INFLUENCE OF SODIUM CHLORIDE SALINITY ON GROWTH AND ORGANIC CONSTITUENTS OF *CATHARANTHUS ROSEUS* G. DON.

B.A. KARADGE* AND P.V. GAIKWAD

Department of Botany, Shivaji University, Kolhapur-416 004

Received on 3 Sept., 2002, Revised on 22 Sept., 2003

SUMMARY

Effect of NaCl salinity (25, 50, 100 and 200 mM NaCl added to soil in pot culture) were investigated on growth and some organic constituents in different parts of *Catharanthus roseus* G. Don, an important medicinal plant. It was observed that NaCl salinity reduced overall growth of this plant, which was highly significant at higher salinity levels only. The level of organic acids and total nitrogen in the leaves declined due to salt treatment. There was accumulation of soluble sugars in the leaves, particularly at lower salinity regimes and in the stem and roots at all salinity levels. Polyphenol metabolism in the plant appeared less influenced by salinity. NaCl salinity,induced both qualitative and quantitative changes in the alkaloids of *C. roseus*.

Key words: **Alkaloids, *Catharanthus roseus*, salinity, salt tolerance.**

INTRODUCTION

Among the several approaches to solve the problem of saline soils, biological approach to identify and grow salt tolerant plants in such soils to bring about soil reclamation is promising. Lot of work has already been carried out in this direction in a number of crop species. Medicinal plants, however, have been neglected in this regard. It is, therefore, essential to test important medicinal plants for their salinity tolerance for the economic exploitation of such problem soils.

In the present investigation an attempt has been made to study the salinity tolerance of *Catharanthus roseus* G. Don, a promising medicinal plant in the pot culture.

MATERIAL AND METHODS

Catharanthus roseus G. Don plants were raised in pot culture from seeds collected from the Botanical Garden of Department of Botany, Shivaji University, Kolhapur. After a month, the fully established plants in the pots were subjected to different levels of salinity such as 25, 50, 100, 200 and 300 mM NaCl. The plants were treated with salt solutions twice a week with alternate watering with equal amounts of tap water to check the loss of water due to evapotranspiration and to maintain the salt concentration in the medium. After two months of treatment, plants were carefully uprooted, keeping the root system intact, washed thoroughly with water and surface dried, separated into different parts and used for further analysis.

Plant growth was assessed through various growth parameters like shoot length, root length, leaf area per plant, number of buds, flowers and pods per plant and biomass (dry wt) per plant. Moisture content of various plant parts was determined following the dry weight method. Organic constituents were determined both from fresh as well as dried material. From fresh material, leaf

*Corresponding author

juice acidity (titratable acid number, TAN) (Thomas and Beevers 1949), polyphenols (Folin and Denis 1915) and proline (Bates *et al.* 1973) were determind. Carbohydrates (Nelson 1944), total nitrogen (Hawk *et al.* 1948) and total alkaloids (Thomas and Sharma 1979) were estimated from oven-dried material. Qualitative analysis for alkaloids was done using thin layer chromatography (Mc Laughin *et al.* 1964).

RESULTS AND DISCUSSION

NaCl salinity reduced the plant height of *Catharanthus roseus* G. Don (Table 1). However, the shoot : root ratio was increased with increasing salinity level. Leaf area and number of buds, flowers and pods produced by the plant were also decreased with increase in salinity level. Salt grown plants exhibited low biomass (dry wt) production. This adverse effect of salinity, however, was significant only at higher salinity levels (100 and 200 mM NaCl). A decrease in overall growth of plants due to salinity has been reported by a number of workers (Hoffman *et al.* 1989, Imamul Huq and Larher 1983, Gonia *et al.* 1994). An increase in shoot : root ratio in *Catharanthus* is indicative of an adverse effect of NaCl salinity on the root growth suggesting salt sensitive nature of the plant.

The moisture content of the leaves and stem was slightly decreased with increasing salinity levels (Fig. 1a). However, this decrease was more at higher (200 mM) NaCl concentration. Roots showed an increase in the moisture level upto 100 mM NaCl concentration. Increase in succulence is considered as mechanism for promoting tolerance to high salinity levels (Jennings 1976). *C. roseus* was successful in developing succulence, particularly at lower salinity regimes, but was unable to do so at higher salinity levels. So this species can be categerized as moderately salt tolerant one.

