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Soil salinity alters the morphology in *Catharanthus roseus* and its effects on endogenous mineral constituents

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Abstract
In order to meet the ever increasing demand for medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil salinity, which is prevalent in many parts of the world, pose serious threat to plant production. So it seems valuable, to test the important medicinal plants for their salt tolerance capacity. In the present investigation, experiments were conducted to study the effects of soil salinity on growth and mineral nutrients in *Catharanthus roseus* plants under pot culture. The plants were treated with different concentrations of NaCl, 25, 50, 75 and 100 mM on 30, 45, 60 and 75 days after sowing (DAS). Salinity affected all the morphological parameters and decreased the growth performance. The mineral contents (nitrogen, phosphorus, calcium, magnesium, potassium, iron, manganese and zinc) were analysed from treated as well as the control plants. All the treatments altered the mineral contents when compared to the untreated control plants but a significant change was found in 50 mM NaCl concentration, in which the levels of some minerals increased. Tissue sodium uptake was determined for all the treated plants and was found to have increased to a significant level when compared to the untreated plants.

Keywords: *Catharanthus roseus*, growth, minerals, salinity.

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INTRODUCTION

The growth and development of the plant are the internal processes that are under the control of the environment. Temperature, moisture, radiation, nutrients and gases can either enhance or retard the growth and development of the plant. Some times these factors may act as stress leading to injury and in extreme cases the death of the plant (Jaleel et al. 2007a). Salinity effects are more conspicuous in arid and semiarid regions, where limited rainfall, high evapotranspiration and high temperature associated with poor water and soil management contributes to the salinity problem and is also of great importance to the agricultural production in these regions. Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern (Jaleel et al. 2007b). Every year more and more land becomes non-productive owing to salt accumulation. Salinization plays a major role in soil degradation. It affects 19.5% of irrigated land and 2.1% of dry land agriculture in the world. In India, out of 9.38 million ha of salt-affected soils, 3.88 million ha are alkali soil and 5.5 million ha (including coastal lands) are saline soils (Anonymous 2000). The use of saline soils and brackish groundwater for growing plants of varying economic importance like

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those used in traditional medicines assumes utmost importance (Manivannan et al. 2008).

The earliest response to salt stress in plants is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress intensifies (Parida and Das 2005). The deleterious effect of salinity on plant growth is attributed to the decrease in the osmotic potential of the growing medium, specifically the ion toxicity and nutrient ion deficiency (Luo et al. 2005). The extent of plant growth depression under saline conditions varies with salt composition, salt concentration, the physiological stage of the plant and the plant species (Jaleel et al. 2007c, 2008a). The general pattern of plant response to salinity is growth suppression more or less proportional to the solute concentration. Plants growing under such saline conditions accumulate various solutes by altering their metabolic pathways (Younis et al. 1993). A saline environment can reduce a wide number of responses in plants ranging from readjustment of transport and metabolic processes leading to growth inhibition (Jaleel et al. 2007d). Na^+ is the predominant soluble cation in most saline soils and water, particularly in the coastal area. Most crop plants exhibit considerable hypersensitivity to a saline environment because the inter cellular accumulation of Na^+ is toxic to the cellular metabolism and for many salt sensitive plants, a major part of the growth inhibition was caused by excess Na^+ in the soil (Jaleel et al. 2007e). High amounts of sodium disturb potassium (K) nutrition and when accumulated in cytoplasm, inhibit many enzymes (Sharma et al. 1997). These effects are also due to a combination of adverse osmotic gradients and inhibitory effects of salts and ions on cell metabolism and of nutrient imbalance and secondary stresses such as an oxidative stress linked to the production of toxic reactive oxygen intermediates (Parida and Das 2005).

Ion uptake and compartmentalization are crucial not only for normal growth but also for growth under saline conditions, because stress disturbs ion homeostasis (Adams et al. 1992). Glycophytes limit sodium uptake or partition sodium in older tissues that serve as storage compartments that are eventually sacrificed (Iqbal et al. 2006). Selective accumulation of ions or solutes enables the plants to make osmotic adjustments, which occur through mass action, and results in increased water retention and/or sodium exclusion (Parida and Das 2005).

