

Does salinity stress amend the morphology and physiological appearance of young betel nut (*Areca catechu* L.) seedlings?

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Arecanut or betel nut (*Areca catechu* L.), one of the most important economically cultivated plants of the family Arecaceae, is an unbranched, medium-sized or long, erect, monoecious flora growing in almost all arid and semi-arid tropical regions. Naturally, the species is exposed to various stresses when raised in nursery or in field planted conditions. The excess salinization of soil, which is more conspicuous by its presence in both arid and semi-arid regions, is one of the main factors limiting crop and agricultural outputs around the world (Panta *et al.*, 2014). Salinity is one of the major environmental stresses tremendously affecting the morphological and yield-constraint of plants, besides inducing oxidative stress by the over production of reactive oxygen species (Kholova *et al.*, 2009). In plant cells, both non-enzymatic and enzymatic antioxidant protection systems are well equipped to succeed in controlling this oxidative stress under various stress conditions (Scandalios, 2002; Arbona *et al.*, 2003; Upadhyay and Panda, 2005a). The growth of individual plant organs, leading to altering general plant morphology, such as a change in the root:shoot ratio, is reduced by salinity stress with varying magnitude (Munns and Tester, 2008). The impact of excess salinity on plant growth causes ion toxicity, osmotic stress and mineral deficiencies leading to morphological, physiological and biochemical perturbations (Hasegawa *et al.*, 2000). The capacity of plants to acclimatize to salt or salinity stress can be achieved through the morphological and physiological

plasticity (Richards *et al.*, 2008). However, with this perspective, the present investigation was made to understand the effect of soil salinity and tolerance under controlled condition as there is a dearth of information on this aspect with respect to arecanut.

Eight months old betel nut (*Areca catechu* L.) seedlings were collected locally from Haflong (93°43' E longitude and 25°47' N latitude), Dima Hasao (erstwhile North Cachar Hills) district of Assam, India, and were brought to the laboratory. Seedlings were grown in laboratory condition at 25 °C, using light/dark cycle at relative humidity of 55-75 per cent with half strength Hoagland solution for watering in the pots for two weeks. After two weeks, they were analysed for salinity treatments of 100 mM and 300 mM NaCl (four seedlings per treatment) to study morphological and physiological parameters.

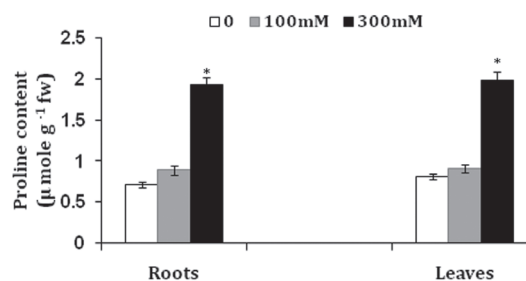


Fig. 1. Effect of NaCl concentrations on proline content of *Areca catechu* L. seedlings.

(The data presented are mean of three replication)

* indicates significant difference at $p \leq 0.05$

Growth, in terms of root and leaf biomass and plant height was measured in control and treated seedlings. Chlorophyll and carotenoid contents were determined and calculated according to Lichtenthaler (1987) and expressed as μ mole gram^{-1} fresh weight. Total anthocyanin content was determined as per the method of Laby *et al.* (2001) and expressed as μ mole gram^{-1} fresh weight. Proline content was determined according to Bates *et al.* (1973) and finally expressed as μ mole gram^{-1} fresh weight. In all the observations, data presented are the mean $\pm$ SE of at least three independent experiments. Differences between treated and control seedlings were analysed through ANOVA by SPSS 7.5.

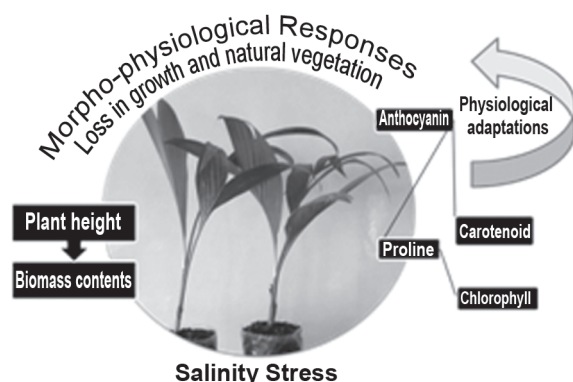


Fig. 2. Morphological and physiological responses of salinity stress on young arecanut seedlings

The effect and tolerance of soil salinity in arecanut seedlings was found to be significant as summarized in Figure 2. Among the morphological parameters studied, the growth of roots was largely inhibited compared to the growth of leaves. The growth inhibition was seen from the exposure to lower concentrations. Seedlings exposed to higher concentration (300 mM) showed uniform decrease in growth of the seedlings, nearly 50 per cent decrease in roots and leaves, suggesting growth arrest of plant at higher concentrations. Salinity had unfavourable effects on plant height, as well. It decreased substantially with higher concentration (300 mM) of NaCl (Table 1). The effect of salinity on changes in physiological parameters also showed apparent results. However, studies have found that anthocyanin, a non-chloroplastic pigment, is produced in response to various types of stresses, including salt, and are believed to increase the

Table 1. Effects of NaCl stress on biomass content of seedlings and their height

NaCl (mM)	Root biomass (g)	Leaf biomass (g)	Seedling height (cm)
0	0.80 $\pm$ 0.03	0.90 $\pm$ 0.04	40 $\pm$ 1.45
100	0.50 $\pm$ 0.03	0.61 $\pm$ 0.001 ^a	42 $\pm$ 0.77 ^a
300	0.39 $\pm$ 0.02 ^a	0.46 $\pm$ 0.003 ^a	38 $\pm$ 1.23 ^a

