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ACCUMULATION OF FREE AMINO ACIDS AND DISTRIBUTION OF Na^+, Cl^- AND K^+ IN RICE VARIETIES EXPOSED TO NaCl STRESS

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SUMMARY

The effect of 100 mM NaCl concentration in the nutrient solution on length, dry matter production and levels of Na^+, Cl^-, K^+ and free amino acids in shoots and roots of five rice varieties was studied in a hydroponic experiment. The CSC 1 and CSC 2 avoided the Na^+ toxicity by maintaining the high level of K^+. TKM 4, TKM 9 and CO 43 appear less able to maintain a basal K^+ level in the presence of Na^+. Varieties TKM 9 and CO 43 accumulated high levels of sodium and exhibited less reduction of vegetative growth and efficient mechanism of maintaining high levels of free proline and protein-bound proline in their shoots and roots under saline conditions.

INTRODUCTION

It has been established that a striking accumulation of the free amino acids occurs in many plants in response to changing osmotic potentials in their external environment by osmotic adjustment of their cellular contents (Greenway and Munns, 1980). Free amino acids have been reported to accumulate in both glycophytes (Bar-Nun and Poljakoff-Mayber, 1977; Chu *et al.*,) and halophytes (Stewart and Lee, 1974;) subjected to saline stresses. Salt resistant variety of rice excludes Na^+ by maintaining a much higher level of K^+ (Hegde and Joshi, 1974). Our earlier reports on rice stated the relationship of salt tolerance with organic acids (Krishnamurthy *et al.*, 1986). This paper describes in detail the effect of NaCl on internal concentration of Na^+, Cl^- and K^+, and on free amino acids accumulation at different growth periods.

MATERIALS AND METHODS

Seeds of rice varieties TKM 4, TKM 9, CSC 1, CSC 2 and CO 43 were germinated on moistened filter paper for 6 days. Seven days old seedlings were transferred to 150 ml of half strength unaerared Hoagland's solution (Hoagland

and Arnon, 1950) containing 100 mM. NaCl was omitted in control nutrient solution. The nutrient solution was renewed twice a week. All plants were grown under conditions of 10 hr light at 11,000-16,000 lux with incandescent lamps, and 14 hr dark at temperatures of 30-32°C.

Shoots and roots were harvested at 7, 14, 23 and 30 days after initial salinization and growth, aminoacid, Na^+, Cl^- and K^+ analyses were conducted. Free amino acids, free proline and protein-bound proline were determined by the method of Singh *et al.* (1973). Sodium and potassium were determined by flame photometry method (Storey and Wyn Jones, 1977) and chloride was assayed by titration method (Chapman and Pratt, 1961). Growth studies were replicated five times and analysed statistically using analysis of variance. Other assays were averages of two determinations.

RESULTS AND DISCUSSION

The effect of 100 mM NaCl on vegetative growth was examined at different gorwth periods. The reduction in the shoot and root length was less pronounced in TKM 9 and CO 43 while it was more in CSC 1, CSC 2 and TKM 4 at different periods of observation. The dry matter accumulation of shoot was reduced by 31 to 56 per cent in TKM 9 and CO 43 and 62 to 74 per cent in CSC 2, TKM 4 and CSC 1 compared to the control at thirty days after initial salinization (Fig. 1). From the vegetative growth studies it was evident that the TKM 9 and CO 43 were more resistant in terms of shoot and root growth under saline treatment.

There was a marked varietal difference in the accumulation of Na^+, K^+ and Cl^- in shoot and root system of rice in response to salinity. Varieties TKM 9 and CO 43 accumulated high levels of chloride in their shoot and root at thirty days after initial salinization (Fig. 2). The NaCl treatment lowered the potassium level in the shoot and root systems of TKM 4, TKM 9 and CO 43 and shoots of CSC 1 when compared to the control. Interestingly the CSC 2 maintained high level of potassium in their shoots and roots in comparison with the control (Fig. 2 g and h), Generally the TKM 9 and CO 43 accumulated high levels of sodium and exhibited less inhibition of shoot and root growth by saline treatment. A direct correlation between massive salt accumulation at low salinity levels and an increase in osmotic pressure, fresh weight and dry weight was found in the extremely salt resistant *Suaeda monoica* (Storey and Wyn Jones, 1979). It has been reported that some varieties of rice exclude the sodium toxicity by rainsing the potassium levels in the tissue (Hegde and Joshi, 1974). Greenway, (1973) suggested that avoidance of salt accumulation and maintenance

of high potassinm level in the growing shoots roots was of fundamental importance in conferring resistance in rice varieties.