Reduction in organic acid content under salt stress condition has been reported by Azizbekova and Rasulova (1972) in cotton leaves, Abdel Resooul *et al.* (1980) in maize, Morishita *et al.* (1986) in tomato leaf sap and Perez-Alfocea *et al.* (1993) in the leaves of *Lycopersicon esculentum* and *L. pennellii*. A decrease in acidity status in the leaves of *C. roseus* (Fig. 1b) indicates that this species has failed to develop the mechanism of salt tolerance when grown under saline condition.

The accumulation of starch in the young and mature leaves of this plant was observed at lower salt concentrations (25 and 50 mM NaCl), while there was decrease at higher salinity levels (100 and 200 mM NaCl). The level of starch increased in the stem and roots linearly with increasing salt level in the medium (Fig. 2a). Gauch and Eaton (1942) have reported an increase in starch content in the leaves of barley under salinity of chlorides of Na, Mg and Ca, which was due to lack of utilization of starch rather than the adverse effect of salinity on photosynthesis. Yang *et al.* (1990) and Heuer (1991) have reported increased sucrose levels under saline conditions in *Sorghum* and sugarbeet respectively. Osmotic adaptation to salinity via increased sucrose accumulation in the leaves and roots is common in plants (Hellebust 1976). From the present study it is evident that

Table 1. Effect of NaCl salinity on growth and development of *C. roseus* G. Don grown in pot culture.

Growth parameter	NaCl treatment (mM)				
	00(Control)	25	50	100	200
Shoot length (S, cm)	14.74±0.65	14.00±0.25	13.40±0.46	12.50±0.28	12.06±0.81
Root length (R, cm)	17.40±0.53	14.70±0.82	14.00±0.58	12.00±0.46	10.18±0.44
Shoot/root ratio	0.85±0.03	0.95±0.04	0.96±0.05	1.04±0.02	1.18±0.03
Leaf area plant^{-1}(cm^2)	114.24	99.38	92.85	73.99	67.69
No. buds plant^{-1}	4.00±1.73	3.00±2.00	3.00±1.00	2.00±1.00	2.00±0.00
No. flowers plant^{-1}	1.40±0.55	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
No. pods plant^{-1}	6.00±2.64	5.00±1.73	4.00±1.00	3.30±1.53	3.00±1.00
Dry wt. plant^{-1} (g)	1.38±0.25	0.98±0.12	0.88±0.18	0.76±0.21	0.67±0.16

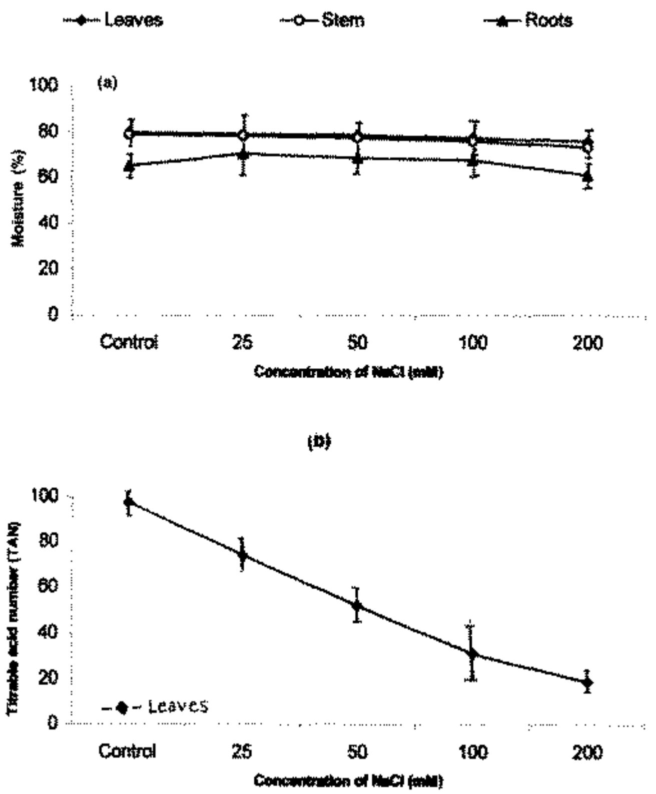

Fig. 1. Effect of NaCl salinity on moisture content and organic acid content (TAN) in different parts of *C. roseus* G. Don. grown in pot culture

