INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-4, Issue-1, Jan-Mar-2013 Coden : IJABPT Copyrights@2013

ISSN : 0976-4550

Received: 10th Dec-2012 *Revised: 15th Dec-2012* *Accepted: 18th Dec-2012*

Research Article

BIOCHEMICAL AND MOLECULAR ANALYSIS OF SUGARCANE GENOTYPES RESPONSE TO SALINITY AND DROUGHT

P. Merlin Christy[1], R. Dharani Preetha[1], S. Vasantha[2], D. Divya[1]

[1]Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women, Trichengode, Namakkal (dt), Tamil Nadu, India.

[2]Plant Physiology, Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India.

Corresponding author – P. Merlin Christy, Ph.no. +917639493337. E-mail: pmerlinchristy@gmail.com.

ABSTRACT: Sugarcane genotypes such as tolerant (Co 99004), and sensitive (Co 97010) were subjected to NaCl (170 and 250mM) and Poly ethylene glycol (-0.8Mpa and -1.7Mpa) treatment and evaluated through biochemical and molecular analysis to assess the commonness between salinity and drought stress. Among the biochemical characters analyzed total chlorophyll decreased and proline, lipid peroxidase, xanthophylls increased with severe stress (both salinity and drought). Peroxidase and superoxide dismutase activity increased with isozymes showing over expression under stress condition. In Reverse Transcriptase-PCR the genes p5cs and ADC1 showed differential amplification with tolerant genotypes recording up-regulation. The results showed that the tolerant genotype (Co 99004) expressed better stability under the saline and water stress conditions where as the sensitive genotype (97010) failed to perform under the stress conditions.

Key words: Tolerant, sensitive, salinity, drought, Poly ethylene glycol, Reverse transcriptase.

INTRODUCTION:

Sugarcane is an important commercial crop occupying about 4 million hectares of cultivable land. The crop requires adequate sunlight, warm weather, and soil rich in nutrients for high productivity. One of the causes of low yield is abiotic stress like drought, salinity, and water logging. Soil salinity and alkalinity / poor quality irrigation water coupled with moisture stress during peak growth phases result in low yield. Yield losses of 10-40% have been reported due to salinity (Anonymous, 1995). Salinity stress negatively impacts agriculture yield throughout the world production whether it is for subsistence or economic gain. The plant response to salinity consists of numerous processes that must function in coordination to alleviate both cellular hyper osmolarity and ion disequilibria (Shuji, *et. al.*, 2002). Plant responses to salt and water stress have much in common. Salinity reduces the ability of plants water uptake and causes reduction in growth. Soluble salts like NaCl Na_2So_4 enters the plant system along with water causing imbalance of ionic content of the plant. Salt tolerant plants differ from salt sensitive ones in having a low rate of Na^+ and Cl^- transport to leaves and the ability to compartmentalize these ions in vacuoles to prevent their build up in cytoplasm or cell walls and thus avoid salt toxicity (Munns, 2002).

Sugarcane is a glycophyte and grows poorly in saline soil /saline water. Varied response in terms of growth, physiological and biochemical parameters were reported for sugarcane varieties. Plant responses to salinity stress are reviewed with emphasis on molecular mechanisms of signal transduction and on the physiological consequences of altered gene expression that effect biochemical reactions downstream of stress sensing (Hasegawa *et al*., 2000). Under either salinity or water stress, plant exhibit lower-leaf water potential. Despite this obvious similarity, some clear distinction exists between plant responses to salt and water stress. One common observation is the lack of wilting under salt stress at water potentials that cause wilting under water stress. In addition, the osmotic adjustment processes under these two stresses differ. Osmoregulation in response to salinity may utilize ions from the soil, particularly those ions in excess, whereas under drought in the absence of salinity, the necessary solutes have to be produced mostly within the plant and thus causes differential energy expenditure (Hsiao, 1973).

