Pak. J. Bot., 43(3): 1655-1658, 2011.

CERTAIN GROWTH RELATED ATTRIBUTES OF MICROPROPAGATED BANANA UNDER DIFFERENT SALINITY LEVELS

IKRAM-UL HAQ[*], FAHEEDA SOOMRO, NAZIA PARVEEN[1], MUHAMMAD UMAR DAHOT AND AMEER AHMED MIRBAHAR[2]

Plant Biotechnology Laboratory, Institute of Biotechnology and Genetic Engineering (IBGE), University of Sindh, Jamshoro-76080, Pakistan
[1]Department of Statistics, University of Sindh, Jamshoro-76080, Pakistan
[2]Plant Tissue Culture & Biotechnology Division, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi-75270, Karachi

Abstract

The effect of salinity (NaCl) was assessed on banana (*Musa* spp.) cv., Sindhri Banana (Basrai) propagating plantlets in aseptic condition. Four different NaCl levels [0 (control) 50, 100 and 150 mM] were maintained at shoot multiplication stage for 6-weeks. Salinity reduced the number of plantlets per explants and plant biomass significantly. A proportional relationship was observed for Na^+ and Cl^- but K^+, Ca^{2+} and NO_3^- were observed to be inversely proportioned with NaCl stress. Similarly, total proteins as well as carbohydrate contents were decreased significantly. Increasing mode of secondary metabolites (proline, betaine contents and reducing sugars) were showing a negative relationship of saline stress with plant micro-propagation efficiency. Among photosynthetic pigments, total carotenoids were increased while chlorophyll contents (Chl *a* & *b*) decreased. Similarly, *nitrate reductase* activity also reduced. Overall, vegetative propagation of banana was affected significantly by NaCl stress under *in-vitro* conditions.

Introduction

Banana is one of the most important table fruit as well as staple food crop all over the world. Its yield has been losing because of a number of environmental stresses. Salinity is one of the major growth limiting abiotic factors, decreasing propagation rate of plant under natural as well as artificial conditions. Meanwhile, plant propagation rate has been considered quite higher under *in-vitro* than *ex-vitro* conditions (Kozai, 1991; Jeong *et al*., 1995; Chen *et al*., 2006).

Today, agricultural land area is losing its fertility because of soil salinity increasing with the passage of time in arid to semi-arid regions of the world. It is resulting either by natural processes or crop irrigation with saline water (Luchli & Epstein, 1990). Normal plant growth is assumed, when cultured under normal and balance nutritional conditions. While, if any of the nutritional components (may be inorganic or organic) is not properly supplied (deficient or exceeded) than it leads to abnormal expression in them (Hake & Grimes, 2009).

Most of the salinity problems are caused by excess NaCl. It imposes diffrent three types of problems, like as to exceed external osmotic pressure than internal. Disruptions of balanced nutritional ions in cells could develop toxic effects on membranes and enzymes directly. Moreover, plant growth depends on proper metabolic processes. Metabolic dysfunction is caused by ion toxicity, osmotic stress and nutritional deficiency (Greenway & Munns, 1980; Zhu, 2001; Hussain, 2008). Under stressed conditions, plants have to synthesis and accumulate various compatible solutes such as free amino acids (proline, betaine etc), sugars and polyols. These are playing a pivotal role to compensate developed osmotic stress due to salinity in different organisms including plants (Hasegawa *et al*., 2000; Ashraf & Foolad, 2007).

The fluctuations in metabolites are observable in growing plantlets through micro-propagation (Alvard *et al*., 1993). Among physical conditions, salinity is a critical factor, limiting plant propagation efficiency in *ex-vitro* as well as *In-vitro*. So plant tissue culture system has been acting as a useful tool to assess effectiveness of various salts on plant growth related characters. However, salt tolerance mechanisms involved at the whole plant level growing under natural conditions could be quite different from *In-vitro* cultures but goal is possible (Adams *et al*., 1992; Lacerda *et al*., 2001; Grennan, 2006; Lopez *et al*., 2006; Wang, 2006).

