed a marked decrease (31%) in sugar content, as well as, in glucose content (32%), as compared to canes of CP-77-400, which gave 65% and 96% recovery for sugar content and glucose content, respectively. Interestingly, CP-77-400 grown at 100 mM salinity level gave 1.54-fold increase in glucose content, while sugar content remained almost unchanged. On the other hand, COJ-84 showed about 50 % reduction, both in glucose and sugar content yield.

The percent yield of sugar content and glucose content for CP-77-400 was slightly affected at 150 mM NaCl treatment, whereas COJ-84 again presented 50% loss for both the parameters. At 200 mM salinity level, COJ-84 showed better recovery for glucose content, as well as, for sugar content as compared to CP-77-400 (Table-1 and 2).

The absorption of metal ions (Na^+ and K^+) at different levels of salinity also differed significantly in both cultivars. CP-77-400 showed less metal ions (Na^+ and K^+) absorption at all the salinity levels as compared to COJ-84. The absorption rate in CP-77-400 was 157 and 125% for Na^+ and K^+, respectively at 150 mM level of salinity, while, COJ-84 at only 50 mM salinity level showed 265 and 233% absorption for Na^+ and K^+, respectively, as compared to the control. The percent increase in Na^+ and K^+ became very high for COJ-84 at 200 mM treatment as compared to CP-77-400 (Table-1 and 2).

The catalytic power of invertases produced under saline conditions also varied significantly. The invertases produced by CP-77-400 at 100 mM level of salinity showed very high activity and gave 7.59 U ml^{-1} as compared to control having only 0.737 U ml^{-1}. The activity of invertases produced by canes at 150 and 200 mM NaCl concentrations also showed activation, while at 50 mM level of salinity, the activity was decreased. In view of some earlier studies, it is evident that under stress conditions, protein content in plant tissues, may increase (Sachs and Ho, 1986; Singh *et al.*, 1987; Bonham-Smith *et al.*, 1988). Similarly, in the present study, total soluble proteins, went on increasing with the increase in salinity level. Specific activity of invertases, i.e., units mg^{-1} protein, of CP-77-400 increased at 100 and 150 mM NaCl treatments, while for other two treatments, it was decreased. The *in vitro* activity of invertases from COJ-84 grown under saline conditions, like CP-77-400, also showed activation trend against all salinity levels. Maximum activation was for invertases, which were obtained from the canes grown at 100 mM NaCl salinity level because they showed highest specific activity. Total proteins were comparatively high for canes grown at 50 mM level of salinity, while all the other treatments showed low amount of proteins. The specific activity values for invertases were reasonably high at 100 and 200 mM level of salinity (Table-1 and 2).

Effect of metal ions on activity of invertases:

The *in vitro* activity of soluble acid invertases from both cultivars was highly inhibited by monovalent (Na^+ and K^+) and divalent (Ca^{2+}) metal ions. The *in vitro* activity of invertases from CP-77-400 showed a sudden fall (60%) at only 0.5 mM concentration of all metal ions while it remained constant at 70 % with a rising and falling trend in activity, up to 9 mM metal ions concentration (Fig-1). Similarly, COJ-84 invertases also showed high inhibition at 0.5 mM metal ions concentration and their activity decreased to only 40%. At higher metal ions concentrations their activity went on increasing and decreasing within a range of 40-60% residual activity (Fig-2).

Sugar accumulation in canes during maturity stage largely depends upon the level of neutral and acid invertases (Venkataramana *et al.*, 1991). Moreover, activity of invertases decreases with the maturation of internodes (Dendsay *et al.*, 1992, Zhu *et al*., 1997).The hydrolysis of sucrose by cell-wall invertases and the subsequent import of hexoses into target cells appeared to be crucial for appropriate metabolism, growth and differentiation in plants (Sherson *et al*., 2003).

Table-1 Biochemical properties of sugarcane juice of cultivars CP-77-400 and COJ-84 grown under saline conditions.