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Past efforts to improve plant tolerance to drought, high salinity and low temperature through breeding and genetic engineering have had limited success owing to the genetic complexity of stress responses. Progress is now anticipated through comparative genomics studies of an evolutionarily diverse set of model organism, through the use of techniques such as high throughput analysis of expressed sequence tags, large scale parallel analysis of gene expression, targeted or random mutagenesis, and gain of function or mutant complementation. The discovery of novel genes, determination of their expression patterns in response to abiotic stress, an improved understanding of their role in stress adaptation will provide the basis of effective engineering strategies leading to greater stress tolerance. (Cushman and Boehnert, 2000).

MATERIALS AND METHODS:

Sugarcane genotypes (Co 99004, Co 97010) were obtained from sugarcane breeding institute, Coimbatore, Tamilnadu, India. An experiment was conducted to study the biochemical and molecular changes in two sugarcane cultivars Co 99004 (tolerant), Co 97010 (sensitive) responses to salt and dehydration treatment. Single bud setts (12 numbers each/tray) were planted in plastic trays filled with field soil. Three replications were maintained for each variety and treatment. When the settlings reached 3-4 leaved stage the treatments were imposed. The treatments included were T1 -control, T2 –NaCl 170mM, T3 -NaCl 250mM, T4 -Poly ethylene glycol (PEG) - 0.8Mpa, T5 -PEG 1.7Mpa. The experiments were carried out when the drying symptoms were first noticed in sensitive genotype.

Biochemical parameters

Leaf pigments (chlorophyll and carotenoids) were estimated in single extraction following Weybrew's method (Weybrow, 1957). Protein was estimated by colorimetric method described by Lowry, *et. al.*, (1951). Proline content of the leaves was estimated by the method described by Bates, *et. al,*. (1973). The peroxidase activity was estimated by using method of Dhindsa *et al.* (1981). Super oxide dismutase (SOD) activity was determined by using nitroblue tetrazolium (NBT) salt as described by Beau Champ and Fridovich (1971). Lipid peroxidation is measured by estimating thiobarbituric acid reactive compounds described by Heath and Packer, (1968).

Molecular analysis

Native – PAGE analysis was done to identify the qualitative and quantitative differences of leaf and root proteins from control and flooding treatments as per the method of Laemmli (1970). Isozyme analysis of peroxidase was done by Sadasivam and Manikam (1996), and superoxide dismutase was done by Beau Champ and Fridovich (1971). Total RNA was isolated as per Powlowski, *et. al.*, (1994) and the total RNA isolated was quantified by UV spectroscopy. Reverse transcription reaction was carried out with four different gene specific primers such as ADC1, p5cs, Apx, hva1.

RESULTS

Chlorophyll

The major components of chlorophyll pigments are chlorophyll a and chlorophyll b. The reduction in chlorophyll a was 52% and 33% under salt and PEG treatment respectively in Co 97010, while it was nil and 37% in case of variety Co 99004. On the contrary under mild stress the pigment content increased in both treatments in Co99004. Reduction in chlorophyll b content was 27% (only in dehydration treatment) in Co 99004 while in Co 97010 the reduction was only under salt treatment (table 1).

Table -1: Effect of Salinity and Drought on Chlorophyll Pigments in Sugarcane Genotypes (mg/g)

Treatments	Total Chlorophyll*		Chlorophyll a*		Chlorophyll b*	
	Co 97010	Co 99004	Co 97010	Co 99004	Co 97010	Co 99004
CONTROL	0.865	1.730	0.683	1.38	0.158	0.315
NaCl-1(170mM)	0.449	2.080	0.352	1.60	0.102	0.397
NaCl2(250mM)	0.380	1.72	0.348	1.432	0.072	0.358
PEG1(-0.8MPa)	0.348	2.186	0.249	1.474	0.072	0.389
PEG2 (-1.7MPa)	0.777	1.191	0.404	0.888	0.168	0.249

* Mean of three replications

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Total carotenoids

Total carotenoids include carotenes, xanthophylls and some minor pigments. Total carotenoid content was 0.238mg/g (Co 97010) and 0.360 mg/g in control samples. With increasing salt concentration in the medium the carotenoids content increased in both the varieties with Co 99004 recording higher levels. Salinity treatment increased xanthophylls content in tolerant (from 0.250mg/g to 0.310 mg/g) while it was reduced in sensitive genotype (Co 97010) (table 2).