The aim of the present study was to assess, aseptic NaCl stress responses of banana (*Musa* spp) cv. Sindhri Banana (Basrai). During study differential growth was observed among cultures. These findings may be useful for marking various vegetative parameters as well as bio-chemical contents showing differential characters in stressed banana plantlets. It could be of great value for developing salt resistance in banana crop through modern studies for future.

Materials and Methods

Meristematic micro-stem cuttings (explants) of young banana (*Musa* spp.) cv., Sindhri Banana (Basrai) were excised from banana suckers. They were stirred in 70 % ethanol for 1 min and then 30 % commercial Robin Bleach® [5.25 % Sodium hypo-chlorite (NaOCl)] for 30 min. After washing with sterile distilled water, explants were cultured on MS_1 [MS_0 (Murashige & Skoog, 1962) basal medium, B_5 vitamins (Gamborg *et al*., 1968), 3 % sucrose, 10 µM IAA, 15 µM BA, 3.60 g L^{-1} phytagel] nutrient medium for organogenesis, shoot induction [MS_{2a} (MS_0, 15 µM BA, 1.0 g L^{-1} phytagel)], shoot multiplication [MS_{2b} (MS_0, 15 µM BA, 2.0 g L^{-1} phytagel)] as by Haq & Dahot (2007). Almost 4-weeks old multiplying plantlets were sub-cultured again on shoot multiplication (MS_{2b}) medium supplied with 0 (MS_{2b}), 50 (MS_{2c}), 100 (MS_{2d}) and 150 mM NaCl (MS_{2e}) in light conditions (~1000 lux).

Data was collected after 6-weeks of culture. The growing plantlets were removed and washed with running tap-water. The numbers of plantlets per explant, pseudostem diameter, and plant height, plant biomass from each culture were measured. Fresh weight (F.Wt) was measured by weighing a cluster of developed plantlets. Dry weight (D.Wt) was taken after drying them at 72°C for 48 hours. The relative water contents (RWC) and leaf area were measured.

[*]E-mail: rao.ikram@yahoo.com

Chlorophyll contents were determined by agitating 0.5 g young fresh leaflets in 10 mL acetone (80 %) as described by Arnon (1949). Betaine contents were determined as by Grieve & Grattan (1983) while proline and ascorbic acids by using methods of Bates *et al*., (1973) and Shalata & Neumann (2001). Total carbohydrates were extracted (Ciha & Brun, 1978) and quantified (Dubois *et al*., 1956). Reducing sugars (Miller, 1959), protein (Bradford, 1976) and phenolics (Ozyigit *et al*., 2007) were also determined. For nitrates, method of Morris & Riley (1963) was followed while *nitrate redutase* activity (NRA) was determined by following Klepper *et al*., (1971) method. Briefly, 100 mg young leaf tissue was homogenized in a buffer [0.1 mM potassium phosphate, 0.05 M KNO_3, 1 % iso-propanol (v/v) and pH 7.5] at 30ºC in dark. After 1 h, 1.0 mL sulfanilamide (1%) and 1.0 mL 1-Naphthyl ethylene-diamine dihydrochloride (0.02 %) were also added. The mixture was quantified by taking absorbance at 540 nm against $NaNO_2$.

Chloride contents were extracted by boiling dried plant material in dH_2O at 121°C for 5min. The volume of the extract was maintained to 50 mL the chloride contents were evaluated by Chloro-counter, by following the instruction in the instrument-operating manual (Marius Instrumenten, Utrecht, and The Netherlands). For Na^+, K^+, Ca^{2+} contents determination dried plant material was digested by acidic digestion as described by Wolf (1982). Sample extract was subjected to cationic analysis as described by Malavolta *et al*., (1989). Statistical significance of data was performed at 5 % by using *COSTAT* computer package (*CoHort Software,* Berkeley, USA).