Cultivar	NaCl levels (mM)				
	(Control)	50	100	150	200
Sugars (mg ml^{-1} juice)					
CP-77-400	214.6	191.4	228.7	285.5	233.8
COJ-84	259.0	138.1	155.08	203.16	231.6
Sugars content % (g $cane^{-1}$ juice)					
CP-77-400	24	15.6 (65)	23.52 (98)	23 (96)	17 (71)
COJ-84	38.7	12 (31)	20.2 (52)	18.62 (48)	24 (62)
Glucose (mg ml^{-1} juice)					
CP-77-400	95.02	125.12	160	112.32	98.1
COJ-84	159.86	88.24	86.1	132	198.04
Glucose content % (g $cane^{-1}$ juice)					
CP-77-400	10.7	10.3 (96)	16.5 (154)	9.1 (85)	7.2 (67)
COJ-84	24	7.7 (32)	11.2 (47)	12.2 (51)	20.6 (86)
Invertase activity (U ml^{-1})					
CP-77-400	0.737	0.65	7.59	2.31	1.57
COJ-84	0.13	0.28	0.26	0.17	0.19
Total proteins (mg ml^{-1})					
CP-77-400	0.38	0.42	0.61	0.65	1.00
COJ-84	0.66	1.00	0.54	0.56	0.41
Specific activity (U mg^{-1} protein)					
CP-77-400	1.94	1.55	12.44	3.55	1.57
COJ-84	0.20	0.28	0.48	0.30	0.46
Na^+ (mg ml^{-1})					
CP-77-400	0.84	1.32(157)	1.38(164)	1.32(157)	2.00(238)
COJ-84	0.52	1.38(265)	1.31(252)	1.4(269)	1.84(354)
K^+ (mg ml^{-1})					
CP-77-400	7.52	9.44(125)	8.63(114)	9.44(125)	14.4(192)
COJ-84	3.36	7.84(233)	8.32(248)	9.12(271)	9.61(286)

Values in parentheses are percent of control

Table-2 Mean squares from ANOVA for different biochemical parameters of two varieties of sugarcane.

Source of variation	df	Mean squares								
		Sugars (mg/ml juice)	Sugars content % (g/cane juice)	Glucose (mg/ml juice)	Glucose content % (g/cane juice)	Invertase activity (u/ml)	Total proteins (mg/ml)	Specific activity (U/mg protein)	Na^+ (mg/ml)	K^+ (mg/ml)
Replications (R)	4	334.666^{ns}	1.541^{ns}	71.370^{ns}	0.562^{ns}	0.208^{ns}	0.025^{ns}	0.321^{ns}	0.510*	1.005**
Varieties (V)	1	13556.458***	54.372***	2715.845**	239.805***	69.698***	0.0001^{ns}	186.940***	0.078^{ns}	62.496***
Error (E_1)	4	234.138	0.524	81.373	0.928	0.196	0.007	0.299	0.034	0.020
Treatments (T)	4	11743.081***	393.918***	2210.340***	104.912***	21.116***	0.101***	56.296***	1.931***	54.810***
VxT	4	7159.207***	178.941***	12697.956***	190.618***	20.383***	0.582***	52.946***	0.071^{ns}	11.245***
Error (E_2)	32	250.884	4.197	118.429	2.040	0.159	0.017	0.399	0.081	0.740

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively
ns = non-significant

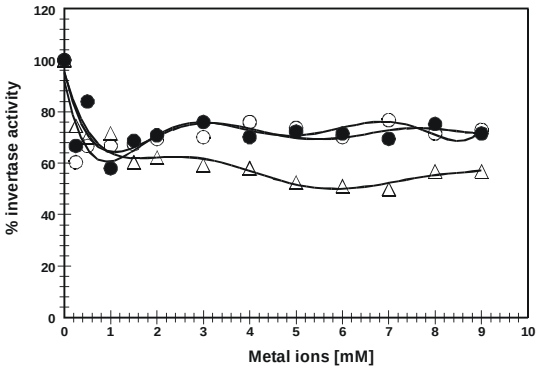

Fig-1: Effect of metal ions on *in vitro* activity of invertases from sugarcane cultivar CP-77-400: where open circle = Na^+, closed circle = K^+ and open triangle Ca^{2+}.