Table -2: Carotenoid Pigments in Sugarcane Genotype (mg/g)

Treatments	Total Carotenoids*		Carotenes*		Xanthophylls*	
	Co 97010	Co 99004	Co 97010	Co 99004	Co 97010	Co 99004
CONTROL	0.237	0.366	0.046	0.109	0.152	0.256
NaCl 1(170mM)	0.133	0.450	0.029	0.129	0.093	0.311
NaCl 2(250mM)	0.119	0.403	0.027	0.112	0.09	0.309
PEG1(-0.8MPa)	0.107	0.475	0.031	0.10	0.066	0.353
PEG2 (-1.7MPa)	0.17	0.239	0.037	0.070	0.099	0.168

* Mean of three replications

Protein and RNA content

The Genotype Co 97010 showed a reduction in protein content in both salt and dehydration treatment. Co 99004 showed a marginal increase in protein content in salt treatment (60.21and 62.21mg/g for mild and severe stress) and significantly higher content in dehydration treatment (62.9 and 96.6 mg/g for mild and severe stress). RNA concentration varied from 268.7 to 1378µg/ml in genotype Co 99004 and 455 to 651µg/ml in Co 97010 (table 3).

Table - 3: Effect of Salinity and Drought on Protein Content and Conentration of RNA

Treatments	Protein content(mg/g)*		Concentration of RNA(µg/ml)*	
	Co 97010	Co 99004	Co 97010	Co 99004
Control	79.87 (±5.58)	50.26 (±4.625)	651.45 (±45.45)	1378.15 (±96.15)
NaCl 1(170Mm)	55.06 (±2.13)	60.6 (±8.52)	617.05 (±43.05)	625.65 (±43.65)
NaCl 2(250Mm)	49.39 (±2.97).	62.21 (±9.56)	518.15 (±36.15)	591.25 (±41.25)
PEG1(-0.8Mpa)	43.03 (±0.53)	62.9 (±1.17)	496.65 (±34.65)	574.05 (±40.05)
PEG2 (-1.7Mpa)	62.32 (±3.17)	96.64(±10.4)	455.8(±31.8)	268.75(±18.75)

* Mean of three replications

Proline content

Free proline, an amino acid that accumulates in response to abiotic stresses, increased in both salt and PEG treatment with marked variation between tolerant and sensitive genotype (fig 1). In Co 97010 (1.03 µmol/g) the proline content was gradually increased under the influence of NaCl treatment. With mild dehydration (-0.8 Mpa) the proline content (0.6µmol/g) was on par with control however, under severe stress (-1.7Mpa), the proline content increased (1.89µmol/g). In Co 99004 the proline content showed gradual increase from 0.75 (control) to 2.4µmol/g under salinity. Higher proline content was obtained in mild dehydration (2.78µmol/g). Proline content stabilized with increased intensity of dehydration stress.

Peroxidase activity

Peroxidase activity estimated as oxidation of O-dianisidine increased over two fold in sensitive genotype (Co 97010) under salinity and dehydration treatment (fig 2). The increase in activity of peroxidase was five times that of control in higher salinity conditions (250mM NaCl). However, under dehydration stress the increase in activity was not multifold. In tolerant genotype (Co 99004) though the peroxidase activity increased, but it was only marginal.

