Full Length Research Article

γ-AMINOBUTYRATE (GABA) SHUNT UNDER SALT STRESS IN *Saccharum officinarum*

[1],*Gireesh Babu, K. and [2]Naik, G. R.

[1],*Bio Genics, Veena Plaza, P.B. Road, Unkal, Hubli, Karnataka, India
[2]Department of Biotechnology, Gulbarga University, Gulbarga 585 106, Karnataka, India

ABSTRACT

The GAD extracted from salt stress sensitive sugarcane variety CoC 671 showed increased activity with increased concentrations of NaCl treatment. The natural GAD activity was found 141% more in salt tolerant variety Co 86032, compared to sensitive variety. The GOT activities displayed specific induction at higher salt concentrations in CoC 671 variety. The GOT activity was 840% more in tolerant variety than in sensitive variety and the same activity was observed at initial concentrations of salt treatment. The activity staining of GOT and GPT did not show any difference in the banding pattern but, expression of a single isoenzyme was detected in control and 0.5% NaCl treated CoC 671 extracts and in 0.75% NaCl treated Co 86032 extracts. The SSADH activity in both varieties decreased as the salt treatment increased, unlike GAD and GOT. The SSADH activity was found less in salt tolerant variety than that of sensitive variety. The activity staining of SSADH extracts from both varieties treated with different concentrations of NaCl did not reveal any difference.

INTRODUCTION

Environmental factors that impose water-deficit stress, such as drought, salinity and temperature extremes, place major limits on the plant productivity (Boyer, 1982). Among all abiotic stresses, salinity is growing as a major factor that limits the agricultural production. Over 397 million ha of land throughout the world is salt-affected (FAO, 2005), which is over 6% of the world's total land area. Of the 1,500 million ha of land farmed by dry land agriculture, 32 million ha (2%) are affected by secondary salinity of varying degrees. Of the current 230 million ha of irrigated land, 45million ha (20%) are salt affected (FAO, 2005). In fact, it is estimated that 50% of the arable lands will be salinised by the year 2050. Sugarcane, *Saccharum officinarum* L., belongs to the tribe *Andropogoneae*, sub-tribe *Saccharinae* of the family Poaceae (Watson *et al*., 1985). Sugarcane is a staple source of sweetening agent grown in tropical and subtropical regions of over 127 countries and is cultivated on about 20 million ha (Cardeiro, 2000). Earlier works have noted that sugarcane is sensitive to saline stress (Naik *et al.*, 1997). Salinity and drought are the major abiotic stresses restricting cane yield by 30-40%. Plant responds to salinity at molecular, cellular and whole plant levels (Greenway and Munns, 1980; Zhu *et al*.,

1996; Yeo, 1998; Bohnert *et al*., 1999; Hasegawa *et al*., 2000). Accumulation of γ-aminobutyric acid (GABA) in response to various biological and mechanical stress conditions is universal (Streeter and Thompson, 1972; Wallace *et al*., 1984; Reggiani *et al*., 1988; Crawford *et al*., 1994), but its role in plants remains uncertain (Satyanarayan and Nair, 1990; Bown and Shelp, 1997). GABA is a four carbon, non-protein ω-amino acid that is ubiquitous in plants, animals, bacteria and fungi (Lawrence and Grant, 1963; Selman and Cooper, 1978; Secor and Schrader, l984; Desmaison and Tixier, 1986; Bown and Shelp, 1989, 1997; Satyanarayan and Nair, 1990). It was discovered in plants more than half a century ago (Steward *et al*., 1949). It occupies a significant component of cytosolic free amino acid pool. Because Glutamate decarboxylase (GAD) activity consumes H^{+}, GABA synthesis contributes to pH regulation of acidified cytosol under stress (Bown and Shelp, 1989). Under stress, the enhanced conversion of glutamate to GABA has been observed, suggesting the considerable importance of GABA in the nitrogen economy of plants (Satyanarayan and Nair, 1990). The similarities of GABA with proline and glycine betaine clearly indicate that it has a role of a compatible osmolyte (Yancey, 1994). GABA stabilizes and protects isolated thylakoids against freezing damage in the presence of salt, exceeding the cryoprotective properties of proline (Heber *et al*., 1971). In addition, GABA possesses *in vitro* hydroxyl-radical-scavenging activity than proline and Glycine (Smirnoff *et al*., 1989).

Corresponding author: Gireesh Babu, K.
Bio Genics, Veena Plaza, P.B. Road, Unkal, Hubli, Karnataka, India

GABA has been successfully tested against oblique-banded leaf-roller larvae (Ramputh and Bown, 1996) and northern root-knot nematode (McLean *et al.*, 2003) in plants. Together, these observations suggest that the endogenous synthesis of GABA serves as a plant defense mechanism. Many commercially employed insecticides are antagonists and agonists of the GABA-gated Cl⁻ current and are thought to inhibit normal neuromuscular activity; ingested GABA might have a similar effect (Shelp *et al.*, 1999). The GABA shunt has been characterized in many plants as influenced by various stresses like hypoxia, drought, osmotic, cold, heat and touch. The reports have clearly indicated the accumulation of GABA and have even put forth the possible roles of GABA under stress. In the present study, we opted to study the status of GABA shunt response to salt stress in sugarcane.

