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Soil Salinity Alters Growth, Chlorophyll Content, and Secondary Metabolite Accumulation in *Catharanthus roseus*

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Abstract: The effect of salinity on growth, photosynthetic pigment content, and alkaloid secondary metabolite accumulation were studied in an economically important medicinal plant, *Catharanthus roseus* (L.) G. Don., under pot culture conditions. Plants were treated with different concentrations of NaCl, (e.g. 50 and 100 mM) 30, 45, 60, and 75 days after sowing (DAS). The plants were uprooted randomly 90 DAS to analyse growth, and chlorophyll and alkaloid content. Salinity affected all the morphological parameters and decreased growth performance. At low salinity regimes, a slight decrease was noted in chlorophyll a and b, and total chlorophyll content, but under high salinity conditions a significant reduction in the content of these pigments was observed. The chlorophyll a:chlorophyll b ratio also varied significantly under salinity stress. Alkaloid content increased under saline soil conditions.

Key Words: *Catharanthus roseus*; growth; chlorophyll; salinity; alkaloid

Catharanthus roseus Bitkisinde Toprak Tuzluluğunun Büyüme, Klorofil Miktarı ve İkincil Metabolit Birikimi Üzerine Etkisi

Özet: Ekonomik öneme sahip *Catharanthus roseus* (L.) tıbbi bitkisinin büyüme, fotosentetik pigment muhtevası ve alkoloit ikincil metabolit birikimi üzerinde tuzluluğun etkisi çalışılmıştır. Bitki tohumları 50 ve 100 mM lık tuz ile muamele edilmiş ve tohumlar ekildikten sonra 30, 45, 60, 75 gün aralıklarla deiyonize suyla sulanmıştır. 90 günlük bitkiler alınarak büyüme, klorofil ve alkoloit muhteva analizi yapılmıştır. Tuzluluğun bütün morfolojik parametreleri etkilediği ve büyüme performansını azaltığı gözlenmiştir. Az tuzluluk rejiminde klorofil a, b ve toplam klorofil muhtevasında çok az bir artış olmasına karşın, yüksek tuzluluk durumlarında pigment muhtevasında önemli bir azalma olmuştur. Klorofil a / klorofil b oranı tuz stresinde oldukça fazla değişkenlik göstermiştir. Toprağın tuzluluğu alkaloid muhtevasında artışa neden olmuştur.

Anahtar Sözcükler: *Catharanthus roseus*, büyüme, klorofil, tuzluluk, alkaloid

Introduction

Agricultural productivity is severely affected by soil salinity and the damaging effect of salt accumulation in agricultural soils has become an important environmental concern (1). Every year more and more land becomes non-productive due to salt accumulation. In India, of the 9.38 million ha of salt-affected soil, 3.88 million ha are alkali soil and 5.5 million ha (including coastal lands) are saline soil. The use of saline soil and brackish groundwater for growing plants of varying economic importance assumes utmost importance (2).

The earliest plant response of salt stress is a reduction in the rate of leaf surface expansion, followed by cessation of expansion as the stress intensifies (3). The deleterious effect of salinity on plant growth is attributed to the decreased osmotic potential of the growing medium, specific ion toxicity, and nutrient ion deficiency (4). Saline environments can reduce a wide number of responses in plants, including readjustment of transport and metabolic processes, leading to growth inhibition (5). Na^+ is the predominant soluble cation in most saline soils and water, particularly in coastal areas. Most crop plants exhibit considerable hypersensitivity to saline environments because inter cellular accumulation of Na^+ is toxic to cellular metabolism, and for many salt-sensitive plants excess Na^+ in the soil plays a major role in growth inhibition (6). High sodium levels disturb potassium (K^+) nutrition and when accumulated in cytoplasm it inhibits many enzymes (7). These effects are also due to a combination of adverse osmotic gradients, and the inhibitory effects of salts and ions on cell metabolism, as well as nutrient imbalance and such secondary stresses as oxidative stress linked to the production of toxic reactive oxygen intermediates (8).