Super oxide dismutase activity

Super oxide dismutase (SOD) activity increased under mild salinity and stabilized with the severity and of the stress (fig 3). In genotype Co 97010 dehydration stress resulted in increased activity and stabilizes at severe stress. While in Co 99004 it was reverse (3.5 and 5.5 unit/g/min) under mild and severe stress. In sensitive genotype the SOD activity increased with the intensity of salinity while the tolerant genotype showed an initial increase and later stabilizing with higher salinity level.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

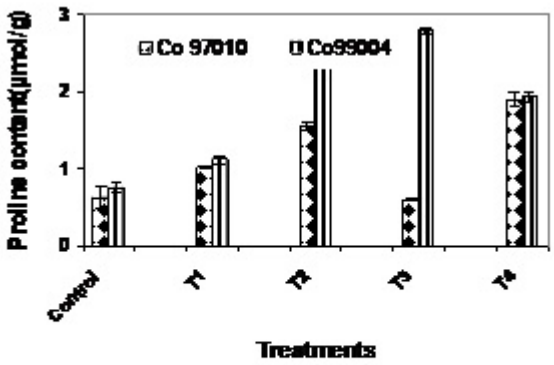
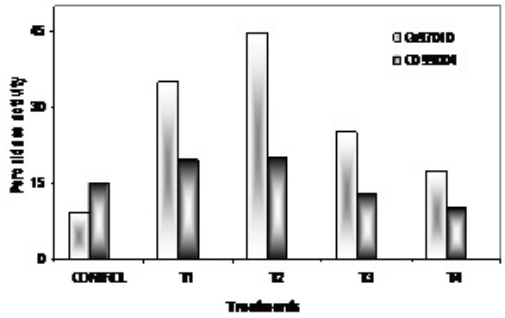


Fig1: Proline content as influenced by salinity and drought

T1 (170mM NaCl) salt treatment: T2 (250mM NaCl) salt treatment

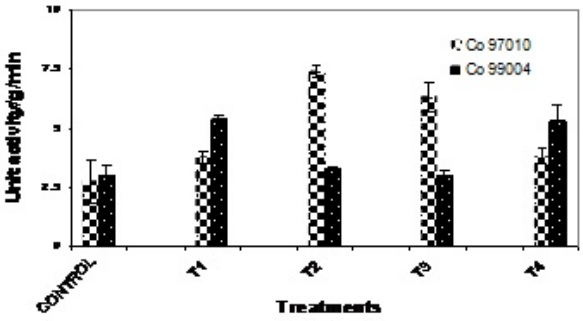
T3 (-0.8Mpa) PEG treatment : T4 (-1.7 Mpa) PEG treatment.



T1 (170mM NaCl) salt treatment: T2 (250mM NaCl) salt treatment

T3 (-0.8Mpa) PEG treatment :T4 (-1.7 Mpa) PEG treatment.

Fig2: Peroxidase activity in sugarcane genotypes subjected to salinity and drought

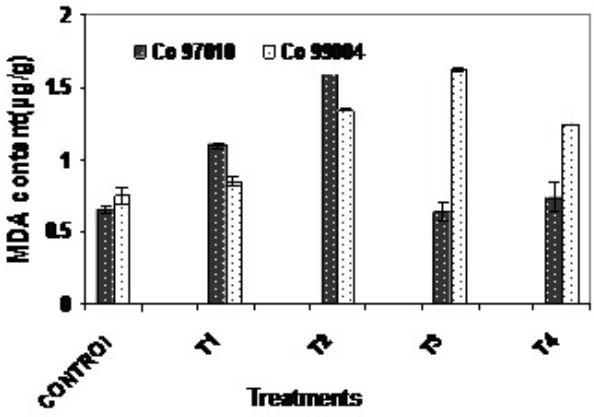


T1 (170mM NaCl) salt treatment: T2 (250mM NaCl) salt treatment:

T3 (-0.8Mpa) PEG treatment: T4 (-1.7 Mpa) PEG treatment.