MATERIALS AND METHODS

Induction of Salt Stress

The sugarcane varieties CoC 671 and Co 86032 were selected for the salt treatment as CoC 671 is saline susceptible and later is saline tolerant (Sreenivasan *et al.*, 1995). Study was targeted to compare the GABA shunt responses in saline stress tolerant and susceptible varieties of sugarcane. The seed materials of sugarcane varieties were collected from BSSK, Bidar and grown in pots (containing 1:1 sand: soil) for 40 days under green house conditions, with regular watering. After 40 days both the varieties were treated with 0.25%, 0.5%, 0.75% and 1% (w/v) NaCl, with control. The 500 ml salt solution was provided every week, with normal water irrigation was given alternatively. The treatment was carried for 4 weeks, till symptoms were clearly observed.

Detection of Soil Conductance

After 4 weeks of salt treatment, the soil was tested for its conductance to determine the concentration of dissolved salts. The conductance was measured by conductometer (Systronics). The 10 g soil was dissolved in 100 ml of deionized water, mixed well and kept undisturbed overnight. The clear solution above the soil layer was used for the conductance analysis.

Analysis of GABA Shunt Enzymes under Salt Stress

The salt treated sugarcane varieties were analyzed for the GABA shunt enzymes and compared with the activities in control, non stressed plants. After the salt treatment, the young and green leaves from both the varieties were collected and the GABA shunt enzymes were isolated and assayed.

GAD

10 g of young and green leaves collected from varying salt treated sugarcane varieties, CoC 671 and Co 86032 were ground in equal amount of cold GAD extraction buffer, mentioned before. The crude extract was filtered through 4 layers of cheese cloth and centrifuged at 12,000 g for 10 min at 4⁰C. The supernatant was ammonium sulfate saturated (80%) to precipitate the proteins followed by centrifugation at 10,000 g for 10 min at 4⁰C. The protein precipitate was dissolved in 1 ml of GAD extraction buffer and dialyzed against 1/10 concentration of GAD extraction buffer for 4 h, at 4⁰C. This protein extract was used as the enzyme source and

the activity of GAD was assayed according to Brandon et al, (1997) at pH 6.0. Total protein was estimated according to Bradford (1976). The GAD activity under salt stress was compared with control. The total protein in each extract was estimated as mentioned above.

GABA-T

The young and green leaves (10 g) from different salt treated varieties were ground at 4⁰C in equal volume of GABA-T extraction buffer. The crude extract was filtered through 4 layers of cheese cloth and centrifuged at 12,000 g for 10 min at 4⁰C. The supernatant obtained was saturated with 80% ammonium sulphate to precipitate the proteins under cold conditions and the proteins were separated by centrifuging at 10,000 g for 10 min at 4⁰C. The protein pellet was dissolved in 1 ml of GABA-T extraction buffer and dialyzed against 1/10 concentration of GABA-T extraction buffer for 4 h and used as GABA-T source. Sugarcane GABA-T isoforms (GPT and GOT) were assayed according to Yonaha *et al.*, (1983). and compared with activities in control, non stressed plants. The protein present in each extract was estimated as mentioned above.

SSADH

The 10 g of young and green leaves collected from each salt treated variety was homogenized with the equal volume of SSADH extraction buffer. The extract was filtered through 4 layers of cheese cloth and centrifuged at 12,000 g for 10 min at 4⁰C. The supernatant was saturated with ammonium sulphate (80%) and the protein precipitate was separated by centrifugation at 10,000 g for 10 min at 4⁰C. The pellet of protein was dissolved in 1 ml SSADH extraction buffer and dialyzed against 1/10 concentration of SSADH extraction buffer for 4 h at 4⁰C. The dialyzed enzyme was used to assay according to Baush and Fromm, (1999) the SSADH and the activities were compared with control. From each extract, the total protein was estimated according to the method said above.

Analysis of GABA-T and SSADH Isoforms under Salt Stress

The possible variation in the expression of isoforms of GABA-T and SSADH in the stress induced sugarcane tissue were analyzed.

GABA-T

The extract from the stress induced leaf tissue was electrophoressed under non-denaturing conditions of PAGE, for 8 h at 4⁰C. The expression analysis of GPT and GOT was carried by detecting the NADP produced. The activity staining of GOT and GPT was performed according to Van Cauwenberghe *et al.* (2002) in 10% non-denaturing polyacryalimide gel.

SSADH

The possible variation in the SSADH isoforms expression was analyzed by running the extract from salt treated tissue in non-denaturing PAGE. The activity staining of SSADH was performed according to Alisa and Arp (2001).