Catharanthus roseus (L.) G. Don (Apocynaceae) derives its economic importance from its highly valued leaf anticancer alkaloids vincristine and vinblastine, and its antihypertensive root alkaloid ajmalicine (9,10). All parts of the plant are rich in alkaloids, with maximum concentrations found in the root bark, particularly during flowering. An infusion of the leaves is used to treat menorrhagia. The juice of the leaves is applied externally to treat wasp stings. All parts of the plant are credited with hypoglycaemic properties and are used to treat diabetes (11). Extensive research has already been conducted concerning this plant's medicinal importance (10,1,12) and growth regulator effects (13,14), and how it is affected by drought (13,15-17). A deeper understanding of the mechanism of salt stress tolerance is necessary for breeders to identify which plants are suitable to saline areas (18), which will also be very useful for cultivating this medicinal plant, particularly in salt-affected areas following natural calamities like tsunami. Moreover, it is important to use a quick and reliable index of salt tolerance that will enable the screening of varieties (9,12). In the present study the effect of salinity on growth and photosynthetic pigment of this plant, in relation to total indole alkaloid concentration, under pot culture conditions was investigated in order to evaluate the adverse effect of soil salinity in medicinal plant cultivation.

Materials and Methods

Plant Cultivation and Salt Stress Induction

Catharanthus roseus (L.) G. Don. seeds were collected from the herb garden of the Department of Botany, Annamalai University for use in the experiments. The experiments were conducted at the Botanical Garden and Stress Physiology Laboratory, Department of Botany, Annamalai University. The seeds were surface sterilized in a 0.2% $HgCl_2$ solution for 5 min (with frequent shaking) and then thoroughly washed with tap water. The seeds were pre-soaked in 500 ml of deionised water (control), then in 50 and 100 mM NaCl solutions for 12 h. Seeds were sown in plastic pots (300 mm diameter) filled with a soil mixture containing red soil, sand, and farm yard manure (FYM) (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. Seeds were sown in pots and watered to field capacity with deionised water for up to 90 days after sowing (DAS); all possible precautions were 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 30, 45, 60, and 75 DAS. The plants were uprooted 90 DAS to estimate growth, and chlorophyll and alkaloid content.

Analysis of Morphological Parameters

Morphological parameters, such as root length and plant height, were measured in fresh samples. Total leaf area was calculated with a LICOR photoelectric area meter (Model 41-3100, Lincoln, USA) and expressed in cm^2 $plant^{-1}$. Fresh and dry weights were measured with an electronic balance (Model-Citizen XK3190-A7M).

Estimation of Chlorophyll Content

Chlorophyll a, chlorophyll b, and total chlorophyll were extracted and estimated from fresh leaves, following the standard method of Arnon (19).

Estimation of Total Indole Alkaloid Content

Ajmalicine extraction from the roots was carried out according to the standard extraction method (20). Identification and quantification of ajmalicine were performed by preparative thin layer chromatography, using silica gel G (Merck) in chloroform:methanol (98:2 v/v) by comparison of R_f values to an authentic ajmalicine standard (Himedia, Mumbai) (Figure 1). Ajmalicine was spotted with Dragendorff's reagent (10).

Statistical Analysis

The experiment used a randomized block design with 7 replicates. Each treatment was analysed with at least 7 replications and a standard deviation (SD) was calculated; data are expressed as mean ± SD of 7 replicates.

Results and Discussion

The Effect of Increasing NaCl Concentration on Growth Parameters

Growth performance of the plants was estimated by measuring plant height, leaf area, root length, and total fresh weight and dry weight 90 DAS. Under 100 mM NaCl stress plant growth was negatively affected,

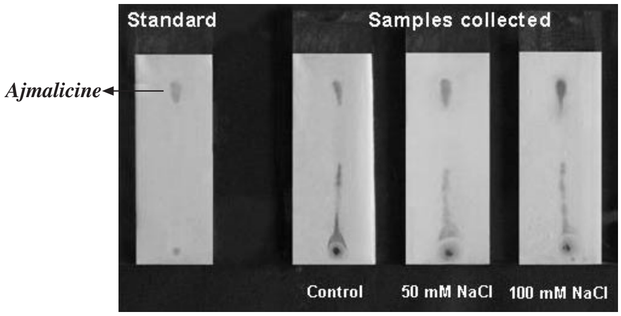

Figure 1. TLC profile of ajmalicine standard and ajmalicine samples from controls and NaCl-stressed *Catharanthus roseus* (L.) G. Don 90 DAS.