Fig3: Super oxide dismutase activity as influenced by salinity and drought

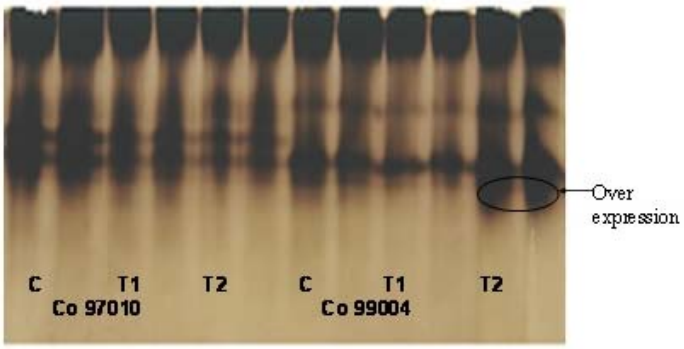
Coden : IJABPT Copyrights@2013 ISSN : 0976-4550



T1 (170mM NaCl) salt treatment:T2 (250mM NaCl) salt treatment:

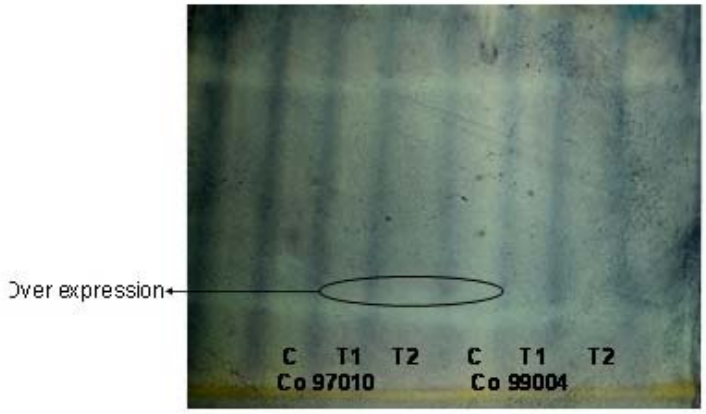
T3 (-0.8Mpa) PEG treatment: T4 (-1.7 Mpa) PEG treatment.

Fig4: Lipid peroxidation under salinity and drought in sugarcane



C-Control, T1-NaCl (250Mm), T2-PEG (-1.7Mpa)

Plate-1: Peroxidase-isozyme pattern



C-Control, T1-NaCl (250Mm), T2-PEG (-1.7Mpa)

Plate-2: Super oxide dismutase- isozyme pattern

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Lipid peroxidation in response to stresses

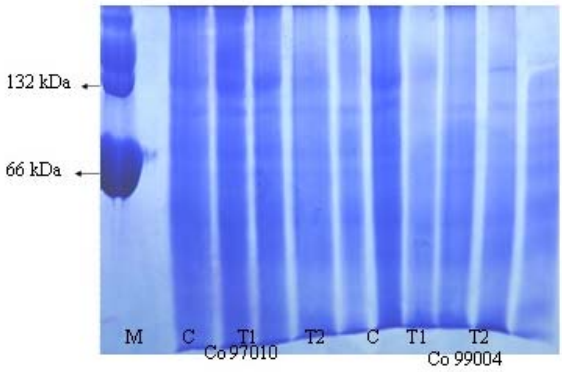
Malondialdehyde (MDA) content increased in both the stresses. In tolerant genotype (Co 99004) the lipid peroxidation was higher under dehydration as compared to salinity (fig 4). MDA content increased in severe level of salinity in sensitive genotype (Co 97010), while the increase was marginal under dehydration stress.

Isozyme pattern and Native - Poly Acrylamide Gel Electrophoresis (PAGE)

Isozyme patterns for peroxidase revealed over expression of a low molecular form in genotype Co 99004 in PEG (dehydration treatment) while such an induced over expression was not detected in sensitive genotype Co 97010 (plate1). SOD isozyme pattern also showed induced over expression under stress situation (plate 2). Crude proteins separated through non denaturizing PAGE showed an array of protein bands whose molecular weight ranges from 14kDa to 230kDa (plate 3).

