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# Effect of Salinity and Drought on Growth Criteria and Biochemical Analysis of *Catharanthus roseus* Shoot

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**Abstract:** The present research was carried out to evaluate the effects of different salinity levels and drought durations on growth (shoot length, fresh and dry weights) as well as pigments, photosynthetic activity, transpiration rate and carbohydrate content of shoot system of *Catharanthus roseus*. Five levels of salinity (0, 50, 100, 150 and 200 mM of NaCl solution) and drought (control, 1, 2, 3 and 4 weeks water regime) were applied for 4 months. The plants could not tolerate the highest salinity level and severe drought duration until the end of the experiment. The impairment of shoot length, shoot fresh and dry weights after 3 and 4 months of salinity and drought treatment were attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and/or decrease in many metabolic activities that could be considered an adaptive feature for plant survival under stress. The reduction in chlorophyll a and chlorophyll b associated with reduced photosynthetic activity and transpiration rate in *C. roseus* plants subjected to all salinity levels were considered a defense mechanism against damaging reactive oxygen species by diminishing light absorbing capacity that reduce the flow of electrons through the photosystems. The plants acclimated to mild drought conditions by maintaining photosynthesis and transpiration rate as appeared from the accumulation of carbohydrates while responded to severe drought by reducing photosynthesis and transpiration.

**Key words:** *Catharanthus roseus*, salinity, drought, photosynthesis

## INTRODUCTION

The last decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products (Briskin, 2000). *Catharanthus roseus* (L.) G. Don. (Family: Apocynaceae) is one of the highly exploited and studied medicinal plants. The plant contains alkaloids vindoline and catharanthine which are valuable source of antitumour agents like vinblastine and vincristine used in chemotherapy of leukemia and in the treatment of Hodgkin's disease. It is also a popular ornamental plant (Filippini *et al*., 2003; Jaleel *et al*., 2006). Unfortunately, *C. roseus* produces a complex array of indole alkaloids of which vinblastine and vincristine represent a very small proportion (about 0.00025% of leaf dry weight; Misawa and Goodbody, 1996). Since vindoline typically occurs in the aerial parts of *Catharanthus* plants (Westekemper *et al*., 1980) large scale mass production requires commercial cultivation of this medicinal plants especially in areas that is not suitable for traditional farming systems. Such cultivation is restricted by unfavorable conditions as soil salinity and drought.

Salinity and drought are the most important abiotic stresses that adversely affect plant growth and productivity (Mahajan and Tuteja, 2005). They result in dehydration and osmotic imbalance of the cell. As the soil salinity increases, water is osmotically held in the soil and water becomes less accessible to the plant (Marschner, 1986). Drought stress can occur for a variety of reasons, such as limited water availability or intense evaporation. Among the several approaches to solve the problems of salinity and drought is to identify and grow stress tolerant plants. Most of the interest was paid to crop plants (Flowers, 2004; Chinnusamy *et al*., 2005). With only a few exceptions, many widely used medicinal plants have not received the extensive plant physiological characterization received by food crops or model plant systems (Briskin, 2000).

Despite being intensively studied, most of the attention paid to *C. roseus* focused on the effect of salinity on seedling stage or suspension cell (Karadge and Gaikwad, 2003; Elkahoui *et al*., 2004, 2005; Misra and Gupta, 2005) and the induction of salt tolerant mutants (Rai and Kumar, 2000; Rai *et al*., 2003). It is therefore, essential to test *C. roseus* for their salinity and drought tolerance during plant development for the economic exploitation. The present investigation was carried out to evaluate the effects of different salinity levels and drought durations on growth (shoot length, fresh and dry

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weights) as well as pigments, photosynthetic activity, transpiration rate and carbohydrate content of shoot system of *Catharanthus roseus*.

## MATERIALS AND METHODS

**Plant material:** *Catharanthus roseus* plantlets (two months old) were planted in 150 pots with dimensions 15 cm width × 10 cm height (2 individuals per pot) filled with soil (3 clay: 1 perlite). The pots were divided into two sets and salinity and drought stresses were separately applied for each set.