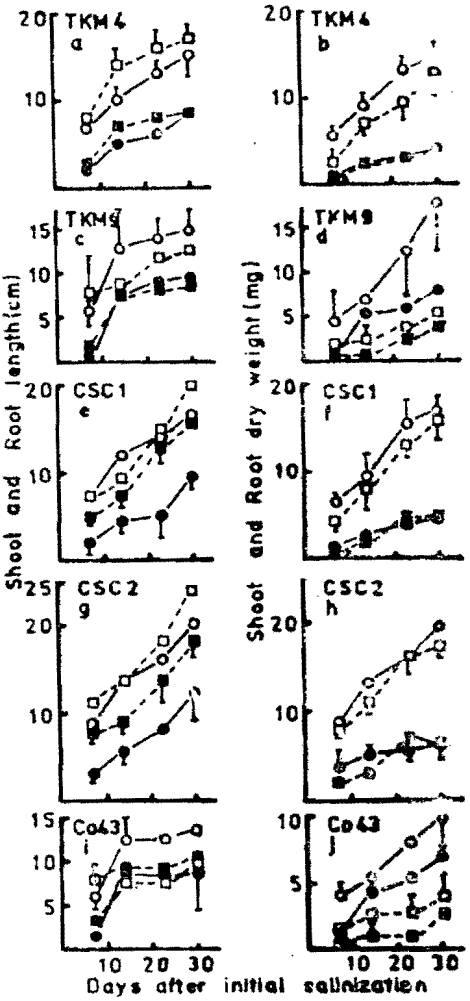

Fig. 1. Effect of 100 mM NaCl on the vegetative growth of rice varieties 7, 14, 23, and 30 days after initial salinization. The vertical indicate L.S.D. at 0.05 ievel.
○—○ : Control, shoots; ●—● : Saline, Shoots; □—□ : Control, roots; ■—■ : Saline, roots.

There was a considerable varietal difference in the accumulation of free amino acids in response to salinization. The TKM 9 and CO 43 were highly

efficient in maintaining high level of free proline than the others in their shoots and roots in response to salinity (Fig. 3 c, d, i and j) and exhibited less inhibition of vegetative growth. In TKM 4, CSC 1 and CSC 2 the proline concentration increased with increasing amount of sodium. Stewart and Lee (1974) suggsted that the capacity of species to accumulate proline was correlated with salt tolerance and thus, the accumulated proline acted as an intracellular osmoticum. It has been reported that possibly proline which accumulated under osmotic

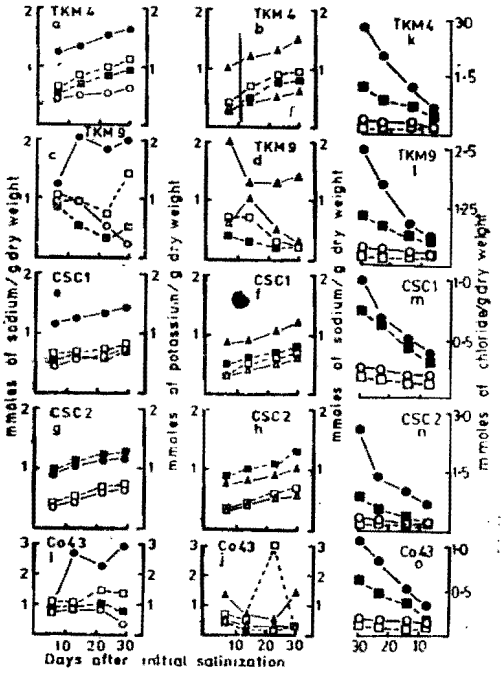

Fig. 2. Effect of 100 mM NaCl on the sodium, chloride and potassium content 7, 14, 23 and 30 days after initial salinization.

○—○ : Control, sodium and chloride content of shoots; ●—● : Saline, sodium and chloride content of shoots; △—△ : Control, sodium content of roots; ▲—▲ : Saline, sodium content of roots; □—□ : Control, potassium content of shoots and roots, chloride content of roots; ■—■ : Saline, potassium content of shoots and roots, chloride content of root.