RESULTS

This section deals with the results of study of the GABA shunt responses to salt stress. The sugarcane varieties CoC 671 and Co 86032, drought tolerant and sensitive, respectively were exposed to different concentrations of NaCl (Fig.1) and the variations in the GABA shunt enzyme activities were assayed.

at a particular concentration of salt was almost similar and this tendency was observed at all the salt concentrations. It indicated the equal influence of stress treatment for both the varieties. The GABA shunt enzymes were extracted from both the varieties, at various concentrations of stress and assayed and results are presented below.
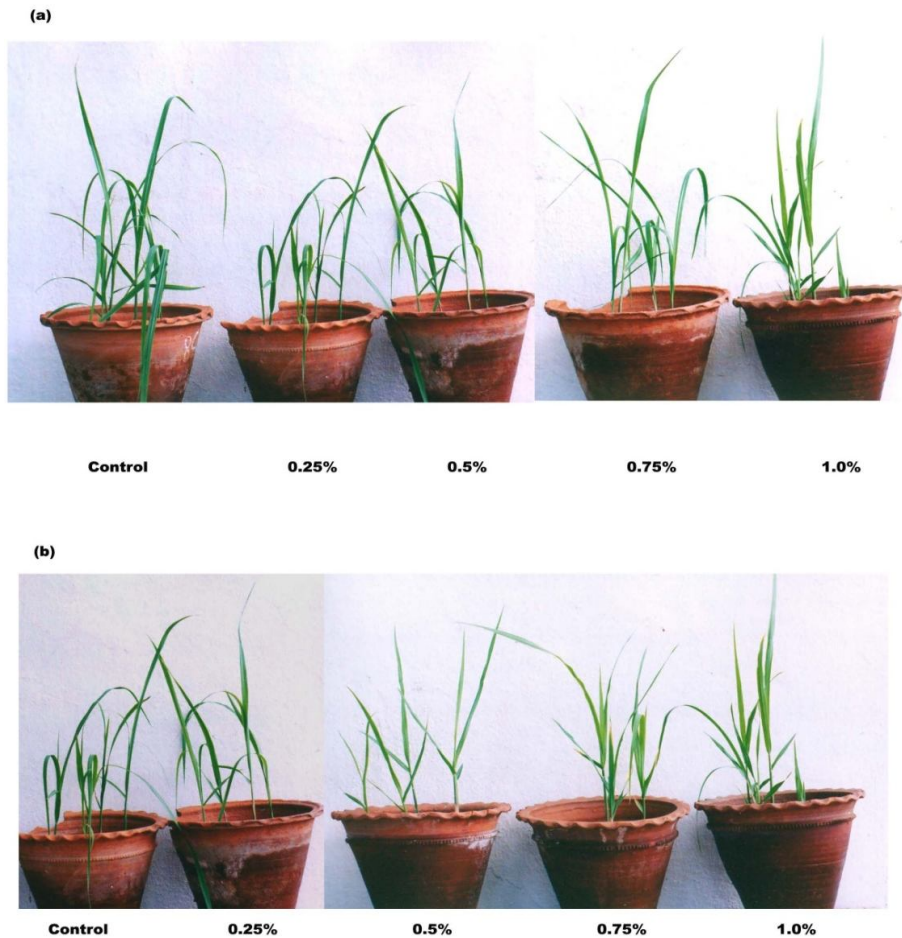

Fig.1- Sugarcane plantlets (40days old) treated with various concentrations of salt a) Co 671; b) Co 86032

Detection of Soil Conductance

Initially, the conductance of the soil solution used for treating the sugarcane plants was determined (Table 1). As the concentration of NaCl increased, the increase in the conductance of the soil solution was observed. The conductance of the soil sample used to grow both the varieties,

Table 1: The analysis of soil used to grow sugarcane plants

Variety	NaCl Treatment (%)	Conductance (mMohs)	Estimated NaCl (g/100g soil in water)
CoC 671	Control	0.7	0.03
	0.25	1.2	0.06
	0.5	1.8	0.09
	0.75	2.0	0.1
	1.0	2.3	0.12
Co 86032	Control	0.8	0.04
	0.25	1.3	0.06
	0.5	2.0	0.1
	0.75	2.2	0.11
	1.0	2.4	0.02

Analysis of GABA Shunt Enzymes under Stress

GAD Under Salt Stress

The GAD extracted from CoC 671 variety, showed increased activity with increased concentrations of NaCl treatment (Fig. 2a). The induction in GAD activity was compared with control and it was observed that the activity increased by 152%, 170%, 180% and 182% at 0.25%, 0.5%, 0.75% and 1% NaCl concentrations, respectively. The natural GAD activity was found 141% more in Co 86032 variety, compared to CoC 671. This activity increased with the increased salt concentration. The induction of 116%, 131%, 141% and 150% was observed from the extracts of 0.25%, 0.5%. 0.75% and 1% NaCl treated Co 86032 plants.