**Salinity stress:** Five levels of NaCl solution were used: Zero (control), 50, 100, 150 and 200 mM. Pots of each treatment were irrigated every two weeks (WK) for a period of 4 months.

**Drought stress:** Five durations of water-regime were applied as: control (3 days), one-week-regime (irrigation every week), 2-weeks-regime (irrigation every 2 weeks), three-weeks-regime (irrigation every 3 weeks) and four-weeks-regime (irrigation every 4 weeks) for a period of 4 months.

For both treatments, the pots were arranged in randomized complete blocks where all salinity levels and drought durations were represented in each block. Six samples were collected from each treatment every month up to 4 months. Immediately after sample collection growth criteria (shoot length and shoot fresh weight), chlorophyll content, net photosynthesis and respiration rate were measured. Samples were oven dried at 35-40°C for the further analysis of carbohydrate content.

**Chlorophyll contents:** Chlorophyll a and chlorophyll b were determined quantitatively using N, N-dimethylformamide (DMF) as described by Moran and Porath (1980). The Chlorophyll content was expressed as g/100 g fresh weight.

**Photosynthesis and respiration rate:** The photosynthesis and transpiration were measured using an infra red gas analysis system by a clipping single leaf in a Parkinson leaf chamber of a portable ADC-LCA4 system (The Analytical Development Company Ltd., Hoddesdon, Herts, UK) at a photon flux density of 250 $\mu E\ m^{-2}\ s^{-1}$ (PAR).

**Carbohydrates:** Carbohydrates were estimated quantitatively using Phenol-sulfuric acid colorimetric method according to DuBois *et al*. (1956).

**Statistical analysis:** The variation in salinity and drought variables in relation to the period of treatment were assessed using Two-way analysis of the variance (ANOVA) for randomized complete block design using SPSS statistical package (Voelkl and Gerber, 1999).

## RESULTS

**Growth criteria:** As the salinity level increased, shoot length, shoot fresh and dry weights gradually decreased when compared with the control at all treatment periods (Table 1). The maximum percentage of reduction in growth criteria was observed in plants treated with 200 mM NaCl for three months (50, 82 and 84% in shoot length, fresh and dry weights, respectively). However, the plants couldn't tolerate this level of salinity for four months and the maximum percentage of reduction was observed in plants treated with 150 mM NaCl (52, 68 and 71% in shoot length, fresh and dry weights, respectively). The observed reduction in growth criteria due to the effect of salinity levels and the period of treatment as well as their interaction was highly significant ($p \leq 0.001$).

After the first month of drought, growth criteria decreased compared to the values of control as the duration of drought increased from 1 to 4 weeks (Table 1). However, the 2-month-old plants subjected to 1-week-regime were less affected showing values close to those of control. As the period of duration to this water regime (one week) increased to 3 and 4 months, the fresh and dry weights were significantly reduced. The plants couldn't tolerate 4-weeks-regime and the maximum percentages of reduction in shoot length, shoot fresh and dry weights (63, 85 and 87%, respectively) were observed in plants subjected to 3-weeks-regime for 4 month. Statistical analysis indicated that the effect of different drought durations, the effect of period of treatment and their interaction were highly significant ($p \leq 0.001$).

**Biochemical analysis**

**Chlorophyll a and Chlorophyll b:** Generally, the content of both chlorophyll a and chlorophyll b decreased as compared to the control with increasing salinity level (Table 2). The lowest highly significant values of chlorophyll a and chlorophyll b were observed after the 3rd and 4th months of treatment with 200 and 150 mM NaCl, respectively. The chlorophyll a/chlorophyll b ratio increased with increasing the period of treatment reaching the highest values in the 3 and 4 month. Statistical analysis showed that the effect of different salinity levels and the period of treatment on chlorophyll a was highly significant ($p \leq 0.001$) while their interaction

