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THE EFFECT OF SODIUM CHLORIDE ON THE PHYSIOLOGY OF COTYLEDONS AND MOBILIZATION OF RESERVED FOOD IN *CICER ARIETINUM*

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Abstract

The effects of 0, 25, 50 75 and 100 meq.l^{-1} sodium chloride on some physiological proceses of gram was studied in solution culture. Fresh and dry weight of cotyledons significantly changed ($P < 0.05$) at different salinity levels. Concentration of carbohydrate and total nitrogen in cotyledons showed a marked decrease from the 2nd day. Length and weight of root and shoot were affected at different salinity levels. Carbohydrate concentration showed marked reduction at 75 and 100 meq.l^{-1} NaCl, whereas, significant differences were observed in the nitrogen content in control and salinity treatments.

Introduction

Salt affected areas in Pakistan has continued to increase alarmingly during the past few years owing to a number of factors and their interactions. Inspite of numerous physical reclamation techniques, a high percentage of productive agricultural land is being lost due to salinity/waterlogging. It is now generally believed that along with physical/mechanical techniques for soil reclamation, tolerance of crops to salt stress becomes essential for maximizing crop productivity. This involves programmes for selection and screening of genomes/lines, plant breeding, gene transfer, etc. Studies on the basic physiology of each crop to salt stress is therefore essential to determine the basis of tolerance to stress conditions.

The salinity stress on a plant is either an osmotic effect (water stress) or ion stress, or a combination of both (Poljakoff - Mayber, 1982) Osmotic effects are mainly due to presence of salts in solution around the root which reduce or prevent water absorption by plants. Ionic stress is caused as a result of altered ion relations in the cells and accumulation of Na^+ and Cl^- ions. The effects of Cl^- on the ion imbalance (Luttge & Smith, 1984) and that of SO_4 for osmotic effects (Strogonov, 1962) have been reviewed extensively.

Reduction in growth under salinity stress is mainly attributed to the expense of energy on overcoming the stress, rather than on normal growth and development of the plant. Salinity stress increases energy requirement necessary to counteract osmotic and ionic stresses for maintenance of normal cellular function, and less energy is left for normal growth requirements (Nieman & Maas, 1978). It has been observed that under such stress, alternate biochemical pathways are triggered to meet the excess demand for food reserves (eg. CHO, protein, etc.). Experiments were therefore carried out to study the biochemical changes occurring during early stages of salt stress on chick pea (*Cicer arietinum*) and to correlate these changes with growth and development of the plant during the stress period.