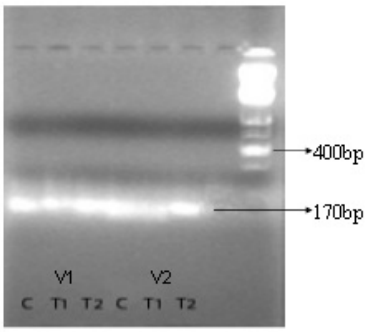
RT-PCR products

The size of RT-PCR product obtained with gene specific primer (GSP) of hva1 was about 170bp (plate 4). GSP of Apx1 amplified a product with a size approximately 120bp (plate 5). Gsp-P5CS has amplified a 150bp sized product in both genotypes and responses to stresses vary among the genotypes. Gsp of ADC1 amplified a product with approximate size of 700bp, with varying intensity among the treatments.



M-marker (BSA): C-control: T1-NaCl(250Mm): T2-PEG(-1.7Mpa)

Plate-3: Separation of proteins -Native-PAGE



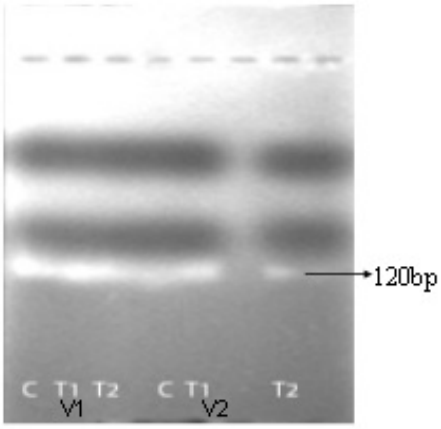
C-control V1-Co 97010

T1-NaCl (250Mm) V2-Co 99004

T2-PEG (-1.7Mpa)

Plate 4: RT-PCR product amplified by gene specific primer (hva1)

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550



C-control V1-Co 97010

T1-NaCl (250Mm) V2-Co 99004

T2-PEG (-1.7Mpa)

Plate -5: RT-PCR product amplified by gene specific primer(Apx1)

DISCUSSION

The plastid pigments decreased in response to both the stresses. Total chlorophyll reduced by 50% in sensitive genotype implying the salt injury on chloroplast system. Under dehydration the reduction were not steep. A significant reduction in chlorophyll a has been recorded. Similar results were reported for sugarcane, (Joshi and Naik 1980; Chandra 1993) barley, wheat, pearl millet and mustard (Reddy and Vora, 1985). The tolerant genotype maintained stability of chloroplast and injury was also marginal. The total carotenoid content increased in tolerant genotype, while there was a significant reduction in sensitive genotype. Carotenoids not only act as secondary/accessory pigment buy also play a role in protecting chlorophyll pigments from photo oxidation. The results indicate that higher carotenoids level protect the chlorophyll pigment and maintain the stability under both the stresses in tolerant genotype (Co 99004), while the reverse has resulted in failure of the pigment system in sensitive genotype. Soluble protein increased under both the stress in the genotypes. The increase was significantly higher in tolerant genotype. Higher protein accumulation in a salt tolerant cultivar and lower protein levels in a sensitive clone was reported for soybean (Elsamad and Shadad, 1997). Proline is a compatible osmolyte that accumulates in greater proportion, without disturbing the metabolic functioning of the cells under abiotic stress. Proline content increased in salt as well as PEG treatment in both the genotypes. Differential pattern of accumulation between genotypes suggests the faster adaptability of the genotype Co 99004 which accumulated more proline under dehydration and relatively less under salinity. This has facilitated better osmoregulation under dehydration and salinity. The oxidative enzymes viz, peroxidase and superoxide dismutase activity increased under the influence of salinity and PEG. Peroxidase activity increased in response to salinity in both the genotypes and similar results were reported for wheat (Srivalli, *et. al.*, 2003), rice. Under dehydration stress the peroxidase activity was on par with control in sensitive genotype, while the increasing trend was observed in tolerant genotype. Superoxide dismutase activity increased in both the genotypes in salt as well as PEG treatment. However, an increasing trend with levels of stress was observed for genotypes, suggesting varied level of oxidative enzymes and cycles involved in scavenging oxyradicals. Malondealdehyde (a lipid peroxidation product) content increased in response to salt and dehydration treatment. The increasing trend in lipid peroxidation differed between the genotypes. In tolerant genotype (Co99004) the lipid peroxidation was much less in salt treatment as compared to dehydration treatment.