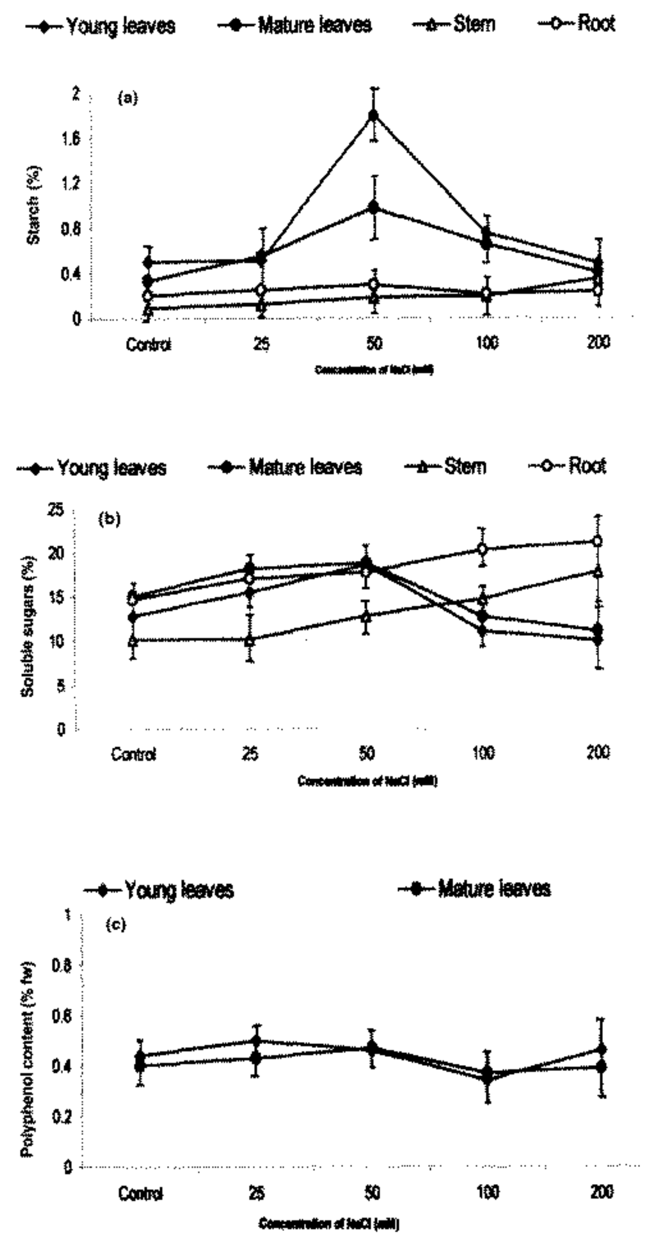

Fig. 2. Effect of NaCl salinity on carbohydrate and polyphenol contents in different parts of *C. roseus* G. Don. grown in pot culture

there was considerable accumulation of soluble sugars (total sugars) in the leaves of *C. roseus* upto 50 mM NaCl level. However, at the higher salinity levels there was not much change in their level. There was accumulation of soluble sugars in the stem and roots at all concentrations of salt (Fig.2b). It appears that accumulation of soluble sugars is an adaptive feature of this species to cope up with adverse saline conditions.

Polyphenol content of young leaves increased with salt concentration, except at 100 mM NaCl salinity, (Fig. 2 c). In the mature leaves its level increased at lower (25 and 50 mM) salt concentrations and decreased at higher (100 and 200 mM) salt concentrations. Shetty (1971) has reported accumulation of polyphenols in the leaves of *Achrosticum aeurum* grown in low salt regimes. It has been reported that the highly evolved halophytes like mangroves are rich in polyphenols (Joshi 1976). An increase in the level of polyphenols in the leaves due to salinity was reported by Nalawade (1988) in niger and Wadkar (1989) in *Crotalaria* species. It appears that polyphenol metabolism in *C. roseus* was less influenced by NaCl salinity.