Alphabet 'a' indicates significant difference at $p \leq 0.05$

antioxidant response of plants in order to support the regular physiological status in tissues directly or indirectly, as affected by these abiotic stresses (Yamasaki *et al.*, 1996; Neill *et al.*, 2002). This, resulted in the accumulation (by 38.9 per cent) of anthocyanin, suggesting the possible involvement of internal detoxification mechanisms in seedlings against salinity (Chalker-Scott, 1999). As changes in pigment content are linked to an ocular symptom of plant illness and of photosynthetic output, the content of chlorophyll in plants was frequently measured for assessing the impact of environmental stresses (Parekh *et al.*, 1990). Decrease in chlorophyll (53%) and carotenoid contents (23%) was noticed, due to salt stress mediated degradation, associated with a slower pigment synthesis (Khan, 2003). Similar studies have been reported in some aquatic model plants under salinity and metal stress (Upadhyay and Panda, 2005b, 2009). However, proline, an effective antioxidant, was induced by 63 per cent (Fig. 1), that may have counteracted directly or indirectly with damaging effects of reactive oxygen species in plant cells generated due to stress (Ghoulam *et al.*, 2002).

This study revealed that an excess salinization of soil inhibits growth of young seedlings due to the obvious involvement in concentration of salts or in dose dependent factor in relation to the alterations in morpho-physiological processes of betel nut seedlings, resulting in decreased growth.

Table 2. Effects of NaCl stress on pigment contents

NaCl (mM)	Total chlorophyll (μ mol g^{-1} fw)	Carotenoid (μ mol g^{-1} fw)	Anthocyanin (μ mol g^{-1} fw)
0	3.71 $\pm$ 0.01	0.454 $\pm$ 0.01	0.234 $\pm$ 0.03
100	2.22 $\pm$ 0.22 ^a	0.459 $\pm$ 0.03	0.266 $\pm$ 0.01
300	1.73 $\pm$ 1.02 ^a	0.351 $\pm$ 0.03 ^a	0.383 $\pm$ 0.02 ^a

Alphabet 'a' indicates significant difference at $p \leq 0.05$

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References

- Arbona, V., Flors, V., Jacas, J., Garcia-Agustin, P. and Gomez-Cadenas, A. 2003. Enzymatic and non-enzymatic antioxidant responses of *Carrizo citrange*, a salt-sensitive citrus rootstock, to different levels of salinity. *Plant Cell Physiology* **44**: 388-394.
- Bates, L.S., Waldren, R.P. and Trare, I.D. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil* **39**: 205-208.
- Chalker-Scott, L. 1999. Environmental significance of anthocyanins in plant stress response. *Photochemistry and Photobiology* **70**: 1-9.
- Ghoulam, C., Foursy, A. and Fares, K. 2002. Effect of salt stress on growth, inorganic ion and proline accumulation in relation to osmotic adjustment in five sugar cultivars. *Environmental and Experimental Botany* **47**: 39-50.
- Hasegawa, P.M., Bressan, R.A. and Zhu, J.H. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**: 463-499.
- Khan, N.A. 2003. NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzyme activities in wheat. *Biologia Plantarum* **47**: 437-440.
- Kholova, J., Sairam, R.K., Meena, R.C. and Srivastava, G.C. 2009. Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. *Biologia Plantarum* **53**: 249-256.
- Laby, R.J., Kincaid, M.S., Kim, D.G. and Gibson, S.I. 2001. The *Arabidopsis* sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *The Plant Journal* **23**: 587-596.
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic bio-membrane. *Methods in Enzymology* **48**: 350-382.
- Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**: 651-681.
- Neill, S.O., Gould, K.S., Kilmartin, P.A., Mitchell, K.A. and Markham, K.R. 2002. Antioxidant activities of red versus green leaves in *Elastostema rugosum* Plant, *Cell and Environment* **25**: 539-547.
- Panta, S., Flower, T., Lane, P., Doyle, R., Haros, G. and Shabala, S. 2014. Halophyte agriculture: Success stories. *Environmental Experimental Botany* **107**: 71-83.
- Parekh, D., Puranik, R.M. and Srivastava, H.S. 1990. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie und Physiologie der Pflanzen* **186**: 239-242.
- Richards, C.L., Walls, R.L., Bailey, J.P., Parameswaran, R., George, T. and Pigliucci, M. 2008. Plasticity in salt tolerance traits allows for invasion of novel habitat by Japanese knotweed s.l. (*Fallopia japonica* and *F. × bohemica*, Polygonaceae). *American Journal of Botany* **95**: 931-942.
- Scandalios, J.G. 2002. The rise of ROS. *Trends in Biochemical Science* **27**: 483-486.
- Upadhyay, R.K. and Panda, S.K. 2005a. Biochemical changes in *Azolla pinnata* L. under chromium toxicity. *Journal of Plant Biology* **32**: 49-52.
- Upadhyay, R.K. and Panda, S.K. 2005b. Salt tolerance of two aquatic macrophytes, *Pistia stratiotes* and *Salvia molesta*. *Biologia Plantarum* **49**: 157-159.
- Upadhyay, R.K. and Panda, S.K. 2009. Copper-induced growth inhibition, oxidative stress and ultrastructural alterations in freshly grown water lettuce (*Pistia stratiotes* L.). *Comptes Rendus Biologies* **332**: 623-632.
- Yamasaki, H., Uefuji, H. and Sakihama, Y. 1996. Bleaching of the red anthocyanin by superoxide radical. *Archives in Biochemistry and Biophysics* **332**: 183-186.