Results and Discussion

Plant growth related aspects are being limited by a number of biotic and abiotic factors while salinity has been remaining a continuous major abiotic constraint (Flower, 2004; Munns *et al*., 2006; Ulfat *et al*., 2007). In present work, aseptic propagation of banana (*Musa* spp.) cv., Sindhri banana (Basrai) under different NaCl stresses was assessed at shoot multiplication stage as shown in Fig. 1 (Haq & Dahot, 2007). Differential modes in growth were observed in these stressed cultures (Table 1). Various morphological parameters such as number of plantlets per explant (6.01±0.13 plantlets) and their heights (3.94±0.15 cm) were decreased with increase in NaCl levels (Fig. 1, Table 1a) significantly. Leaf area, plant biomass and relative water contents were also decreased significantly. Salinity causes to decrease in photosynthetic pigments (Chl *a*, *b* and total chlorophyll) in plants (Table 1b). From data it was observed that Chl *a* is more sensitive than Chl *b* to salinity. The polyphasic abundance of chlorophyll represents a promising tool for reduction of plant tolerance to various environmental stresses. More than 50 % aseptic micro-propagation efficiency reduced under NaCl stress.

Salinity has been adversely affecting quality and quantity of vegetative characters of multiplying plantlets (Table 1). Meanwhile, accumulation of various metabolites (ascorbic acids and total carotenoids) as presented in Table 1, enables plants improve its functioning under salt stressed conditions (Noctor *et al*., 2002). These are non-enzymatic antioxidants that enable the cells against cell's oxidative stress and also perform an additional role in protection of oxidizing carotenoids (Imai *et al*., 1999; Foyer & Noctor, 2000; Conklin, 2001).

Proline contents increases significantly under NaCl stress (Table 1c). Among other organic osmolytes, proline is responsible for protecting plant tissues from stress injury. However, inorganic ions are known to act as first line of defense to combat with NaCl stress before the activation or synthesis of organic components (Hartzendorf & Rolletschek, 2001). Apparently, proline contents increases with the increase in salt (NaCl) stress from low to higher as well as low to higher amounts of Na^+ in growing tissues. Each of them may be acting as an indicator for salt stress. Meanwhile, K^+ concentration decreased significantly (Table 1c). It is responsible for imparting salt tolerance to plant tissue, but proline accumulation seems to be a symptom of cell's injury rather than to an indicator for salinity resistance (Lutts *et al*., 1999; Ottow *et al*., 2005). Incline to decline of proline is also an indication of a critical point for growth, either plantlet is under stress or non-stress conditions (Watanabe *et al*., 2001). Proline may be overproduced only when degree of stress has reached above to that of critical point for plant growth.

Potassium ions are known to be a major component of osmotic adjustment under NaCl stress (Shannon, 1992; Ottow *et al*., 2005). The presence of a high concentration of K^+ in control one, is supposed to be acting as a natural inorganic osmo-regulator (Watad *et al*., 1991; Hasegawa *et al*., 2000; Chen *et al*., 2003). Perhaps, it is allowing Na^+ to enter in tissues, situation of lowering of K^+ content is because of Na^+ shock (Table 1d). So optimum K^+ concentrations narrows with increasing Na^+ (Lutts *et al*., 1999; Lacerda *et al*., 2001).

Under saline stressed conditions, nitrate flux is blocked by reduction in *nitrate reductase* activity (NRA) biosythesis or its degradation (Ferrario *et al*., 1998; Huchzermeyer *et al*., 2004). Nitrate assimilation also reduced and its causes are evolved in parallel to expressed phenomena of plant growth (Table 1c). In agreement with observations of Silveira *et al*., (2001), when plants are subjected to NaCl stress, they present a possible relationship of homeostasis between nitrate assimilation and plant growth (Frechill *et al*., 2001; Zhu, 2001). Even plants have shown slower growth rates, may be compatible with general metabolic processes of nitrate assimilation rates (Table 1c). Among peroxidases, soluble and ionically bounded forms were affected negatively. Both of them were decreased under NaCl stress, while covalently bounded form also decreased non-significantly. However, total carotenoids were increased in the culture from low to higher saline stress (Table 1b), which could be beneficial for developing tolerance in micro-propagating plantlets under stressed conditions.