Total nitrogen content in young leaves of *C. roseus* decreased at 25 mM NaCl concentration, but later gradually increased with further increase in salinity level, and at 200 mM NaCl it was higher than control (Fig. 3a). In mature leaves it decreased at lower salt concentrations (upto 100

Indian J. Plant Physiol., Vol. 8, No. 4, (N.S.) pp. 392-397 (Oct.-Dec., 2003)

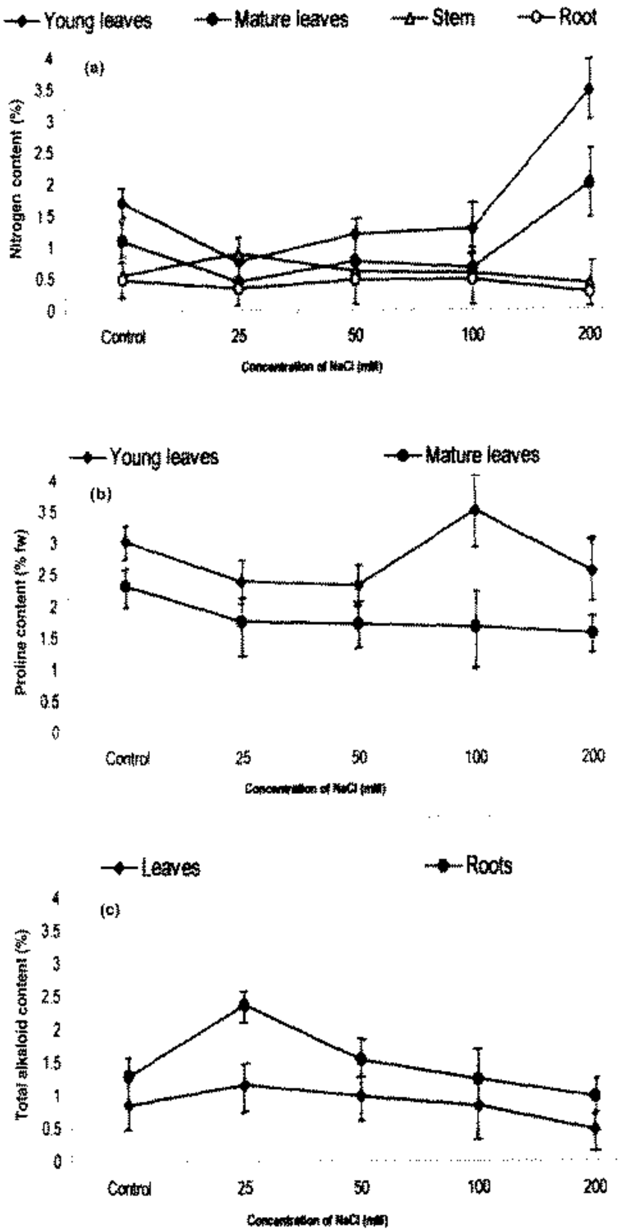

Fig. 3. Effect of NaCl salinity on total nitrogen, proline and alkaloid contents in different parts of *C. roseus* G. Don. grown in pot culture

mM NaCl). Level of total nitrogen content of stem also increased at lower salinity levels but decreased at higher salinity levels. In the roots there was decrease in N-content at all salinity levels. Nirtrogen matabolism is adversely affected by salt stress in plants (Strogonov 1964). A decrease in total nitrogen content due to salinity stress has been reported by Paliwal and Maliwal (1980) in sorghum and green gram, Karadge (1981) in *Portulaca oleracea* and He and Cramer (1992) in three *Brassica* species. Accumulation of nitrogen in the leaves of this plant grown at 200 mM NaCl level, however, indicates a disturbed overall metabolism of the plant at the higher salinity regimes.

The amount of proline in the young leaves of *C. roseus* decreased due to salt stress except at 100 mM NaCl concentration (Fig. 3b) while in the mature leaves there was a linear decrease in it with increasing salinity level in the rooting medium. It indicates that proline content of this plant was not influencd by NaCl salinity and it appears that proline has no role to play in the salt tolerance capacity of this species.