Table 1: Effect of salinity and drought on growth criteria of *Catharanthus roseus* shoots

| | | | Salinity (mM) | | | | Drought (weeks) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Period | Measurements | Control | 50 | 100 | 150 | 200 | 1 | 2 | 3 | 4 |
| **1st month** | | | | | | | | | | |
| | Length | 14.70±1.25 | 10.00±3.00 | 9.30±1.25 | 8.60±1.15 | 7.50±1.50 | 9.80±1.04 | 9.20±1.12 | 8.60±0.20 | 7.50±0.10 |
| | Fresh weight | 3.95±0.35 | 2.99±1.39 | 2.30±0.70 | 2.10±0.60 | 1.85±0.65 | 3.20±0.80 | 2.70±0.05 | 2.16±0.59 | 2.06±0.37 |
| | Dry weight | 0.87±0.324 | 0.44±0.13 | 0.42±0.11 | 0.38±0.13 | 0.24±0.06 | 0.47±0.21 | 0.46±0.18 | 0.29±0.16 | 0.28±0.10 |
| **2nd month** | | | | | | | | | | |
| | Length | 24.90±2.00 | 18.40±1.31 | 16.60±1.82 | 15.60±3.38 | 15.30±0.57 | 23.80±0.56 | 12.60±0.52 | 10.70±0.40 | - |
| | Fresh weight | 8.54±0.07 | 4.69±0.06 | 4.41±0.95 | 4.01±0.65 | 2.82±0.66 | 8.62±0.75 | 3.41±0.27 | 2.71±0.35 | - |
| | Dry weight | 1.15±0.37 | 0.75±0.26 | 0.72±0.50 | 0.57±0.38 | 0.31±0.04 | 1.12±0.03 | 0.56±0.47 | 0.42±0.27 | - |
| **3rd month** | | | | | | | | | | |
| | Length | 33.30±5.41 | 27.80±0.70 | 19.50±0.05 | 18.40±1.10 | 16.60±0.35 | 30.50±0.50 | 19.90±0.55 | 11.80±0.76 | - |
| | Fresh weight | 16.82±1.23 | 9.25±2.33 | 7.02±1.40 | 5.64±1.30 | 3.01±0.65 | 13.31±0.54 | 6.12±1.33 | 3.13±0.34 | - |
| | Dry weight | 2.65±0.01 | 1.37±0.43 | 1.15±0.15 | 1.01±0.55 | 0.42±0.08 | 1.83±0.98 | 0.89±0.33 | 0.53±0.14 | - |
| **4th month** | | | | | | | | | | |
| | Length | 43.00±1.00 | 35.70±0.75 | 25.20±0.75 | 20.40±0.55 | - | 38.90±0.79 | 27.40±0.10 | 15.90±0.70 | - |
| | Fresh weight | 22.3±0.800 | 16.95±0.95 | 8.30±1.25 | 7.02±0.01 | - | 17.00±0.10 | 11.02±2.10 | 3.32±0.98 | - |
| | Dry weight | 4.7±0.930 | 2.99±0.64 | 2.15±0.43 | 1.33±0.28 | - | 3.29±0.67 | 1.89±0.34 | 0.61±0.14 | - |

Table 2: Effect of salinity and drought on biochemical criteria of *Catharanthus roseus* shoots