GABA-T under Salt Stress

The GABA-T activities in salt treated sugarcane varieties showed interesting aspects about its regulation. The GOT activities displayed specific induction at higher salt

concentrations in CoC 671 variety. At 0.25% NaCl no induction in GOT was observed but, a sharp 500%, 650% and 800% induction was seen at 0.5%, 0.75% and 1% salt treated extracts, respectively (Fig.2b). The GOT activity was 840% more in Co 86032 variety than CoC 671 and the same activity was observed at initial concentrations of salt treatment (0.25% and 0.5%). A small induction of 102% and 107% was observed at 0.75% and 1% NaCl. The nature of induction of GPT was similar to GOT in CoC 671 variety. At initial salt concentration of 0.25% no induction was noted but a 300%, 360% and 400% induction was noted at 0.5%, 0.75% and 1% NaCl (Fig.2c). The nature of GPT regulation in Co 86032 was similar to that of GOT at 0.25% and 0.5% NaCl. But at higher salt concentrations the activities decreased and only 96% activity was noted at other two concentrations of NaCl. Both GOT and GPT were analyzed for the variation in their expression by activity staining of non-denaturing PAGE. No difference in the banding pattern between GOT and GPT were observed but presence of an extra single isoenzyme was detected in control and 0.5% NaCl treated CoC 671 extracts (Fig.3a) and in 0.75% NaCl treated Co 86032 extracts (Fig.3b).
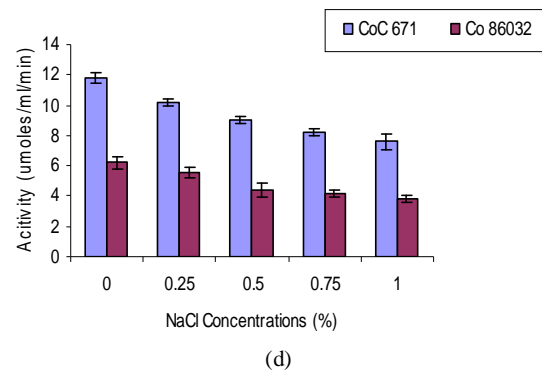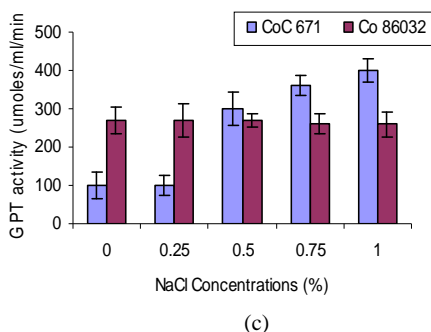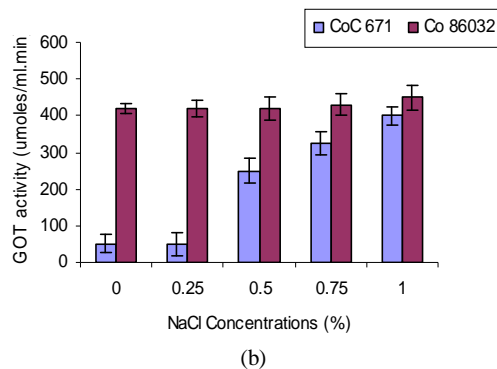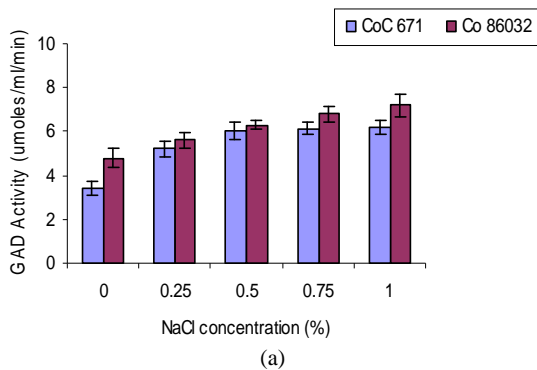

(d)

Fig. 2: Analysis of GABA shunt responses to salt stress

a) GAD activity under salt stress
b) GOT activity under salt stress
c) GPT activity under salt stress
d) SSADH activity under salt stress
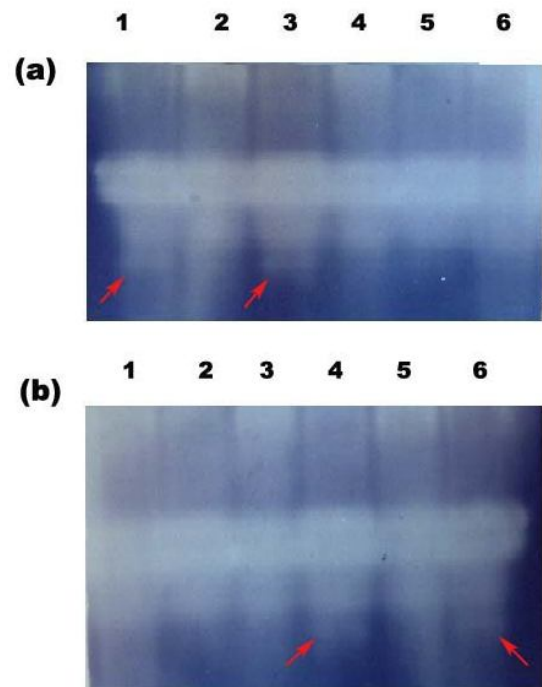

(a)

(b)

Fig. 3- Isoenzyme analysis of GABA-T isoforms isolated from Co671 (a) and Co 86032 (b)