In our work it was observed that saline (NaCl) stresses are acting as critical phenomena for banana micro-propagation efficiency. Plant biomass has been decreased due to decrease in plant growth rate. Meanwhile, presence and biosynthesis of certain bio-chemicals are considered as very important for aseptic developing plantlets especially when under NaCl stresses (Fig 1, Table 1). Their biosynthesis is activated because of the feeling or sensing of applied stress. High salts concentrations develop specific osmotic stress on propagating plantlets. Synthesis of osmo-sensors (proline, betaine and reducing sugar) is biologically very important for detection, tolerance and adaptation against specific stress and acts as osmo-regulators for adjusting osmotic pressure in cells. They are the extent to which a plant can regulate the uptake and compartmentation of salts or to delay its toxic effects on the photosynthesizing apparatus, where assimilation of certain body need metabolites are going on. For future research, breeders and genetic engineers should focus their research on naturally adopted facts to saline conditions.

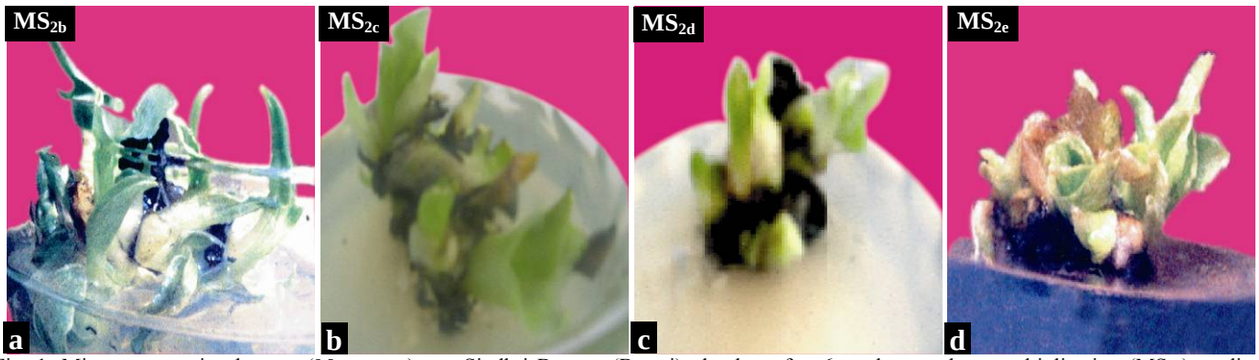

Fig. 1. Micro-propagating banana (*Musa* spp.) cv., Sindhri Banana (Basrai) plantlets after 6-weeks, on shoot multiplication (MS_{2b}) medium supplied with different NaCl [0 mM (a), 50 mM (b), 100 mM (c) & 150mM (d)] stresses.

Table 1. Some morpho-biochemical traits of banana (*Musa* spp.) cv Sindhri Banana (Basrai) micro-propagated under different NaCl stresses for 6-weeks.