With increasing realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and antibiotics, more and more people are interested in the use of plants and plant based drugs being revived through out the world. So exploitation of medicinal plants has become more and more popular (Tan et al. 2006). For the past several years, several scales of physiology have been applied to study responses to salt stress tolerance mechanisms and methods to overcome salt stress in field crops (Sankar et al. 2006, Manivannan et al. 2008). However little information is known about the physiological basis of growth reduction in terms of mineral composition under salt stress in medicinal plants. It seems necessary to do research related to the correlation between medicinal plants and salt stress for the increasing need of medicinal plants. In order to meet the ever increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil salinity and other forms of pollutions pose serious threats to plant production (Qureshi et al. 2005, Jaleel et al. 2007c, 2008a). So it seems valuable, to test the important medicinal plants for their salt tolerance capacity.

Catharanthus roseus (L.) G. Don. (Apocynaceae) is one of the important medicinal plants, due to the presence of the indispensable anti-cancer drugs, vincristine and vinblastine. Roots of this plant are the main source of the anti-hypertension alkaloid ajmalicine (Jaleel et al. 2006a). It is also a popular ornamental plant. There are commonly two varieties of this plant based on the flower colour viz., pink flowered rosea and white flowered alba (Jaleel et al. 2007f). Previous works revealed the influences of triadimefon on the antioxidant metabolism and ajmalicine production (Jaleel et al. 2006a), paclobutrazol mediated growth regulation (Jaleel et al. 2006b), salinity effects on proline metabolism (Jaleel et al. 2007c), salt stress protection by paclobutrazol (Jaleel et al. 2007b) and the effects of different types of stresses and growth regulators (Jaleel et al. 2007g-m, 2008b,c) in *C. roseus*. To the best of our knowledge, no information on the physiological response in terms of nutrient content and mineral composition of *C. roseus* under soil salinity is available. The purpose of this study was to provide additional information on the growth performance with specific emphasis to micro and macro elements accumulating in *C. roseus* under different concentrations of NaCl treatments.

MATERIAL AND METHODS

Plant culture and salt stress induction

The seeds of *Catharanthus roseus* (L.) G. Don. (Apocynaceae) were collected from our Botanical garden and surface sterilized with a 0.2% $HgCl_2$ solution for 5 min with frequent shaking and then thoroughly washed with deionised water. The seeds were pre-soaked in 500 mL of deionised water (control) and 25, 50, 75 and 100 mM NaCl solutions for 12 h. Seeds were sown in plastic pots (300 mm diameter) filled with 3 kg of a soil mixture containing red soil, sand and farm yard manure (FYM) at 1:1:1 ratio. Before sowing the seeds, the pots were irrigated with the respective treatment solutions and the electrical conductivity (EC) of the soil mixture was measured. Four seeds were sown per pot and the pots were watered to the field capacity with deionized water for 90 days after sowing (DAS) and every care was taken to avoid leaching. The initial EC level of the soil was maintained by flushing each pot with the required volume of corresponding treatment solution on the 30th, 45th, 60th and 75th DAS. The plants were uprooted the 90th DAS for estimating the growth, and to perform biochemical and mineral content analyses.

Growth parameters

Morphological parameters like root length, plant height were measured in fresh samples. The total leaf area was calculated with a LICOR Photoelectric Area Meter (Model 41-3100, Lincoln, USA). Fresh and dry weights were calculated by an electronic weighing device (Model-Citizen XK3190-A7M).

Mineral content estimations

Nitrogen (Peach and Tracey 1956), phosphorus, calcium, magnesium (Yoshida et al. 1972), potassium (Williams and Twine 1960), copper, iron, manganese and zinc (De Vries and Tiller 1980) were analyzed from the leaves of treated and control plants on the 90th DAS. Sodium uptake in the NaCl treated plants was determined by using the method of Piper (1952).

Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The values are mean ± SD for the six samples in each group. P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

The growth performance of the plants was estimated by measuring the plant height, leaf area, root length, total fresh weight and dry weight on the 90th DAS. Under 100 mM NaCl stress, the growth of the plants was highly affected and reduced the fresh and dry weight by about 25% and 26% when compared to control plants (Table 1). Plant height was decreased up to 7% and 34% under low and high salinity respectively when compared to the control plants. Root length of the plants showed a reduction up to 30% and 53% under 50 and 100 mM NaCl treatment respectively in comparison with the untreated plants. Leaf area showed the highest value in the control plants whereas, under salt conditions it decreased gradually with the increase in salinity (Table 1). Similar results were found in *Salvodora persica* under salinity conditions (Dagar et al. 2004). The earliest response of salt stress in plants is a reduction in the rate of leaf surface expansion, followed by a cessation of expansion as the stress

Table 1. The effect of increasing NaCl concentration on different growth parameters in *C. roseus* on 90 DAP.