reducing fresh and dry weight by about 25% and 26%, respectively (Table). Plant height decreased by up to 7% and 34% under low and high salinity, respectively, when compared to the control. Root length was reduced by up to 30% and 53% under 50 and 100 mM NaCl treatment, respectively, in comparison to untreated plants. Leaf area showed the highest value in controls, whereas under salt conditions it decreased gradually with increasing salinity (Table). Similar decreases in growth parameters were found in *Withania somnifera* under salt stress (21) and in *Salvodora persica* under saline conditions (22). *Catharanthus* plants act as an absorber of Na and may be incapable of coping with it, leading to the leaves eventually suffering from the toxic effects and resdulting in reduced leaf growth, as reported in *Withania somnifera* (21). Due to abiotic stress from salt, the plant tries to cope with the situation by decreasing its leaf area, hence, conserving energy.

The Effect of Increasing NaCl Concentration on Chlorophyll Content

A decrease in photosynthetic pigment content of *Catharanthus* plants under salt stress was observed. There was a decrease of 11% and 38% of chlorophyll a in response to the 50 and 100-mM NaCl treatment, respectively, when compared to the control 90 DAS (Figure 2). In the case of chlorophyll b, the decrease was 16% and 33% in response to the 50 and 100-mM NaCl treatment, respectively, compared to the control. Total chlorophyll was reduced by 14% and 34% under low and high salinity, respectively (Figure 1). The results obtained in this study are in agreement with those of Azooz et al. (23) for sorghum and Dager et al. (22) for Salvadora *persica*. The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex (24). It is also attributed to the interference of salt ions with the de novo synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (25). It is therefore proven that soil salinity had negative effects on the growth and photosynthetic metabolism of *C. roseus*.

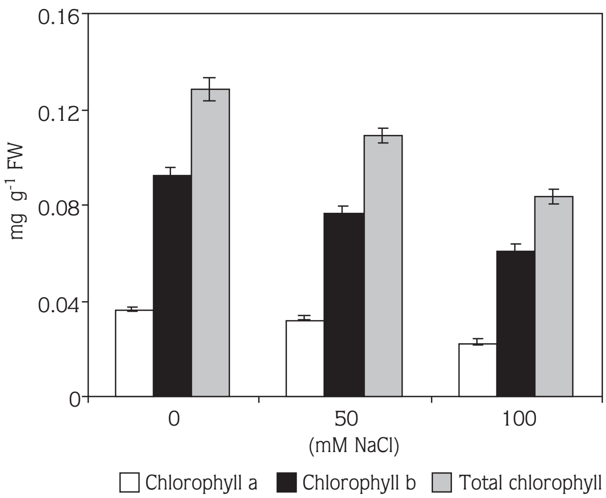

Figure 2. The effect of increasing NaCl concentration on the chlorophyll (mg g^{-1} FW) content of *Catharanthus roseus* (L.) G. Don 90 DAS. The data are the mean ± SD of 7 replicates.

Table. The effect of increasing NaCl concentration on different growth parameters in *C. roseus* (L.) G. Don. 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 $plant^{-1}$)	Indole alkaloid content (mg g^{-1} DW)
0	63 ± 2.33	26 ± 0.87	164 ± 5.86	28.48 ± 0.95	3.64 ± 0.14	2.6±0.09
50	58 ± 2.07	18 ± 0.67	117 ± 4.33	23.17 ±0.83	2.81 ± 0.11	2.8±0.12
100	41 ± 1.37	12 ± 0.40	98 ± 3.50	21.11 ± 0.78	2.69 ± 0.07	3.1±0.22

Values are mean ± SD of 7 replicates.

Effect of Increasing NaCl Concentration on Total Alkaloid Content

Total indole alkaloid content varied with different soil salinity regimes; higher alkaloid content was recorded in NaCl-treated plants when compared to control plants. Previously, we reported alterations in the alkaloid content of *C. roseus* in response to abiotic stresses like drought, as well as under growth regulator treatments (10,13-18).

Conclusion

Based on the present results, it is concluded that *C. roseus* can be cultivated in salt-affected areas, which could increase its production of secondary metabolites at the plant level. Nonetheless, a detailed investigation regarding the suitability of this plant to varying salinity regimes, from very low to high, in addition to detailed field experiments are needed to confirm this conclusion.

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