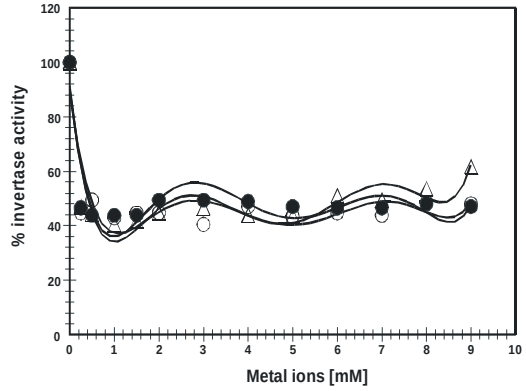

Fig-2: Effect of metal ions on activity of invertases from sugarcane cultivar COJ-84: where open circle = Na^+, closed circle = K^+ and open triangle Ca^{2+}.

Invertases catalyse the irreversible hydrolysis of sucrose and thus are expected to contribute to carbohydrate partitioning. Sucrose acts as a carbon and energy source in sink organs of most plant species and its channeling into sink metabolism requires its cleavage by several isoforms of invertase and sucrose synthase, which are localized in different subcellular compartments. Invertases play important role in regulation of phloem unloading of sucrose in sink organs. Sugars are also considered as regulator of gene expression. Therefore, the sucrose-cleaving enzymes play a fundamental role in controlling cell differentiation and development (Eschrich, 1980; Gibeaut *et al*., 1990; Koch, 1996; Strum and Tang, 1999; Ma *et al*., 2000; Roitsch *et al.*, 2000; Winter and Huber, 2000).

It has been found that the hydrolytic activity of soluble acid invertase (SAI) is strongly correlated with sucrose accumulation in sugarcane (*Saccharum* spp.) and the plants exhibiting SAI activity above a low threshold level do not accumulate high concentrations of sucrose (Zhu *et al.*, 2000). In storage sinks, invertase activity is proposed to be important for creating a sucrose concentration gradient from the phloem to sink tissue, and then maintaining the strong sink for promoting phloem unloading (Oparka and Prior, 1988). Sugarcane invertases have been characterized by Rose and Botha (2000) and they report that neutral invertase had a higher specific activity than soluble acid invertase (apoplastic and vacuolar) in the sucrose accumulation region of the sugarcane stem. Furthermore, a significant correlation between sucrose content and neutral invertases was found and this was largely due to a stronger association between the two components in the bottom of the internodes.

Total sugar content of COJ-84 confirmed that this was highly productive as compared to CP-77-400. The *in vivo* activity of soluble acid invertases was analyzed indirectly by determining the total amount of glucose per cane, because invertases cleave sucrose into glucose and fructose (Sturm, 1999). Total glucose content showed that invertases of COJ-84 were more active than that of CP-77-400. On the other hand, *in vitro* activity of acid invertases of CP-77-400, which was measured using apo-enzyme (metals free), was very high (0.737 units ml^{-1}) as compared to COJ-84 (0.132 units ml^{-1}). Moreover, total proteins secreted by CP-77-400 were also very low than COJ-84. Therefore, based on specific activity it was concluded that the invertases of CP-77-400 were about 9.7-fold more active than those of COJ-84.