| | | | Salinity (mM) | | | | Drought (weeks) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Period | Measurements | Control | 50 | 100 | 150 | 200 | 1 | 2 | 3 | 4 |
| **1st month** | | | | | | | | | | |
| | Chl a | 0.173±0.02 | 0.170±0.01 | 0.168±0.01 | 0.140±0.01 | 0.122±0.02 | 0.197±0.003 | 0.190±0.02 | 0.168±0.009 | 0.146±0.01 |
| | Chl b | 0.064±0.005 | 0.067±0.004 | 0.061±0.01 | 0.047±0.006 | 0.045±0.03 | 0.047±0.006 | 0.046±0.01 | 0.048±0.004 | 0.0510±0.004 |
| | Chl a/b | 2.7 | 2.53 | 2.75 | 2.98 | 2.71 | 4.19 | 4.13 | 3.5 | 2.86 |
| | Photosynthetic activity A | 0.380±0.10 | 0.360±0.03 | 0.240±0.02 | 0.190±0.08 | 0.130±0.02 | 0.580±0.08 | 0.470±0.11 | 0.290±0.06 | 0.110±0.01 |
| | Transpiration E | 0.410±0.06 | 0.280±0.01 | 0.160±0.08 | 0.160±0.01 | 0.100±0.04 | 0.550±0.14 | 0.450±0.01 | 0.340±0.07 | 0.300±0.01 |
| | Carbohydrate content | 4.890±0.82 | 4.990±0.79 | 4.850±0.74 | 4.800±0.04 | 4.700±0.28 | 5.800±1.10 | 6.030±0.46 | 6.260±0.58 | 6.150±0.95 |
| **2nd month** | | | | | | | | | | |
| | Chl a | 0.183±0.02 | 0.162±0.01 | 0.149±0.01 | 0.130±0.006 | 0.092±0.01 | 0.186±0.008 | 0.174±0.01 | 0.165±0.004 | - |
| | Chl b | 0.070±0.005 | 0.055±0.001 | 0.049±0.009 | 0.047±0.002 | 0.031±0.02 | 0.053±0.01 | 0.065±0.009 | 0.062±0.006 | - |
| | Chl a/b | 2.61 | 2.94 | 3.04 | 2.76 | 2.96 | 3.5 | 2.67 | 2.66 | - |
| | Photosynthetic activity A | 0.480±0.13 | 0.380±0.08 | 0.250±0.03 | 0.100±0.03 | 0.050±0.01 | 0.660±0.08 | 0.550±0.11 | 0.380±0.06 | - |
| | Transpiration E | 0.390±0.07 | 0.190±0.08 | 0.160±0.05 | 0.160±0.04 | 0.080±0.01 | 0.340±0.06 | 0.330±0.01 | 0.300±0.15 | - |
| | Carbohydrate content | 6.070±0.53 | 6.400±0.35 | 5.830±0.21 | 5.610±0.58 | 5.460±0.21 | 6.710±0.23 | 5.340±0.34 | 5.300±0.60 | - |
| **3rd month** | | | | | | | | | | |
| | Chl a | 0.189±0.01 | 0.159±0.02 | 0.096±0.03 | 0.078±0.01 | 0.030±0.01 | 0.182±0.01 | 0.175±0.006 | 0.158±0.04 | - |
| | Chl b | 0.047±0.005 | 0.038±0.009 | 0.025±0.01 | 0.018±0.006 | 0.009±0.004 | 0.058±0.008 | 0.043±0.003 | 0.042±0.01 | - |
| | Chl a/b | 4.02 | 4.18 | 3.84 | 4.33 | 3.33 | 3.13 | 4.06 | 3.76 | - |
| | Photosynthetic activity A | 0.520±0.03 | 0.290±0.05 | 0.170±0.02 | 0.100±0.01 | 0.050±0.00 | 0.300±0.01 | 0.290±0.08 | 0.170±0.03 | - |
| | Transpiration E | 0.200±0.05 | 0.140±0.03 | 0.120±0.03 | 0.090±0.006 | 0.080±0.00 | 0.270±0.02 | 0.190±0.06 | 0.120±0.04 | - |
| | Carbohydrate content | 6.530±0.28 | 5.320±0.44 | 5.220±0.46 | 5.130±0.23 | 4.620±0.74 | 6.030±0.34 | 5.700±1.25 | 5.450±1.04 | - |
| **4th month** | | | | | | | | | | |
| | Chl a | 0.198±0.02 | 0.134±0.02 | 0.095±0.03 | 0.091±0.01 | - | 0.180±0.03 | 0.180±0.01 | 0.140±0.03 | - |
| | Chl b | 0.047±0.005 | 0.024±0.009 | 0.023±0.01 | 0.022±0.01 | - | 0.052±0.008 | 0.054±0.005 | 0.037±0.01 | - |
| | Chl a/b | 4.21 | 5.58 | 4.13 | 4.14 | - | 3.46 | 3.33 | 3.78 | - |
| | Photosynthetic activity A | 0.590±0.05 | 0.190±0.04 | 0.050±0.01 | 0.020±0.01 | - | 0.370±0.05 | 0.230±0.03 | 0.110±0.07 | - |
| | Transpiration E | 0.170±0.06 | 0.090±0.02 | 0.080±0.06 | 0.060±0.00 | - | 0.140±0.03 | 0.120±0.01 | 0.110±0.05 | - |
| | Carbohydrate content | 7.660±0.97 | 5.790±0.16 | 5.700±0.46 | 5.360±0.23 | - | 6.610±1.50 | 5.300±1.21 | 5.220±0.11 | - |