SSADH under Salt Stress

The SSADH activity in both varieties decreased as the salt treatment increased, unlike GAD and GOT. Only a 64%, 76%, 69% and 64% activity was observed in the extracts of CoC 671 grown at 0.25%, 0.5%, 0.75% and 1% NaCl (Fig. 2d). The SSADH activity was found less in Co 86032 control extract than that of CoC 671. Only 90%, 70%, 67% and 61% SSADH activity was observed as the salt concentration of NaCl increased by 0.25%, 0.5%, 0.75% and 1%, respectively. The activity staining of SSADH extracts from both varieties treated with different concentrations of NaCl did not reveal any difference (Fig.4 a & b). The banding pattern remained same in all the salt treated extracts of one variety and when the banding was compared among the varieties, no difference was observed.
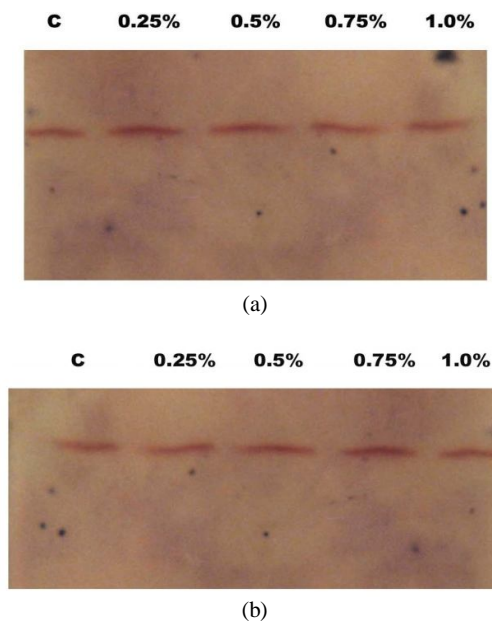

(a)

(b)

(c)

(a)

(b)

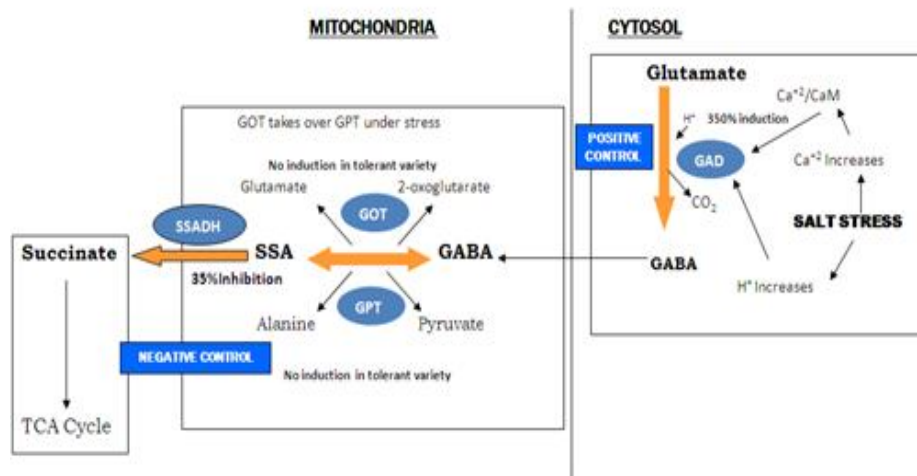**Fig.4- Isoenzme analysis of SSADH isolated from Co671 (a) and Co 86032 (b)**

Fig. 5- Summary of GABA shunt operation in *Saccharum officinarum* under salt stress

DISCUSSION

Analysis of GABA Shunt Responses to Salt Stress

The GABA accumulation in plants under various stresses is well known (Hanower and Brzozowska, 1975; Mayer *et al.,* 1990; Wallace *et al.,* 1984). The accumulated GABA performs various functions towards adopting the cell to stress (Heber *et al.,* 1971; Chung *et al.,* 1992; Crawford *et al.,* 1994; Shelp *et al.,* 1999; Snedden and Fromm, 1999). The analysis of the GABA shunt responses in sugarcane under salt stress has never been done before and in the present study, such characterization was carried, as sugarcane is a salt sensitive plant (Naik and Vedamurthy, 1997). The two sugarcane varieties known for their tolerance limits to salt stress were

exposed for the salinity stress with NaCl treatment. The GABA shunt enzymes were extracted and assayed to note the differences in their regulation.

Response of GAD

The GAD was induced in its activity as the impact of the stress increased (Fig.30a). Naturally, the GAD activity was found more in salt tolerant variety Co. 86032 and at all stages of the experiment, it showed more activity than CoC. 671. The induction of GAD activity means the more accumulation of GABA. Typically, GABA levels in plant tissues are low ranging from 0.03-2 pmol/ g FW (Rhodes *et al.,* 1986; Satyanarayan and Nair, 1990, Fougère *et al.,* 1991) and increase several fold in response to many diverse stimuli (Bown and Shelp, 1989, 1997; Satyanarayan and Nair, 1990; Mayer *et al.,* 1990; Ramputh and Bown, 1996; Streeter and Thompson, 1972; Ford *et al.,* 1996). It has been noted in soybean (Wallace *et al.,* 1984), tomato cells (Rhodes *et al.,* 1986), tobacco (Binzel *et al.,* 1987). The first report of a dramatic increase in GABA accumulation in response to stress was made by Naylor and Tolbert (1956). Interestingly, drought and salt stress induced patterns of GABA accumulation of similar duration.