While in sensitive genotype (Co 97010) the MDA content was higher under salinity suggesting selective impact of salinity in this genotype. In sugarcane, rice and rye increased peroxidation has been reported (Venkataramana, 1987; Shalata and Tai, 1998). The lipid peroxidation level was higher in sensitive genotype as compared to tolerant one indicating stress damage to membrane system. Soluble protein increased under both the stress in the genotypes studied. The increase was significantly higher in tolerant genotype. Although no inductions of new protein bands were recorded in salt and PEG treatment in the genotypes studied, upregulation of protein with molecular weight of 120kDa and 26kDa was observed in both the genotypes. The stress induced over expression may be due to the requirements of changed metabolic function. Induction of new protein with varying molecular weights was reported for NaCl adapted cell lines of tobacco (Singh, *et. al.*, 1985). Ramagopal and Carr (1991) identified 14 new proteins either appear or disappear in response to mild salinity stress in barley. However, the results do not confirm any inducible proteins perhaps the samples were drawn when the injury has already occurred. Isozymes of peroxidase revealed a distinct upregulation of low molecular weight isoform in tolerant genotype in response to dehydration treatment. SOD isoenzyme pattern showed intense band (over expression) in salinity treatment in sensitive genotype. The gene specific primers used in the study designed using clustal-x programme, from gene bank data for hva 1, APX 1, p5cs and ADC 1. The gene hva 1, product is a LEA protein that is reported to be induced by salt, drought, ABA and cold (Hong, *et. al*., 1992). The product size of 170bp amplified through RT-PCR was uniform in intensity in control as well as salt and PEG treatment in both tolerant and sensitive genotypes. APX 1 encodes for ascorbate peroxidase that is involved in the synthesis ascorbate for protection against oxidative stress (Wang, *et. al.*, 1995). Amplification of APX 1 was also uniform and the product size was 120bp. p5cs encodes pyroline-5-carboxylate synthatase enzyme involved in proline biosynthetic pathway. The RT-PCR product with p5cs primer showed differential amplification. In genotype Co 97010 salinity treatment did not amplify p5cs while in genotype Co 99004 the amplification was observed. ADC 1 encodes the enzyme Arginine decarboxylase, involved in the polyamine synthesis cycle and is reported to be induced under salinity (Rajam, 1998). RT-PCR product with ADC 1 primer was 700bp long and under PEG treatment the intensity of the stain was deep in sensitive genotype (Co 97010) while such no disparity was recorded with Co 99004. Biochemical parameters studied indicate the altered metabolic behavior under salt and dehydration stress. The genotypic variation adapting to the stress through biochemical characters viz, pigments, lipid peroxidation, antioxidant enzymes and osmolytes was significant. According to our findings, we concluded that the genotype (Co 97010) with better stability of the aforesaid parameters fail to perform under NaCl stress implies its selective sensitiveness to salinity. The genotype (Co 99004) with better stability, under both NaCl and PEG induced stresses, supports the cross tolerance behavior with commonness of diagnosis and adaption.

ACKNOWLEDGEMENT

I am thankful to the Department of Plant Physiology, Sugarcane Breeding Institute, Coimbatore, Tamil Nadu where I have carried out my Research work.

REFERENCES

Anonymous. (1995). Water stress and salinity management for sugarcane production survey Report. Sugarcane breeding Institute, Coimbatore.P.47.

Bates L.S, Waldern R.P. and Teak T.D. (1973). Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.

Beau Champ C. and Fridovich I. (1971). Superoxide dismutase: Improved assays and assay applicable to acrylamide gels. Analyt. Biochem. 44: 276-287

Chandra P, Singh S.K, Jagdish Singh, and Singh S.B. (1993). Influence of salt stress on photosynthetic pigment and dry weight of sugarcane shoot culture in vitro. Co- operative Sugar: 24(11) 611-612.