was significant ($p \leq 0.05$). The effect of the salinity level and the period of treatment on chlorophyll b was highly significant ($p \leq 0.001$), while their interaction was insignificant.

The chlorophyll a and chlorophyll b contents respond differently to the drought (Table 2). The chlorophyll a content significantly increased after one and 2 weeks of drought for 1 month then insignificantly reduced in as the period increased. The only significant reduction was observed in the 3-weeks-water regime after 3 and 4 month. On the contrary, chlorophyll b content increased in 3-month-old plants subjected to one-week-regime and 4-month-old plants after one and two weeks drought.

**Photosynthetic activity and transpiration:** The photosynthetic activity significantly decreased with increasing salinity level and the period of treatment (Table 2). The lowest activities were evident with the salinity level of 200 mM at the third and fourth months followed by those of 100 and 150 mM at the fourth month. Also, transpiration rate decreased with increasing salinity level with the maximum reduction occurred with 150 mM in the fourth month. Statistical analysis showed that the effect of the duration of treatment on photosynthetic activity was not significant. Yet, it was highly significant ($p \leq 0.001$) regarding transpiration. The effect of different salinity levels on the photosynthetic activity and transpiration was highly significant ($p \leq 0.001$).

Generally, the photosynthetic activity significantly increased in plants treated for one and two weeks of drought for one and two months (Table 2) then significantly decreased in all drought regimes. The respiration rate increased only in plants treated with 1 week drought for one month while it was reduced in all other drought durations.

**Carbohydrates content:** Carbohydrate content gradually decreased with increasing salinity level from 50 to 200 mM during the four months of treatment compared to control samples (Table 2). An exceptional slight increase was observed with the level 50 mM in the first and second month. The reduction in carbohydrate content was more pronounced in the second month (10%) with the level 200 mM. The percentage of reduction increased after three months of treatment with the maximum value of 29% with 200 mM NaCl compared to control sample. After four months of treatment, the plant could not survive the prolonged salinity treatment with 200 mM, so the maximum reduction in carbohydrate content was 30% with the level 150 mM NaCl compared to control sample. Analysis of variance revealed highly significant reduction ($p \leq 0.001$) in carbohydrate content with different salinity levels as well as the period of treatment, while their interaction was not significant.

After one month of treatment, the different drought durations caused a successive increase in total soluble carbohydrate content in the shoot system of *C. roseus*. After two months of treatment, one-week-regime was the only duration that increased carbohydrate content by 10% compared to control sample. There was a successive reduction in carbohydrate content in the third and the fourth month of treatment by the duration to 3-weeks-regime (17 and 31%, respectively). Statistical analysis showed that neither the effect of the different drought durations nor the effect of the period of treatment caused significant decrease in carbohydrate content. The interaction between the two variables was also not significant.