Osmotic and salt stress each induces Ca^{+2} signals of similar magnitude and duration (Sanders *et al.,* 1999). If GABA is involved in stress responses, the pattern of GABA accumulation in stress-resistant and stress-sensitive cultivars should differ. This question has been investigated in leaves and roots of cultivated tomato and its wild salt-tolerant relative *L. pennellii* (Bolarin *et al.,* 1995). Treatment with 140 mM NaCl showed significantly greater GABA accumulation in the salt-sensitive cultivar when compared with the salt tolerant cultivar. Cytosolic levels of Ca^{+2} are elevated in response to cold shock, heat shock, salinity, drought, touch, and osmotic stress (Sanders *et al.,* 1999). Increased cytosolic Ca^{+2} forms complexes with calmodulin (CaM) and Ca^{+2}/CaM complex activates GAD in the physiological pH range. Acidic pH stimulation of GAD occurs in response to stresses that reduce

cellular pH. Anoxia occurs under conditions of flooding, and this stress causes cytosolic acidosis (Roberts *et al.,* 1984; Aurisano *et al.,* 1995), giving the greatest increases in GABA accumulation. The stimulation of GAD by Ca^{+2}/CaM may serve as a rapid or initial response to stress and/or a response to a mild or transient stress. As cytosolic pH decreases due to the extended duration and/or severity of the stress, then GAD activity could be stimulated by acidic pH in a Ca^{+2}/ CaM-independent manner.

The GABA accumulated has been attributed with various functions but, a direct role for GABA in stresses has not been demonstrated. Cellular accumulation of GABA could balance the decrease in water potential that occurs during cellular dehydration. In support of such a protective role for GABA, Heber *et al.,* (1971) showed that GABA can protect biological membranes from inactivation during freezing. Labeling studies have demonstrated that newly synthesized GABA is exported from cells (Chung *et al.,* 1992) and GABA efflux in response to anaerobosis has been reported (Crawford *et al.,* 1994). This is consistent with a role for GABA as an intercellular signaling molecule (Crawford *et al.,* 1994; Shelp *et al.,* 1999). Under unfavorable conditions, GABA can act as an amplifier of the signal, as an inducer of ethylene, mediates mineral acquisition and anaplerotic role in stress related metabolism. Plant genes encoding for proteins with a high degree of amino acid sequence homology to the superfamily of animal ionotropic glutamate receptors (iGLRs) have been identified (Lam *et al.,* 1998). Other preliminary data suggest that the putative plant glutamate receptors (GLRs) may be functionally different from the iGLRs. In animals the iGLRs are exclusively associated with the plasma membrane. Elucidating the function of GABA-like receptors in plants may help clarify the precise roles of GABA in plant responses to stress.

Responses of GABA-T

The GOT and GPT forms were individually analyzed for their responses under salt stress. The GOT response in both varieties was contrasting. The GOT activity in Co 86032 was almost constant in all the experimental plants while, initially at 0.25% salt concentration, no change in the activity was noted in variety CoC 671 but a drastic induction was observed in higher salt concentrations. The GPT activities were not changed at 0.25% salt but decreased as the stress impact increased in Co 86032. But, CoC 671 responded oppositely, though at 0.25% salt no difference in the activity was noted. GABA-T acts as a key for the regulation of GABA shunt under stress. It appears like the cell shows the tendency to accumulate more GABA as stress impact raises. The induction of GABA synthesizing enzyme, GAD, was noted before and either a constant or decreasing GABA-T activity was noted in salt tolerant variety. It indicates that the tolerant variety has a better GABA-T mediated regulation, facilitating the GABA accumulation. A contrasting response was observed in salt sensitive variety, where the GABA-T activity was induced at higher salt concentrations. The increased GABA-T activity hinders the accumulation of GABA by converting it to SSA. At this point it is evident that the tolerant varieties utilize the first regulatory key better than the sensitive varieties, which appears as a major difference for their tolerance capabilities. As it has been predicted that GOT acts when cell is healthy and GPT when cell undergoes stress, an interesting support has evolved in present part of research. The GOT activities of

tolerant varieties remained constant stating that stress has no effect on tolerant varieties and the activity was more than that of sensitive varieties at all the stages. But it was quite reverse in case of GPT, which reduced in activity as the stress increased. The GPT activity of sensitive variety overcomes the activity of enzyme in tolerant variety at higher concentrations of salt showing the inefficiency to control the degradation of GABA to SSA.

Responses of SSADH

The SSADH is the next major key for the regulation of GABA concentrations in the cell. The Figure 30 (d) clearly shows the inhibition of enzyme activity as the stress impact increases. This tendency was observed in both the sugarcane varieties. But, at all the stages the SSADH activity of the sensitive variety was much higher than that of tolerant variety. GABA shunt is negatively regulated by SSADH under stress (Shelp *et al.,* 1999). The higher enzyme activity in sensitive variety shows weak negative regulation than that of tolerant variety. The isoenzyme study of SSADH under salt stress did not reveal any interesting differences. The similar banding pattern suggested that the sugarcane SSADH doest not have isoforms. The GABA shunt in sugarcane is positively regulated by GAD and negatively regulated by SSADH and GABA-T. The regulation mechanism tends to accumulate/maintain more GABA under salt stress, so that GABA performs the above mentioned functions. In many studies, it has been noted that the plant responses to drought and salt stress will be quite similar but in the present study, interestingly, we have clearly noticed that the drought tolerant variety (CoC 671) behaves quite differently than salt tolerant variety (Co. 86032). It means that GABA shunt response to salt stress is unique and the drought tolerant varieties weakly regulate their response to stress through GABA shunt.