Cushman J.C. and Bohnert H.J. (2000) Genomic approaches to plant stress tolerance. Curr Opin Plant Biol 3: 117–124

Dhindsa R.S. Plumb-Dhindsa P. and Thorne T.A. (1981). Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J. Expt. Bot. 32: 93-101.

Coden : IJABPT Copyrights@2013 ISSN : 0976-4550

Elsamad H.M.A. and Shadad M.A.K. (1997). Salt tolerance of soybean cultivars. Biol. Plant. 39: 263-269.
Hasegawa P.M. Bressan R.A. and Pardo J.M. (2000). The dawn of plant salt to tolerance genetics. Trends in Plant Sci. 5: 317-319.
Hsiao T.C. (1973). Plant responses to water stress. Annu. Rev. Plant Physiol. 24: 519-570.
Heath R.L. and Parker L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 125: 189-198.
Hong B. Barg R. and Ho T-HD. (1992). Developmental and organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol Biol. 18: 663–674.
Joshi, G.V. and Naik G.R. (1980). Response of sugarcane to different types of salt stress. Plant and Soil: 56(2) 255-263.
Laemmli U.K. (1970). Cleavage of structure protein during the assembly of head of the head of bacteriophage T4. Nature. 227: 680-685.
Lowry O.H. Rose L. A. Brough and Randall R.J. (1951). Protein measurement with folin phenol reagent. J. Biol.Chem. 193: 265-275.
Munns R. (2002). Comparative physiology of salt and water stress. Plant Physiology: 25(2) 239.
Powlowski k. Kunze R. Devries S. and Bisseling T. (1994). Isolation of total poly (A) and polysomal RNA from plant tissue. Plant molecular biology Mannual. 5:1-13.
Rajam M.V. Dagar S. Waie B. Yadav J.S. Kumar P.A. Shoeb F. and Kumaria R. (1998). Genetic Engineering of Poly amine andCarbohydrate Metabolism for osmotic stress tolerance in higher Plants. J. Biosci. 23(4): 473-482.
Ramgopal S. and J.B.Carr. (1991). Sugarcane proteins and messenger RNAs regulated salt in suspension cells. Plant cell Environ. 14: 47-56.
Reddy M.P. and A.B. Vora. (1985). Effect of salinity on protein metabolism in bajra leaves. Indian J. Pl. Physiol. 28:190-195.
Sadasivam S. and Manickam A. (1996). Biochemical Methods for Agricultural Sciences. New Age International (P) Ltd., New Delhi. 1-97.
Scandalios J.G. (1993). Oxygen stress and superoxide dismutases. Plant Physiology 101:7-12. Scavenging enzymes in environmental stress. Ag. Biotech. News Inform., 7: 189–195.
Shalata A. and Tai M. (1998). The effect of salt stress on lipid perodxidantion and antioxidants in leaf of the cultivated tomato and its wild slat tolerant relative in tomato. Physiol. Plant. 104: 167-174.
Shuji Yokoi., Ray A. Bressan and Paul Mike Hasegawa. (2002). Salt stress tolerance of plants. JIRCAS Working Report. PP:25-33.
Singh N.K. Handa A.K. Hasegawa P.M. and R.A.Bressan. (1985). Proteins associated with adaptation of cultured tobacco cells to NaCl. Plant Physiol. 79: 126-137.
Srivalli, B., Viswanathan Chinnusamy and Renu Khanna-Chopra. (2003). Anti oxidant defense in response to abiotic stresses in plants. J. Plant Biol. 30 (2):121-139.
Venkataramana S. Mohan Naidu K. and Sudama Singh. (1987). Membrane thermostability and nitrate reductase activity in relation to water stress tolerance of young sugarcane plants. Sugarcane Breeding Institute: 335-340.
Wang M.Y. S.H. Qui, Z.J. Hua and G.Q. Han. (1995). Effect of water stress upon the activities of protective enzyme system and the structure of membrane systems in maize. Acta Agric. Bareali Sinica: 10(2) 43-49.
Weybrew J.A. (1957). Estimation of plastid pigments of Tobacco, Tobacco Science 1:1-5.