In order to evaluate biochemical basis of high productivity of COJ-84, the information about the concentration of absorbed metal ions was very important, because metal ions may affect the function of invertases, which are known for their involvement in growth. It was observed that under natural conditions (control) COJ-84 was more salt resistant as compared to CP-77-400 because it absorbed less Na^+ and K^+ (Table-1). The juice of CP-77-400 contained almost double amount of sodium and potassium as compared to COJ-84, which might have significantly inhibited the acid invertases of CP-77-400, thereby resulting into decrease in phloem unloading of sucrose in parenchymatous cells and hence might be possible reason for low productivity of this cultivar. Despite numerous studies, biochemical basis of sucrose accumulation in sugarcane is still poorly understood (Moore, 1995).

The finding, i.e., low productivity of CP-77-400 due to the inhibition of its invertases by high concentration of absorbed metal ions was further evaluated by assessing the effect of metal ions on *in vitro* activity of invertases

from both cultivars. The invertases of CP-77-400 gave about 65% residual activity at 9 mM concentration of all metals (Na^+, K^+ and Ca^{2+}), whereas, COJ-84 showed about 55 % residual activity at the same concentration (Fig-1 and 2). Obviously, it is very difficult to correlate these findings with *in vivo* activities of invertases, but it could provide some evidence because the concentrations of Na^+ (0.84 mg ml^{-1} =14.4 mM) and K^+ (7.5 mg ml^{-1}=101 mM) in juice of CP-77-400 were very high as compared to those of COJ-84 having Na^+ (0.52 mg ml^{-1}=8.9 mM) and K^+ (3.36 mg ml^{-1}=45 mM). Therefore, in the light of above findings, it was concluded that low productivity of CP-77-400 was due to high inhibition of its invertases by large concentration of absorbed metal ions. Earlier work supports this finding, according to Subbarao and Shaw, (1985) sugarcane is a typical glycophyte exhibiting stunted growth or no growth under salinity, with its yield falling to 50% or even more of its true potential. The correlation between culm and leaf lengths become more pronounced when the canes were grown under saline conditions, and it was found that increase in leaf length resulted into concomitant decrease in culm length and hence decrease in total sugars yield. Therefore, under salinity the vegetative growth, i.e., leaf length was triggered. COJ-84, which was salt tolerant under natural conditions behaved strangely under higher levels of applied salinity and showed lower growth rate as compared to CP-77-400. Average internode length of CP-77-400 did not decrease up to 150 mM NaCl level, whereas, COJ-84 showed decrease against all salinity levels. Furthermore, culm diameter was cumulatively increased for COJ-84 and decreased for CP-77-400 at different levels of salinity (effect of salinity on growth attributes of these cultivars is discussed elsewhere). Like growth, production of sugars under different levels of salinity also confirmed that COJ-84 could not withstand high salinity and presented high % decrease in sugar yield as compared to CP-77-400 (Table-1).

Surprisingly, COJ-84 which was salt tolerant under natural conditions and absorbed about half quantity of metal ions as compared to CP-77-400 became inverted at higher levels of salinity. The abrupt increase in absorption rate of metal ions, which reached to about more than double as compared to CP-77-400, made the soluble acid invertases of COJ-84 highly inhibited and hence possible reason for lower growth and total sugars yield. The *in vivo* activities of invertases in terms of total glucose for both cultivars also supported this line of reasoning (Table-1). Vorster and Botha (1998) reported the significant inhibition of sugarcane neutral invertases by $HgCl_2$, $AgNO_3$, $ZnCl_2$, $CuSO_4$ and $CoCl_2$ but not by $CaCl_2$, $MgCl_2$ or $MnCl_2$. The suppression of growth of plants under salinity has been assigned to the accumulation of toxic ions (Shrivastava *et al.,* 1989). Many workers have explored diverse response of plants to salinity (Greenway and Munns, 1980, Flowers, 1985, Isla *et al.*, 1998). More *et al*. (1994) working with sugarcane cultivars, reported that the mixture of NaCl and Na_2SO_4 decreased the reducing and non-reducing sugars, chlorophyll and potassium content but increased the proline content of leaves.