REFERENCES

Alisa, V.S. and Arp, J.D. 2001 An inducible 1-butanol dehydrogenase, a quinohaemoprotein, is involved in the oxidation of butane by *Pseudomonas butanovora*. *Microbiol.* 147:745-756.

Aurisano, N., Bertani, A. and Reggiani, R. 1995 Involvement of calcium and calmodulin in protein and amino acid metabolism in rice roots under anoxia. *Plant Cell Physiol.* 36:1525-1529.

Baush, K.B. and Fromm, H. 1999 Plant succinic semialdehyde dehydrogenase. Cloning, purification, localization in mitochondria and regulation by adenine nucleotides. *Plant Physiol.* 121:589-597.

Binzel, J.L, Hess, F.D., Bressan, R.A. and Hasegawa P.M. 1987 Solute accumulation in tobacco cells adapted to NaCl. *Plant Physiol.* 84:1408-1415.

Bolarin, M. C., Santa-Cruz, A., Cayuela, E. and Perez-Alfocea, F.1995 Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salini*ty. J. Plant Physio*l. 147:463-468.

Bonhnert, H.J., Su, H. and Shen, B. 1999 Molecular mechanisms of salinity tolerance. In: Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants. Shinozaki, K. and Yamaguchi-Shinozaki, K. (Eds.), Landes Company, Austin, TX, pp. 29-62.

Bown, A. and Shelp, B.J. 1989 The metabolism and physiological roles of 4-aminobutyric acid. *Biochem (Life. Sci. Adv.).* 8:21-22.

Bown, A.W. and Shelp, B.J. 1997 The metabolism and function of γ-aminobutyric acid. *Plant Physiol.* 115:1-5.

Boyer, J.S. 1982 Plant productivity and environment. *Science.* 218:443-448

Bradford, M.M. 1976 A Rapid and sensitive method for quantification of microgram quantities of protein utilizing the principles of protein dye-binding. Ann. Biochemistry. 32:248-253.

Brandon S.J., Narendra K.S., Joe H.C., Robert D.L., 1997 Purification and characterization of glutamate decarboxylase from cowpea, Phytochemistry. 46(1)39-44.

Cardeiro, G.M., Taylor, G.O. and Henry, R.J. 2000 Characterization of microsatellite markers from sugarcane (*Saccharum* spp.), a highly polyploidy species. *Plant. Sci.* 155:161-168.

Chung, I., Bown. A.W. and Shelp, B.J. 1992 The production and efflux of 4-aminobutyrate in isolated mesophyll cells. *Plant Physiol.* 99:659-664.

Crawford, L.A., Bown, A.W., Breitkreuz, K.E. and Guinel, F.C. 1994 The synthesis of γ-aminobutyric acid in response to treatments reducing cytosolic pH. *Plant Physiol.* 104:865-871.

Desmaison, A.M. and Tixier, M. 1986 Amino acids content in germinating seeds and seedlings from *Costonea sariva* L. *Plant Physiol.* 81:692-695

FAO 2005 Global Network on Integrated Soil Management for Sustainable Use of Salt-affected Soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. http://www.fao.org/ag/agl/agll/spush

Ford, Y.Y., Ratcliff, R.G. and Robins, R.J. 1996 Phytohormone-induced GABA production in transformed root cultures of *Datura stramonium:* an *in* vivo ^{15}N-NMR study. *J. Exp. Bot.* 47:811-818.

Fougère, F., Le Rudulier, D. and Streeter, J.G 1991 Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids and cytosol of alfalfa (*Medicago sativa* L.). *Plant Physiol.* 96:1228-1236.

Greenway, H. and Munns, R. 1980 Mechanisms of salt tolerance in nonhalophytes. *Annal. Rev. Plant. Physiol.* 31:149 -190.

Hanower, P. and Brzozowska, J. 1975 Influence d'un choc osmotique sur la composition des feuilles de cotonnier en acides amines libres. *Phytochemistry* 14:1691-1694.

Hasegawa, P.M., Bressan, R.A., Zhu, J.-K. and Bohnert, H.J. 2000 Plant cellular and molecular responses to high salinity. *Annl. Rev. Plant Physiol. Plant Mol. Biol.* 51:463-499.

Heber, U., Tyankova, L. and Santarius, K. K. 1971 Stabilization and inactivation of biological membranes during freezing in the presence of amino acids. *Biochem. Biophy*s. *Acta.* 241:578-592.

Lam, H. M., Chiu, J., Hsieh, M. H., Meisel, L., Oliveira, I. C., Shin, M. and Corruzzi, G. 1998. Glutamate-receptor genes in plants. *Nature* 396:125-126.

Lawrence, J.M. and Grant, D.R. 1963 Nitrogen mobilization in pea seedlings. 2. Free amino acids. *Plant Physiol.* 38:561-566.