Parameters	MS_{2b}	MS_{2c}	MS_{2d}	MS_{2e}	Significance
a. Morpho-metrics					
# of plantlets	6.01 ± 0.13^{a}	4.23 ± 0.124^{b}	3.25 ± 0.231^{c}	2.76 ± 0.148^{c}	***
Plant height (cm)	3.94 ± 0.15^{a}	2.86 ± 0.677^{a}	1.64 ± 0.043^{b}	1.48 ± 0.023^{b}	**
P. diameter (cm)	0.26 ± 0.01^{a}	0.24 ± 0.02^{ab}	0.21 ± 0.01^{ab}	0.17 ± 0.01^{b}	*
Fresh weight (g)	0.67 ± 0.03^{a}	0.43 ± 0.02^{b}	0.33 ± 0.021^{bc}	0.21 ± 0.015^{c}	***
Dry weight (g)	0.034 ± 0.01^{a}	0.028 ± 0.002^{ab}	0.024 ± 0.001^{ab}	0.018 ± 0.001^{d}	**
b. Chlorophyll contents (mg g^{-1})					
Chlorophyll a	0.439 ± 0.004^{a}	0.432 ± 0.006^{a}	0.411 ± 0.003^{b}	0.362 ± 0.003^{c}	***
Chlorophyll b	0.225 ± 0.003^{a}	0.212 ± 0.005^{ab}	0.201 ± 0.005^{ab}	0.188 ± 0.004^{b}	*
Chlorophyll ab	0.766 ± 0.103^{a}	0.641 ± 0.003^{b}	0.609 ± 0.002^{c}	0.553 ± 0.003^{d}	***
Total Carotenoids	4.43 ± 0.09^{c}	5.62 ± 0.10^{b}	5.76 ± 0.16^{b}	6.14 ± 0.1^{a}	***
Chl a,/Chl b	1.955 ± 0.023^{a}	2.043 ± 0.08^{a}	2.048 ± 0.04^{a}	1.923 ± 0.026^{a}	ns
c. Organic contents (mg g^{-1})					
Protein contents	3.142 ± 0.003^{a}	2.201 ± 0.004^{b}	1.995 ± 0.057^{b}	1.582 ± 0.099^{c}	***
Reducing sugars	0.372 ± 0.007^{a}	0.418 ± 0.005^{b}	0.433 ± 0.001^{b}	0.484 ± 0.006^{c}	***
Total sugars	2.905 ± 0.006^{a}	1.695 ± 0.025^{b}	1.563 ± 0.009^{b}	1.365 ± 0.095^{b}	***
Proline (µM/mg)	2.925 ± 0.021^{a}	3.126 ± 0.004^{b}	3.254 ± 0.006^{c}	2.351 ± 0.002^{d}	***
Betain (µM/mg)	2.165 ± 0.01^{a}	3.312 ± 0.006^{b}	3.562 ± 0.008^{c}	3.684 ± 0.01^{d}	***
Phenol	0.214 ± 0.005^{a}	0.263 ± 0.003^{b}	0.271 ± 0.003^{b}	0.292 ± 0.002^{c}	***
AA (µmol g^{-1})	1.94 ± 0.08^{c}	2.22 ± 0.12^{bc}	2.61 ± 0.06^{b}	3.14 ± 0.08^{a}	***
RWC (%)	65.62 ± 2.75^{a}	39.22 ± 2.54^{b}	$28.04 \pm 2.74b^{c}$	15.35 ± 2.07^{c}	***
NRA (µM NO_2/mg)	1.464 ± 0.006^{a}	1.305 ± 0.003^{b}	1.196 ± 0.006^{c}	1.014 ± 0.003^{d}	***
d. In-organic contents (mg g^{-1})					
Na^{+}	2.52 ± 0.051^{a}	4.18 ± 0.136^{b}	5.06 ± 0.09^{c}	6.58 ± 0.065^{d}	***
K^{+}	3.122 ± 0.005^{a}	2.182 ± 0.002^{b}	1.965 ± 0.002^{c}	1.658 ± 0.002^{d}	***
Ca^{2+}	2.285 ± 0.02^{a}	1.895 ± 0.03^{b}	1.612 ± 0.007^{c}	1.345 ± 0.004^{d}	***
Cl^{-}	3.392 ± 0.002^{a}	5.174 ± 0.007^{b}	8.681 ± 0.009^{c}	9.213 ± 0.012^{d}	***
NO_3^{-}	1.546 ± 0.006^{a}	1.521 ± 0.011^{ab}	1.497 ± 0.006^{b}	1.257 ± 0.006^{c}	***
K^{+}/Na^{+}	1.07 ± 0.002^{a}	0.605 ± 0.032^{b}	0.389 ± 0.007^{c}	0.252 ± 0.002^{d}	***

The values are the means of three replicates (each replicate comprised on at least three cultured banana) ± standard error with DMR test at 5%. P diameter: Pseudostem diameter. In case of root induction there was simple half strength MS medium while salinity levels are same as for shoot multiplication medium. AA: Ascorbic acid

References

Adams, P., J.C. Thomas, D.M. Vernon, H.J. Bohnert and R.G. Jensen. 1992. Distinct cellular and organismic responses to salt stress. *Plant Cell Physiol.,* 33: 1215-1223.

Alvard, D., F. Cote and C. Teisson. 1993. Comparison of methods of liquid medium culture for banana micro-propagation: Effects of temporary immersion of explants. *Plant Cell Tiss. Org. Cult.,* 32: 55-60.

Arnon, D.T. 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta Vulgaris*. *Plant Physiol.,* 24: 1-15.