Salinity tolerance is a complex process and controlled by many genes, which are expressed only when plants are exposed to salinity (Gracia *et al.*, 1995). The invertases produced by CP-77-400 under 100 mM level of salinity were 10-fold more active than control ones. While almost all invertases produced by both cultivars under saline conditions showed activation. As far as, total proteins are concerned they also went on increasing with the increase in salinity level. Therefore, considering the specific activity values, it is suggested that presence of high concentration of metal ions in the sink cells might have affected the expression of regulatory, as well as, structural genes of invertases, because the specific activity of invertases for canes from both cultivars grown under saline conditions increased reasonably (Table-1).

Conclusion

COJ-84 was found to be more productive as compared to CP-77-400. Low productivity of CP-77-400 might be due to the inhibition of its invertases by high concentration of absorbed metal ions. Specific activity values of invertases under saline conditions suggest that accumulation of high concentration of metal ions in sink cells might have affected the expression of invertase genes.

References

Bonham-Smith, P.C., Kapoor, M. and Bewley, J.D. A comparison of the stress responses of Zea mays seedlings as shown by quantitative changes in protein synthesis. Can. J. Bot., 1988. 66:1883-1890.

Botha, F.C. and Black, K. G. Sucrose phosphate synthase and sucrose synthase activity during maturation of internodal tissue in sugarcane. Aust. J. Plant Physiol., 2000. 27:81-85.

Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 1976. 72:248-254.

Dendsay, J.P.S., Dhawan, A.R. and Sehtiya, H.L. Relationship between invertase activity and internodal elongation and maturity in sugarcane. Plant Physiol. Biochem., 1992. 19:15-18.

Eschrich, W. Free space invertase, Its possible role in phloem unloading. Ber. Dtsch. Bot. Ges., 1980. 93:363-378.

Flowers, T.J. Physiology of halophytes. Plant Sci., 1985. 89:41-56.

Gibeaut, D.M., Karuppiah, N., Chang, S.R., Brock, T.G., Vadlamudi, B., Kim, D., Ghosheh, N.S., Rayle, D.L., Carpita, N.C. and Kaufman, P.B. Cell wall and enzyme changes during graviresponse of the leaf-sheat pulvinus of oat (Avena sativa L.). Plant Physiol., 1990. 94:411-416.

Gracia, A., Senadhira, D., Flowers, T.J. and Yeo, A.R. The effect of selection for sodium transport and for agronomic characteristics upon salt resistance in rice (Oryza sativa L.). Theor. Appl. Genet., 1995. 90:1106-1111.

Greenway, H. and Munns, R. Mechanisms of salt tolerance in non-halophytes. Annu. Rev. Plant Physiol., 1980. 31:149-194.

Hatch, M.D. A uridine diphosphatase from sugarcane. Biochem. J., 1963. 88:423-427.

Isla, R., Agragues, R. and Royo, A. validity of various physiological traits as screening criteria for salt tolerance in barley. Field Crops Res., 1998. 58: 97-107.

Koch, K.E. Carbohydrate-modulated gene expression in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 1996. 47:509-540.

Ma, H., Albert, H.H., Paul, R. and Moore, P.H. Metabolic engineering of invertase activities in different sub-cellular compartments affects sucrose accumulation in sugarcane cells. Aust. J. Plant Physiol., 2000. 27:1021-1030.

Miller, G.I. Use of Dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 1959. 31:426-428.

Ming, R., Liu, S.C., Bowers, J.E., Moore, P.H., Irvine, J.E. and Paterson, A.H. Construction of a Saccharum consensum genetic map from two interspecific crosses. Crop Sci., 2002. 42(2):570-583.

Ming, R., Liu, S.C., Moore, P. H. Irvine, J.E. and Paterson, A.H. QTL analysis in a complex autopolyploid: genetic control of sugar content in sugarcane. Genome Res., 2001. 11:2075-2084.