The total alkaloid content was more in the roots of *C. roseus* than leaves and it increased in both upto 100 mM NaCl salinity (Fig. 3c). Shain (1988) reported increased alkaloid content in *Atropa belladonna, C. roseus* and *Solanum laciniatum* when subjected to water stress. Saenz *et al.* (1993) observed two fold increase in the alkaloid content of mature leaves of *C. roseus* under severe water stress. Addition of NaCl, KCl or sorbitol to 5-day-old cell suspension culture of *C. roseus* stimulated an increase in intracellular accumulation of Catharanthine and other alkaloids within 48-72 h. The magnitude of response depends upon the concentration of compound added (Smith *et al.* 1987). Accumulation of secondary metabolites like alkaloids in the tissue under stress condition could have a bearing on the adaptability of the plant to stress condition. The preliminary studies on alkaloid composition of the roots and leaves (Table 2) indicated that NaCl salinity exerts a remarkable influence on individual alkaloids of these plant parts. It is, therefore, suggested that mild stress (water or salinity stress, upto 100 mM NaCl) at the time of maturity of the plant can have a commercial application to obtain maximum amount of secondary metabolites of medicinal importance from both roots and leaves of *C. roseus.*

Table 2. Effect of NaCl salinity on alkaloid composition (with relative amount of each alkaloid) in roots and leaves of *C. roseus* G. Don.

Type of Alkaloid with Rf value on TLC		NaCl treatment (mM) 00	50	100	200	300
			Roots			
1	0.26	+	+	+	+	+
2	0.34	+	+	-	-	+
3	0.48	+	+	+	+	+
4	0.53	-	++	-	-	++
5	0.60	-	+++	-	-	-
6	0.64	-	+	-	-	++
7	0.68	-	++	-	-	++
8	0.73	++	+	+	+	+
9	0.85	++	+	++	++	+
1	0.91	+	+	+	+	+
			Leaves			
1	0.03	+	+	+	+	+
2	0.47	-	+	-	+	-
3	0.54	+	+	-	+	-
4	0.57	+	+	++	-	-
5	0.61	++	+	+	++	++
6	0.66	+	+	+	++	++
7	0.78	+	+	+	++	++
8	0.88	++	++	++	+	+
9	0.93	+++	++	++	+++	+
10	0.96	++	++	++	+++	+++

+ Present ++ Present in appreciable amounts +++ Present in large amounts (accumulation)

ACKNOWLEDGEMENTS

One of the authors (PVG) acknowledges UGC for the award of Teacher Fellowship. Authors sincerely thank Head, Department of Botany, Shivaji University, Kolhapur for providing facilities in the Department.

REFERENCES

Abdel-Rasoul, M., Hussein, M.M. and Ashoub, M.A. (1980). Effect of some growth regulators on the concentration of organic acids and amino acids in maize (*Zea mays*) seedlings under different levels of salinity. I. The concentration of organic acids. *Shams Univ. Fac. Agric. Res. Bull.* (1330) : 1-9.

Azizbekova, Z.S. and Rasulova, D.A. (1972). The influence of nitrogenous fertililzers on the content of organic acids in cotton leaves under the chloride salinity. *Doklady Akademii nauk Azerbeizhankoi SSR.* **5** : 71-73.

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

Folin, O. and Denis, W. (115). A calorimetric estimation of phenols and phenol derivatives in urine. *J. Biol. Chem.* **22** : 305-308.

Gauch, H.G. and Eaton, F.M. (1942). Effects of saline substrate on hourly levels of carbohydrates and inorganic constituents of barley plants. *Plant Physiol.* **17** : 347-365.

Gonia, H., Ghorbal, M.H. and Touraine, B. (1994). Effect of NaCl on flows of N and mineral ions and on NO_3 reduction rate within whole plants of salt sensitive bean and salt tolerant cotton. *Plant Physiol.* **105** : 1409-1418.

Hawk, P.B., Oser, B.L. and Summerson, W.H. (1948). Practical Physiological Chemistry. The Blakiston Company, U.S.A.

He, T. and Cramer, G.R. (1992). Growth and mineral nutrition of six rapid cycling *Brassica* species in response to seawater salinity. *Plant Soil* **139** : 285-294.

Hellebust, J.A. (1976). Osmoregulation. *Ann Rev. Plant Physiol.* **27** : 485-505.

Heuer, B. (1991). Response of photosynthesis, translocation and partitioning in sugarbeet plants exposed to an osmotic shock. *Israel J. Bot.* **40** : 275-282.