## DISCUSSION

In the present study, *C. roseus* plants could not tolerate the highest salinity level (200 mM) and severe drought duration (4-weeks-water regime) until the end of the experiment (4 months) (Table 1 and 2). Both salinity (100 and 150 mM) and drought (2 and 3-week-water regime) impair shoot length, shoot fresh and dry weights after 3 and 4 months. Similar observations were reported by Rai and Kumar (2000) and Rai *et al.* (2003) on comparing *C. roseus* salt-sensitive wild type with their salt-tolerant mutant. The reduction in plant growth has been attributed to reduced water absorption due to osmotic effect, nutritional deficiency on account of ionic imbalance and/or decrease in many metabolic activities (Kumar *et al.*, 2005). Besides, the reduction in growth could also be attributed to reduction in cell division and/or in cell enlargement (Hopkins, 1999; Zhu, 2001). It has been suggested that slower growth could be considered an adaptive feature for plant survival under stress, because it allows plants to divert assimilates and energy, otherwise used for shoot growth, into protective molecules to fight stress (Zhu, 2002).

Pigmentation reflects photosynthetic properties of phototrophic organisms as it indicates the size of light-harvesting capacity. Both chlorophyll a and chlorophyll b were reduced in *C. roseus* plants subjected to all salinity levels with more reduction in chlorophyll b (Table 2). Salinity, like any other abiotic stress, leads to oxidative stress. If too much light energy hits the photosystems, they can react with substrates other than the normal electron carriers generating very damaging reactive oxygen species (Qureshi *et al.*, 2005). The reduced photosynthetic machinery (such as reduction of the chlorophyll content) can diminish the flow of electrons through the photosystems (Koyro, 2006; Sicher, 1999).

The reduction in chlorophyll content subsequent to salinity was correlated with reduced photosynthetic activity and transpiration rate (Table 2). Several studies on halophytes (e.g., *Plantago coronopus*, *Beta vulgaris* sp. *maritima* and *Spartina townsendii*) revealed at their threshold salinity tolerance a reduced photosynthesis, minimum transpiration, high stomatal resistance and minimum internal $CO_2$-concentration (Koyro, 2000, 2003, 2006). It was concluded that such behavior tends to reduce the salt loading into the leaves and helps to increase the longevity by maintaining salts at subtoxic levels longer than it would occur if transpiration rates were not diminished (Everard *et al.*, 1994). So that, the stomatal closure, reduced photosynthetic activity and

transpiration rate could be considered as adaptive mechanisms to cope with excessive salt, rather than merely a negative consequence of it (Flanagan and Jefferies, 1988; Clark *et al*., 1999).

*C. roseus* plants responded differently when subjected to drought (Table 2). The chlorophyll a content of plants subjected to mild drought (1 and 2 weeks water regime) was maintained at levels similar to those of control. Besides, an increase in chlorophyll b content was observed after 3 and 4 month at the same water regime. This pattern was correlated with increase in photosynthetic activity and transpiration rate in *C. roseus* plants subjected to the mild drought (1 and 2 weeks water regime) for short periods (one and two months). It was found that in coffee plants if drought progresses slowly, they can acclimate to water shortage and thus, plants could minimize the deleterious effects of drought, e.g., by partially maintaining photosynthetic and transpiration rates (Praxedes *et al*., 2006). The drought-induced accumulation of carbohydrates after 1 and 2 weeks water regime for 1 and 2 months should support this suggestion.

As the severity of drought increased to 3 to 4 weeks water regime for 3 to 4 month period, the photosynthetic activity and transpiration rate fall apart (Table 2). It has been proved that plants respond to water stress by closing stomata, which reduces $CO_2$ availability in the chloroplasts and thus photosynthesis and photosynthetic capacity are progressively decreased under severe drought treatment (Cornic, 2000; Galmes *et al*., 2007).

In conclusion, the results may suggest that *C. roseus* tolerates salinity up to 150 mM NaCl and drought up to 3-weeks water regime. The plants appeared to tolerate drought more than salinity. More investigation is needed to correlate both stresses with the alkaloid production of this plant.

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