NaCl (mM)	Plant height (cm plant $^{-1}$)	Root length (cm plant $^{-1}$)	Leaf area (cm^2 plant $^{-1}$)	Total fresh weight (g plant $^{-1}$)	Total dry weight (g plant $^{-1}$)
0	63 ± 2.333^{a}	26 ± 0.866^{a}	164 ± 5.857^{a}	28.48 ± 0.949^{a}	3.64 ± 0.135^{a}
25	64 ± 2.353^{a}	23 ± 2.336^{b}	163 ± 5.333^{a}	23.00 ± 0.333^{b}	2.80 ± 2.333^{b}
50	58 ± 2.071^{b}	18 ± 0.666^{c}	117 ± 4.333^{b}	23.17 ± 0.827^{b}	2.81 ± 0.108^{b}
75	$50 + 2.633^{c}$	$16 + 0.333^{d}$	$110 + 4.333^{c}$	$22.33 + 1.030^{b}$	$2.77 + 2.333^{b}$
100	41 ± 1.366^{d}	12 ± 0.400^{c}	98 ± 3.500^{d}	21.11 ± 0.781^{b}	2.69 ± 0.072^{b}

Values are given as mean ± SD of seven experiments in each group. Bar values are not sharing a common superscript (a,b,c,d,e) differ significantly at $P \leq 0.05$ (DMRT).

intensifies (Parida and Das 2005). The Catharanthus plants acts as an absorber of Na and may not be able to cope with it, leading to leaves eventually suffering from the toxic effects and hence leads to damage in leaf growth as in the marigold (Chaparzadeh et al. 2004) might be the reason for retarded growth. Due to the abiotic stress from salinity, the plant tries to cope with the situation by decreasing its leaf area and hence allows the conservation of energy.

The treatments with NaCl altered the mirco and macro nutrient elements in all parts of the *Catharanthus* plants significantly when compared to the control plants. The treatments decreased the potassium content in the leaves of the treated plants. Increasing salt treatments decreased the potassium content in all parts of the *Catharanthus* plants seedlings on the 90th DAS (Fig. 1b). The reduction of tissue potassium content was high in the shoot followed by the root. Reduced potassium content was also reported in NaCl stressed grasses (Warwick and Hallovan 1991) and *Vicia faba* (Gadallah 1999). The reduction in nitrogen content (Fig 1b) under NaCl treatments was comparable with the results of Rother et al. (1983) in heavy metal treatments like cadmium, lead and zinc. Flores et al. (2000) emphasized that Cl^- sharply decrease the NO_3 uptake by the roots, incorporation of nitrogen into organic compounds and translocation of nitrogen to the leaves.

The phosphorus content (Fig. 1a) of the shoot decreased with an increase in the NaCl concentration. Excess amounts of trace elements usually affect the mineral nutrition of plants (Bollard 1983). The decrease in mineral level due to NaCl stress may be due to the competition of Na^+ and Cl^- with other mineral ions in the uptake (Khan 2001). Metal toxicity in general was associated with reduced absorption and accumulation of potassium (Siddiqi and Glass 1983). Potassium is one of the essential macronutrients, taken up by the roots is generally transported to the shoot through the xylem and this transport seems to be controlled by the shoot growth (Lidon and Henriques 1993). A decrease in potassium content of *Catharanthus* due to NaCl treatments may be due to the toxic effect of NaCl on plant growth or competition by other ions, which in turn exercised a regulatory control on potassium uptake.