Hoffman, G.J., Shalhevet, J. and Meiri, A. (1980). Leaf age and salinity influence on water relations of pepper leaves. *Physiol. Plant.* **48** : 463-469.

Imamul Huq, S.M. and Larher, F. (1983). Osmoregulation in higher plants : Effects of NaCl salinity on non-nodulated *Phaseolus aureus* L. II. Changes in organic solutes. *New Phytol.* **93** : 209-216.

Jennings, D.H. (1976). The effect of sodium chloride on higher plants. *Biol. Rev. Cambridge Philos.* Soc. **51** : 453-486.

Joshi, G.V. (1976). Studies in photosynthesis under saline conditions. 3. Photosynthesis in mangroves. *Final Report on PL-480 Project.* Shivaji University, Kolhapur.

Karadge, B.A. (1981). Physiological Studies in *Portulaca oleracea* Linn. Ph. D thesis submitted to Shivaji University, Kolhapur. India.

McLaughine, J.E., Goyan, J.E. and Paul, A.G. (1964). Thin layer chromatography of argot alkaloids. *J. Pharm Sci.* **53** : 306-310.

Morishita, J., Shiraouna, N., Segawa, M. and Ohta, Y. (1986). Changes in the concentration of organic acids and mineral ions in saps of cucumber and tomato plants under excess supply of sodium chloride. *Soil. Sci. Plant Nutr.* **32** : 469-473.

Nalawade, B.B. (1988). Studies on Physiology of an Oilseed Crop. Niger (*Guezotia abyssinica* Cass.). Ph.D. Thesis submitted to Shivaji University, Kolhapur. India.

Nelson, N. (1944). A photometric adaptation of the Somgyi method for the determination of glucose. *J. Biol. Chem.* **153** : 375-380.

Paliwal, K.N. and Maliwal, G.L. (1980). Growth and nutrient uptake relationships of some crops in saline substrate. *Ann. Arid Zone.* **19** : 251-257.

Perz-Alfocea, F., Estan, M.T., Caro, M. and Guerrier, G. (1993). Osmotic adjustment in *Lycopersicon esculentum* and L. *pennellii* under NaCl and polyethylene glycol 6000 iso-osmotic stresses. *Physiol. Plant.* **87** : 493-498.

Saenz, L.A., Santamaria, J.M., Villanueva, M.A., Loyola-Vargas, V.M. and Oropeza, C. (1993). Changes in alkaloid content of *C. roseus* as a result of water stress and treatment with ABA. *J. Plant Physiol.* **142** : 244-247.

Shain, S.S. (1988). Prospects of controlling the productivity of some alkaloid bearing medicinal crops. *Khim. Farm. Zh.* **21**: 587-595.

Shetty, G. (1971). Physiology of Growth and Salt Tolerance of Plants. Ph.D. thesis sumitted to Shivaji University, Kolhapur. India.

Smith, J.I., Smart, N.J., Kurz, W.G.W. and Misawa, M. (1987). Stimulation of indol alkaoid production in cell suspension cultures of *Catharanthus roseus* by A.B.A. *Planta. Med.* **53** : 470-474.

Strogonov, B.P. (1964). Physiological basis of salt tolerance of plants. Transl. A. Poljakoff-Mayber and A.M. Mayer, pp. 279, Israel Program Sci. Jerusalem.

Thomas, M. and Beerers, M. (1949). Physiological studies in acid metabolism in green plants-II. Evidence of CO_2 fixation in *Bryophyllum calycium* and the study of diurnal fluctuations in the genus. *New Phytol.* **48** : 421-447.

Thomas, R. and Sharma, A. (1979). Assessment of alkaloids in mutant of *Catharanthus roseus* through chromatography. *J. Indian Bot. Soc.* **58** : 69-74.

Wadkar, P.R. (1989). Studies in Salt Tolerance of *Crotalaria* L. Species Ph.D. thesis submitted to Shivaji University, Kolhapur. India.

Yang, Y.W., Newton, R.J. and Miller, F.R. (1990). Salinity tolerance in *Sorghum*. I. Whole plant response to NaCl in *S. bicolor* and *S. halepense*. *Crop Sci.* **30** : 775-781.