Ashraf, M. and M.R. Foolad. 2007. Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.,* 59: 206-216.

Bates, L.S., R.P. Waldren and I.D. Tear. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil,* 39: 205-207.

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

Chen, S., J. LI, S. Wang, E. Fritz, A. Huttermann and A. Altman. 2003. Effects of NaCl on shoot growth, transpiration, ion compartmentation, and transport in regenerated plants of *Populus euphratica* and *Populus tomentosa*. *Canad. J. Forest Res.,* 33: 967-975.

Chen, U., C. Hsia, M. Yeh, D.C. Agrawal and H. Tsay. 2006. *In vitro* micro-propagation and *ex vitro* acclimation of *bupleurum kaoi* – an endangered medicinal plant native to taiwan. *In Vitro Cell. Dev. Biol. -Plant*, 42: 128-133.

Ciha, A.J. and W.A. Brun. 1978. Effect of pod removal on nonstructural carbohydrate concentration in soybean tissue. *Crop*

Sci., 18: 773-776.

Conklin, P.I. 2001. Recent advances in the role and biosynthesis of ascorbic acid in plants. *Plant Cell Environ.,* 24: 383-394.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Ann. Chem.,* 28: 350-356.

Ferrario, S., M. Valadier and C.H. Foyer. 1998. Over-expression of *nitrate reductase* in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. *Plant Physiol.,* 117: 293-302.

Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot.,* 55: 307-319.

Foyer, C.H. and G. Noctor. 2000. Oxygen processing in photosynthesis: regulation and signaling. *NewPhytologist* 146: 359-88.

Frechill, S., B. Lasa, L. Ibarretxe, C. Lamsfus and P. Aparicio-Trejo. 2001. Pea responses to saline stress is affected by the source of nitrogen nutrition (ammonium or nitrate). *Plant Growth Regul.,* 35: 171-179.

Gamborg, O.L., R.A. Miller and K. Ojima. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.,* 50: 151-158.

Greenway, H. and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. *Ann. Rev. Plant Physiol.,* 31: 149-90.

Grennan, A.K. 2006. Abiotic stress in rice. An ‘‘Omic’’ approach. *Plant Physiol.,* 140: 1139-1141.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water soluble quaternary-amino compounds. *Plant and Soil*., 70: 303-307.

Hake, K.D. and D.W. Grimes. 2009. Crop Water Management to Optimize Growth and Yield. *In*: *Physiology of Cotton*, (Eds.): James McD. Stewart, Derrick M. Oosterhuis, James J. Heitholt and Jackson R. Mauney, Springer Netherlands, pp. 255-264.

Haq, I. and M.U. Dahot. 2007. Effect of permanent and temporary immersion systems on banana micro-propagation. *Pak. J. Bot.,* 39: 1763-1772.

Hartzendorf, T. and H. Rolletschek. 2001. Effects of NaCl salinity on amino acid and carbohydrate contents of *Phragmites australis*. *Aq. Bot.,* 69: 195-208.

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

Huchzermeyer, B., N. Hausmann, F. Paquet-Durant and H.W. Koyro. 2004. Biochemical and physiological mechanisms leading to salt tolerance. *Trop. Ecol.,* 45: 141-150.

Hussain, T.M., T. Chandrasekhar, M. Hazara, Z. Sultan, B.K. Saleh and G.R. Gopal. 2008. Recent advances in salt stress biology – a review. *Biotechnol. Mol. Biol. Rev.,* 3: 1008-1013.

Imai, T., A.H. Kingston-Smith and C.H. Foyer. 1999. Inhibition of endogenous ascorbate synthesis in potato leaves supplied with exogenous ascorbate. *Free Rad. Res.,* 31: 171-179.

Jeong, B.R., K. Fujiwara and T. Kozai. 1995. Environment control and photoautotrophic micropropagation. *Hort. Rev.,* 17: 125-172.

Klepper, L., D. Flesher and R.H. Hageman. 1971. Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. *Plant Physiol.,* 20: 580-590.

Kozai, T. 1991. Micropropagation under photoautotrophic conditions. In: Micropropagation: technology and application. (Eds.): P.C. Debergh and R.H. Zimmerman. Dordrecht. Kluwer Academic Publishers, pp. 447-469.

Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz. 2001. Plant growth and solute accumulation and distribution in two sorghum genotypes, under NaCl stress. *Rev. Brassica Fisiol. Veg.,* 13: 270-284.

Lopez, M., J.A. Herrera-Cervera, C. Luch and N.A. Tejera. 2006. Trehalose metabolism in root nodules of the model legume *Lotus japonicus* in response to salt stress. *Physiol. Plant.,* 128: 701-709.

Luchli, A. and E. Epstein. 1990. Plant responses to saline and sodic conditions. In: Tanji KK (ed), *Agricultural Salinity Assessment and Management*, ASCE, New York, pp. 113-137.

Lutts, S., V. Majerus and J.M. Kinet. 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.,* 105: 450-458.

Malavolta, E., G.C. Vitti and S.A. Oliveira. 1989. The evaluation of the nutritional state of the plants: principles and applications. *Piracicaba*, Brazilian Association for Research of Potash and Phosphate, pp. 201.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. *Anal Chem.,* 31: 426-429.

Morris, A.W. and J.P. Riley. 1963. The determination of nitrate in sea water. *Anals of Chemica Acta*, 29: 272-279.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant, Cell Environ.,* 25: 239-250.

Munns, R., R.A. James and A. Luchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.,* 57: 1025-1043.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.,* 15: 473-497.

Noctor, G., L. Gomez, H. Vanacker and C.H. Foyer. 2002. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. *J. Exp. Bot.,* 53: 1283-304.

Ottow, E.A., M. Brinker, T. Teichmann, E. Fritz, W. Kaiser, M. Brosche, K. Kangasjarvi, X. Jiang and A. Polle. 2005. *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf succulence under salt stress. *Plant Physiol.,* 139: 1762-1772.

Ozyigit, I.I., M.V. Kahraman and O. Ercan. 2007. Relation between explant age, total phenols and regeneration response in tissue cultured cotton (*Gossypium hirsutum* L.) *Afr J. Biotechnol.,* 6: 3-8.

Papageorgiou, G.C. and N. Morata. 1995. The usually strong stabilizing effects of glycine betaine on the structure and function in the oxygen evolving photosystem-II complex. *Photosynth. Res.,* 44: 243-252.

Shalata, A. and M. Neumann. 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. *J. Exp. Bot.,* 52: 2207-2211.

Shannon, M.C. 1992. The effects of salinity on cellular and biochemical process associated with salt tolerance in tropical plants. In: (Eds.): T.L. Davenport and H.M. Harrington. *Proceedings Plant Stress in the Tropical Environment*, Florida University, Gainesville, USA, pp. 55-63.

Silveira, J.A.G., A.R.B. Melo, R.A. Viegas and J.T.A. Oliveira. 2001. Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. *Environ. Exp. Bot.,* 46: 171-179.

Ulfat, M., H.R. Athar, M. Ashraf, N.A. Akram and A. Jamil. 2007. Appraisal of physiological and biochemical selection criteria for evaluation of salt tolerance in canola (*Brassica napus* L.). *Pak. J. Bot.,* 39: 1593-1608.

Wang, Q., C.H. Wang, B. Zhao, Z.J. Ma, Y.Q. Luo, J.K. Chen and B. Li. 2006. Effects of growing conditions on the growth of and interactions between salt marsh plants: implications for invisibility of habitats. *Biol. Inv.,* 8: 1547-1560.

Watad, A.E.A., M. Reuveni, R.A. Bressan and P.M. Hasegawa. 1991. Enhanced net K^+ uptake capacity of NaCl adapted cells. *Plant Physiol.,* 95: 1265-1269.

Watanabe, S., K. Katsumi, I. Yuji and S. Sasaki. 2001. Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica In vitro*. *Plant Cell Tiss. Org. Cult.,* 63: 199-206.

Wolf, B.1982. A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Comm. Soil Sci. Plant Ann.,* 13: 1035-1059.

Zhu, J.K. 2001. Plant salt tolerance. *Trend Plant Sci.,* 6: 66-71.

(Received for publication 14 February, 2010)