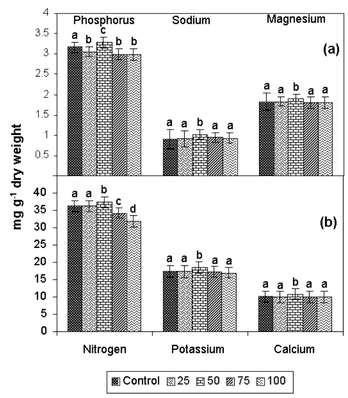

Fig. 1. Effects of NaCl at different concentrations (0, 25, 50, 75 and 100 mM) on (a) phosphorus, sodium, magnesium and (b) nitrogen, potassium, calcium contents of *C. roseus* on 90 DAS. Values are given as mean SD of seven experiments in each group. ± Bar values are not sharing a common superscript (a,b,c,d,e) differ significantly at $P \leq 0.05$ (DMRT).

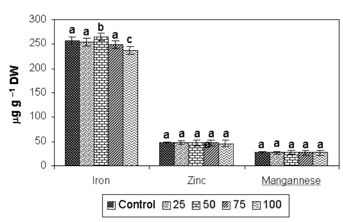

Fig. 2. Effects of NaCl at different concentrations (0, 25, 50, 75 and 100 mM) on iron, zinc and manganese contents of *C. roseus* on 90 DAS. Values are given as mean ± SD of seven experiments in each group. Bar values are not sharing a common superscript (a,b,c,d,e) differ significantly at $P \leq 0.05$ (DMRT).

Sodium uptake was more or less equal in the control and treated plants. A significant uptake was foud in the 50 mM NaCl concentration only (Fig. 1a). The iron content (Fig. 2) of the *Catharanthus* plants decreased with the increase in NaCl concentration in the soil, but increased under the 50 mM NaCl concentration. Zinc and manganese were not affected by the NaCl treatment significantly. Wallace and Abou-Zam Zam (1989) found that the inhibition of iron absorption was due to occupation of interfering ions in the iron absorbing sites. Their efficient translocation appears to depend on chelation or complexing by organic acids in the plant. The manganese content (Fig. 2) of the shoot of the *Catharanthus* plants under NaCl treatment decreased gradually with the increase in the NaCl level in the soil. Inhibition of manganese is in accordance with the findings of Parida et al. (2004) in *Bruguiera parviflora* under salt stress.

CONCLUSION

From the results of this investigation, it is clear that, the NaCl treatment caused significant accumulation in the micro and macronutrients in the medicinal plant *Catharanthus roseus* though it reduces the plant growth. This data is of great economic significance, hence it was previously reported that (Sreevalli et al. 2004), abiotic stresses like water stress, salt stress, metal stress etc., can cause an increase in the active principle contents in medicinal plants and this will be helpful for the cultivation of this plant in salt affected areas.

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Toprak Tuzlulugunun *Catharanthus roseus*'un Morfolojisini Degistirmesi ve Endojen Mineral Icerigine Etkileri

Ozet

Tibbi bitkiler, yoresel tibbi uygulamalari ve eczacilik endustrisi icin gittikce artmakta olan talebi karsilamak amaciyla, bazi tibbi bitkiler ticari olarak uretilmelidirler. Ancak dunyanin bir cok yerinde yaygin olan toprak tuzlulugu, bitki uretimi acisindan buyuk bir tehdit olusturmaktadir. Bu yuzden onemli tibbi bitkileri tuza toleranslari acisindan test etmek onem arz etmektedir. Bu arastirmada, toprak tuzlulugunun *Catharanthus roseus*'da buyume ve mineral maddeler uzerine etkilerini arastirmak amaciyla deneyler yapilmistir. Bitkiler ekimden sonra 30, 45, 60 ve 75. gunlerde 25, 50, 75 ve 100 mM NaCl konsantrasyonlari ile muamele edildiler. Tuzluluk butun morfolojik parametreleri etkiledi ve buyume performansini dusurdu. Mineral icerigi (nitrojen, fosfor, kalsiyum, magnezyum, potasyum, demir, manganez ve cinko) muamele edilmis bitkiler ve kontrol bitkilerinde analiz edildi. Kontrol bitkileri ile karsilastirildiginda, butun muameleler mineral icerigini degistirdi, fakat bazi minerallerin seviyelerinin arttigi, 50 mM NaCl konsantrasyonunda onemli bir degisiklik tespit edildi. Doku sodyum alimi, butun muamele edilmis bitkiler icin belirlendi ve kontrol bitkileriyle kiyaslandiginda onemli oranda arttigi goruldu.

Anahtar Kelimeler: Buyume, *Catharanthus roseus*, mineral, tuzluluk.