Mayer, R. R., Cherry, J. L. and Rhodes, D. 1990 Effects of heat shock on amino acid metabolism of cowpea cells. *Phytochemistry.* 94:796-810.

McLean, M.D., Yevtushenko, D.P., Deschene, A., Van Cauwenberghe, O.R., Makhmoudova, A., Potter, J.W., Bown, A.W. and Shelp, B.J. 2003 Over expression of glutamate decarboxylase in transgenic tobacco plants confers resistance to the northern root-knot nematode. *Mol. Breeding.* 11:277-285.

Naik, G.R. and Vedamurthy, A.B. 1997 *In vitro* evaluation of red rot toxin influence on sugarcane (*Saccharum officinarum* Var. CoC 671). *Curr. Sci.* 73(4): 367-369.

Naylor, A. W. and Tolbert N. E. 1956 Glutamic acid metabolism in green and etiolated barley leaves. *Physiol. Plan*t. 9:220-229.

Ramputh, A. I. and Bown, A. W. 1996 Rapid γ -aminobutyric acid synthesis and the inhibition of the growth and development of oblique-banded leaf-roller larvae. *Plant Physio*l. 111:1349-1352.

Reggiani, R., Cantu, C.A., Brambilla, A. and Bertani, A. 1988 Accumulation and interconversion of amino acid in rice roots under anoxia. *Plant Cell Physiol*. 29:981-987.

Rhodes, D., Handa, S. and Bressan, R.A. 1986 Metabolic changes associated with adaptation of plant cells to water stress. *Plant Physiol.* 82:890-903.

Roberts, J. K. M., Calfis, J., Wemmer, D., Walbot, V. and Jardetzhy, O. 1984 Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. *Proc. Nat. Acad. Sci. USA.* 81:3379-3383.

Sanders, D., Brownlee, C. and Harper, J. F. 1999 Communicating with calcium. *Plant Cell* 11:691-706.

Satyanarayan, V. and Nair, P.M. 1990 Metabolism, enzymology and possible roles of 4-aminobutyrate in higher plants. *Phytochemistry.* 29:367-375.

Secor, J. and Schrader, L.E. 1984 Characterization of amino acid efflux from isolated soybean cells. *Plant Physiol.* 74:26-31.

Selman, W. and Cooper, P. 1978 Changes in the free amino compounds in young tomato plants in light and darkness with particular reference to gamma-aminobutyric acid. *Ann. Bot.* 42:627-636.

Shelp, B.J., Bown, A.W. and Mclean, M.D. 1999 Metabolism and function of Gamma-aminobutyric acid. *Trends. Plant. Sci.* 4:446-452.

Smirnoff, N. and Cumbes, Q.J. 1989 Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry.* 28:1057-1060.

Snedden, W. A. and Fromm, H. 1999 Regulation of the γ-aminobutyrate-synthesizing enzyme, glutamate decarboxylase, by calcium-calmodulin: a mechanism for rapid activation in response to stress. In: Plant Responses to Environmental Stresses, pp. 549-576.

Sreenivasan, T.V., Bhagyalakshmi, K.V. and Sahi, B.K. 1995 Sugarcane varieties. In: Sugarcane production manual. Alexander, K.C and Arulraj, S. (Eds.). SBI (ICAR) pp. 7-16.

Steward, F. C., Thompson, J. F. and Dent, C. E. 1949 γ -aminobutyric acid: a constituent of the potato tuber? *Science.* 110:439-440.

Streeter, J.G. and Thompson, J.F. 1972 Anaerobic accumulation of gamma-aminobutyric acid and alanine in radish leaves (*Raphanus sativus* L.). *Plant Physiol.* 49:572-578.

Van Cauwenberghe, O.R., Amina, M., McLean, M.D., Clark, S.M. and Shelp, B.J. 2002 Plant pyruvate-dependent gamma-aminobutyrate transaminase: identification of an *Arabidopsis* cDNA and its expression in *Escherichia coli. Can. J. Bot.* 80:933-941.

Wallace, W., Secor, J. and Schrader, L. 1984 Rapid accumulation of γ-aminobutyric acid and alanine in soybean leaves in response to an abrupt transfer to lower temperature, darkness, or mechanical manipulation. *Plant Physiol.* 75:170-175.

Watson, L., Cliffort, H.T. and Dallwitz, M.J. 1985 The classification of Poaceae; subfamilies and supertribes. *Aust. J. Bot.* 33:433-484.

Yancey, P.H. 1994 Compatible and counteracting solutes. In: Cellular and Molecular Physiology of Cell Volume Regulation. Strange, K. (Eds.) CRC Press, pp. 81-109.

Yeo, A. 1998 Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exptl. Bot.* 49:913-929.

Yonaha, K., Suzuki, K., Minei, H. and Toyama, S. 1983 Distribution of ω-amino acid: pyruvate transaminase and aminobutyrate: α-ketoglutarate transaminase in microorganisms. *Agric. Biol. Chem.* 47:2257-2265.

Zhu, J.K., Hasegawa, P.M. and Bressan, R.A. 1996 Molecular aspects of osmotic stress in plants. *Crit. Rev. Plant Sci.* 16:253-77.

****