Moore, P.H. Temporal and spatial regulation of sucrose accumulation in the sugarcane stem. Aust. J. Plant Physiol., 1995. 22:661-679.

More, P.V., Desai, B.B. and Chavan, U.D. Effect of salt stress on biochemical parameters of sugarcane. J. Maharashtra Agric. Univ., 1994. 19: 431-433.

Oparka, K. J. and Prior, D.A.M. Movement of Lucifer Yellow C.H. in potato tuber storage tissues: a comparision of symplastic and apoplastic transport. Planta, 1988. 176:533-540.

Roitsch, T., Ehness, R., Goetz, M., Hause, B., Hofman, M. and Sinha, A.K. Regulation and function of extracellular invertase from higher plants in relation to assimilate partitioning, stress responses and sugar signaling. Aust. J. Plant Physiol., 2000. 27(815-825.

Rose, S. and Botha, F.C. Distribution patterns of neutral invertase and sugar content in sugarcane internodal tissues. Plant Physiol. Biochem., 2000. 38:819-824.

Sachs, M.M. and Ho, D.T.H. Alternation of gene expression during environmental stress in plants. Annu. Rev. Plant. Physiol., 1986. 37:363-376.

Sherson, S.M., Alford, H.L., Forbes, S.M. Wallace, G. and Smith, S.M. Roles of cell-wall invertases and monosaccharide transporters in the growth and development of Arabidopsis. J. Exp. Bot., 2003. 54(382): 525-531.

Shrivastava, A.K., Singh, K., Ghosha, A.K., Darash, R., Rai, R.K., Shunkla, S.P. and Singh, K. Uptake and partitioning of sodium and chloride ions in sugarcane. Sugarcane, 1989. 4:3-6.

Singh, N.K., LaRosa, P.C., Handa, A.K., Hasegawa, P.M. and Bressan, R.A. Hormonal regulation of protein synthesis associated with salt tolerance in plant cells. Proc. Natl. Acad. Sci. USA, 1987. 84:739-743.

Sturm, A. and Tang, G.Q. The sucrose cleaving enzymes of plants are crucial for development, growth and carbon partitioning. Trends Plant Sci,. 1999. 4:401-407.

Sturm, A., Hess, D., Lee, H.S. and Lienhard, S. Neutral invertase is a novel type of sucrose-cleaving enzyme. Physiol. Plant., 1999. 107:159-165.

Sturm, A. Invertases: Primary structures, function, and roles in plant development and sucrose partitioning. Plant Physiol., 1999. 121:1-8.

Subbarao, M. and Shaw, M.A.E. A review of research on sugarcane soils, of Jamaica. Proc. Meeting West Indies Sugar Technol., 1985. 2:343-355.

Venkataramana, S., Naidu, K.M. and Singh, S. Invertase and growth factors dependent sucrose accumulation in sugarcane. Plant Sci., 1991. 74:65-72.

Vorster, D.J. and Botha, F.C. Partial purification and characterization of sugarcane neutral invertase. Phytochemistry, 1998. 49:651-655.

Wiegand, C.L., Escobar, D.E. and Lingle, S.E. Growth and yield responses of sugarcane to saline soils:I. Sensing and mapping using aerial videography. Proc. Inter-American Sugarcane Seminars. pp. 15-17. 1996.

Winter, H. and Huber, S.C. Regulation of sucrose metabolism in higher plants: Localization and regulation of activity of key enzymes. Crit. Rev. Plant Sci., 2000. 19:31-67.

Zhu, Y.J., Komor, E. and Moore, P.H. Sucrose accumulation in the sugarcane is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. Plant Physiol., 1997. 115:609-615.

Zhu, Y.J., Albert, H.H. and Moore, P.H. Differential expression of soluble acid invertase genes in the shoots of high sucrose and low-sucrose species of Saccharum and their hybrids. Aust. J. Plant Physiol., 2000. 27:193-199.