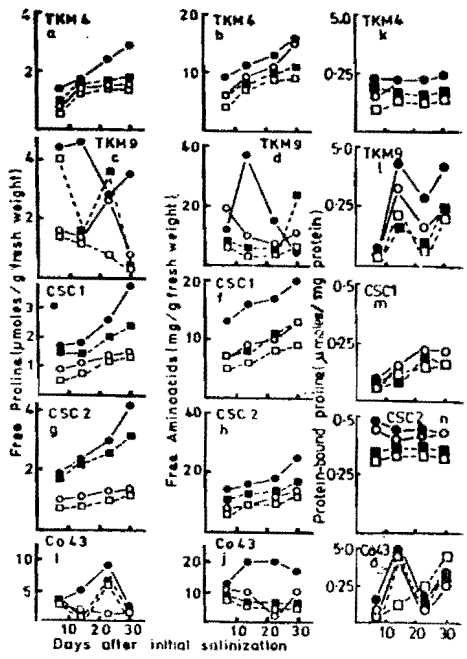

Fig. 3. Effect of 100 mM NaCl on the amino acid content 7, 14, 23 and 30 days after initial salinization.

○—○ : Control, total free amino acids, free proline and protein-bound proline content of shoots; ●—● : Saline, total free amino acids, free proline and protein-bound proline content of shoots; □—□ : Control, the free amino acids, free proline and protein-bound proline content of roots; ■—■ : Saline total free amino acids, free proline and protein-bound proline content of roots.

stress condition supplies energy for growth and survival under saline conditions and thereby induces salinity resistance to rice varieties (Bal, 1974).

The protein-bound proline content was not significantly changed in CSC 1, CSC 2 and TKM 4 when exposed to salinity. On the contrary, this was highly increased in the shoots and to a certain extent in the roots of TKM 9 and CO 43 due to saline treatment Fig. 3 1 and 0). An unaffected protein-bound proline content with a parallel increase in the free proline pool under salinity, clearly supports the theory of increased proline synthesis in response to salinity as reported by Palfi and Juhaz (1968). The increased protein-bound proline and free proline in rice varieties TKM 9 and CO 43 under salinized conditions clearly indicate an NaCl enhanced proline synthesis in the present study.

The present study therefore, indicates that the TKM 9 and CO 43 accumulated high levels of sodium, efficiently maintained high levels of free proline and protein-bound proline in their shoots and exhibited less reduction of vegetative growth than the others under saline conditions.

REFERENCES

Bal, A. R. (1974). A note on the comparative study of free amino acids content between wild salt tolerance rice and cultivated rice varieties. *Curr. Sci.*, **44** : 194-195.

Bar-Num, N. and Poljakoff-Mayber, A. (1977). Salinity stress and the content of proline in roots of *Pisum sativum* and *Tamarix tetragyna*. *Ann. Bot.*, **41** : 173-179.

Chapman, H. D. and Pratt, P. F. (1961). Chlorine. In 'Methods of analysis for soils, plants and waters' (Chapman, H. D. and Pratt, P. F. eds), pp. 97-100. University of California, Riverside.

Chu, T. M., Aspinall, D. and Paleg, L. C. (1976a). Stress metabolism. VII Salinity and and proline accumulation in barley. *Aust. J. Plant Physiol.*, **3** : 219-228.

Greenway, H. (1973). Salinity, plant growth and metabolism. *J. Aust. Inst. Agric. Sci.*, **39** : 24-34.

Greenway, H. and Munns, R. (1980). Mechanism of salt tolerance in nonhalophytes. *Ann. Rev. Plant Physiol.*, **31** : 149-190.

Hegde, B. A. and Joshi, G. V. (1974). Mineral salt absorption in saline rice variety. *Kala Rata. Plant Soil.*, **41** : 421-424.

Hoagland, D. R. and Arnon, D. I. (1950). The water culture method for growing plants without soil. Circuler 347, Calfornia Agr. Exp. Station Univ. of California, Berkaley.

Krishnamurthy, R., Anbazhagan, M. and Bhagwat, K. A. (1986). Effect of NaCl on the inorganic ions, growth and yield of rice. *Oryza*, **24** (in press).

Palfi, G. and Juhasz, J. (1968). Changes in the amino acid contents and plants caused by qywater deficiency and soil salinity. *Agrokem. Talajt*, **15** : 243-254.

Singh, T. N., Paleg, L. C. and Aspinall, D. (1973). Stress metabolism. I. Nitrogen metabolism in the barley plant during water stress. *Aust. J. Biol. Sci.*, **26** : 45-56.

Stewart, G. R. and Lee, J. A. (1274). The role of proline accumulation in halophytes. *Planta*, **120** : 279-289.

Storey, R. and Wyn Jones, R. C. (1977). Quaternary ammonium compounds in plants in relation to salt resistance. *Phytochem.*, **16** : 447-453.

Marschner, H. and Schafarczyk, W. (1979). Responses of *Atriplex spongiosa* and *Suaeda monoica* to salinity. *Plant Physiol.*, **63** : 156.162.