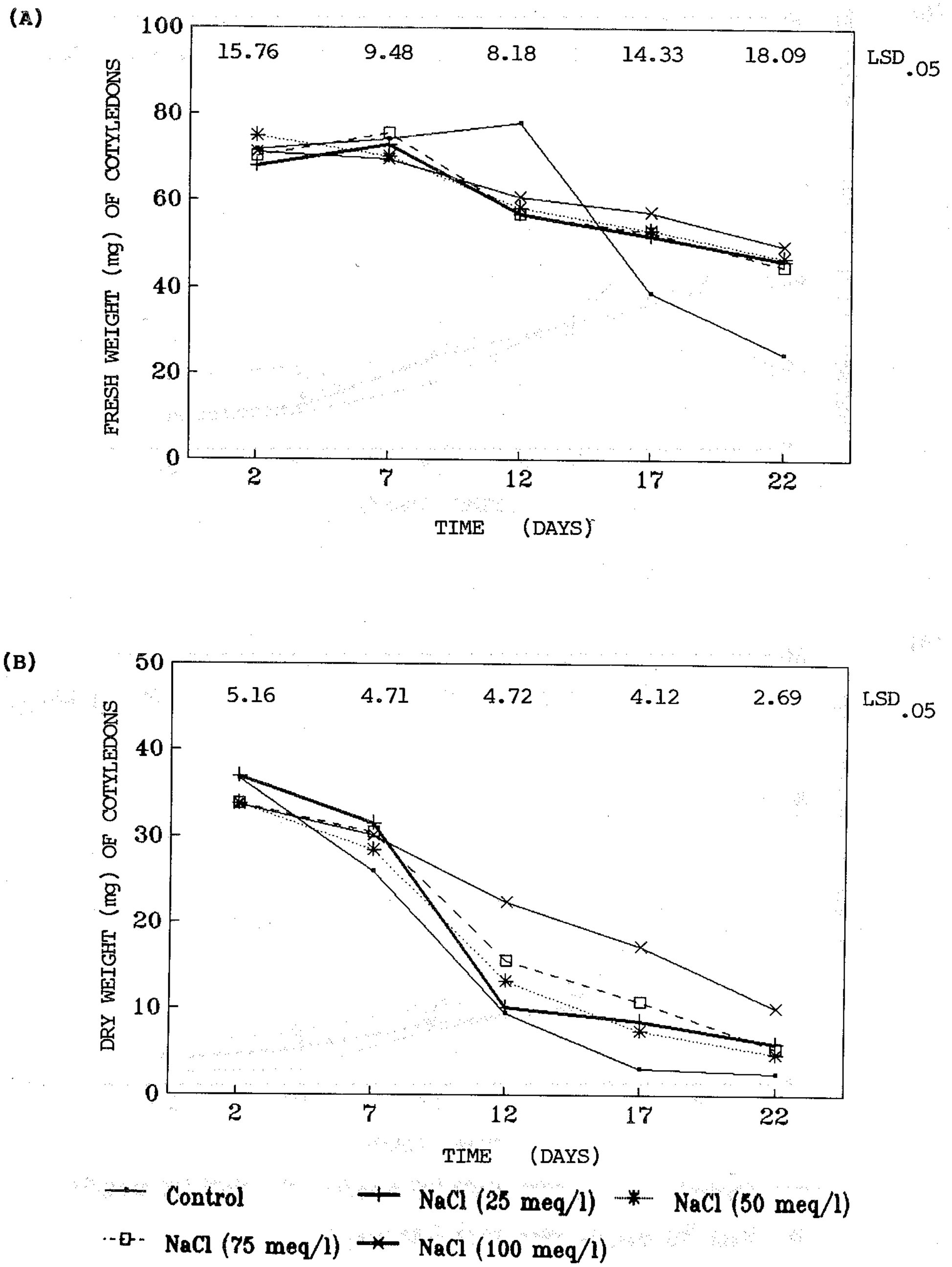

Fig.1. Changes in (a) fresh and (b) dry weight of gram cotyledons exposed to different salinity levels.

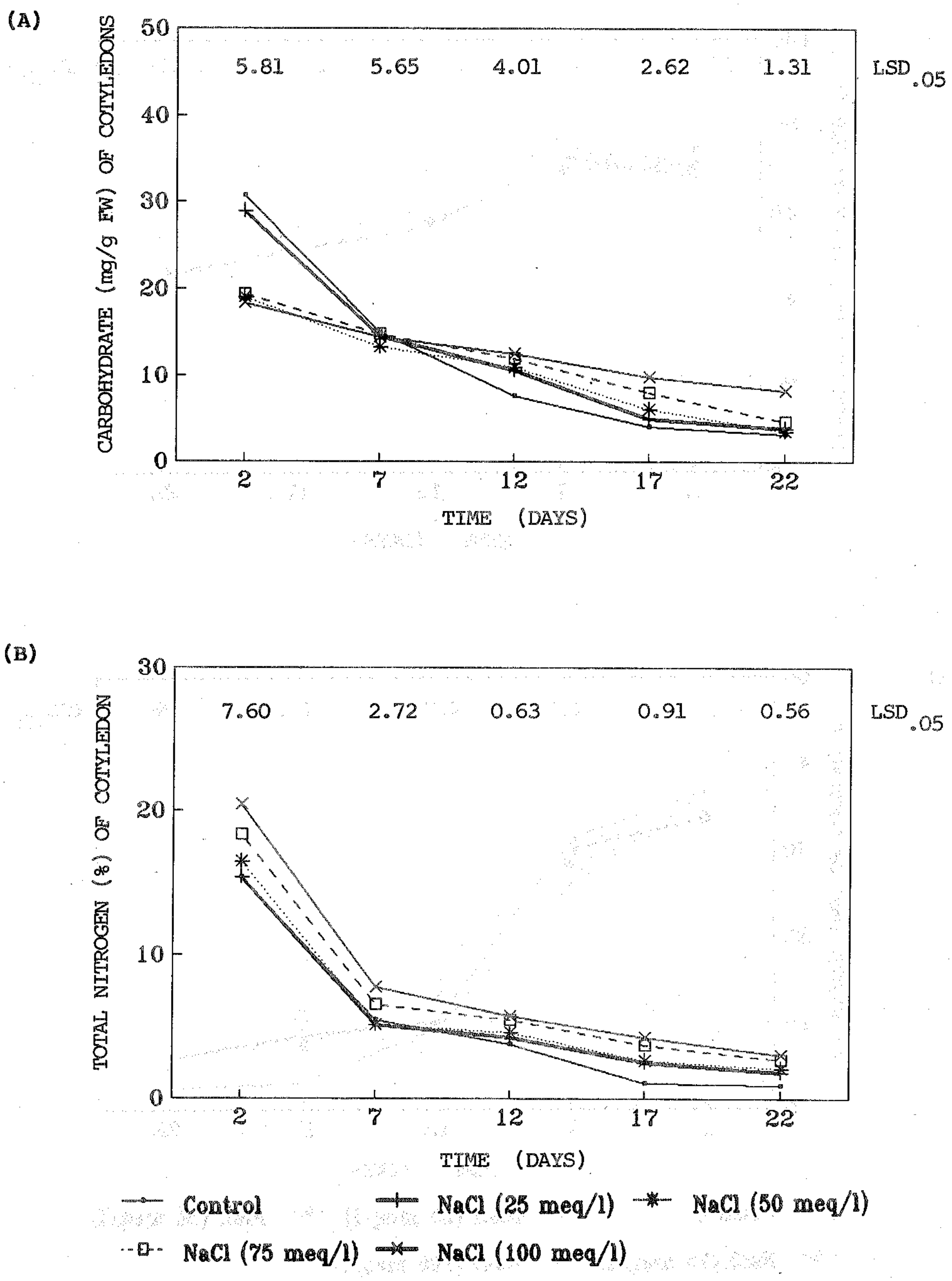

Fig.2. (a) Carbohydrate and (b) nitrogen content of gram cotyledons at different interval of days exposed to different salinity levels.

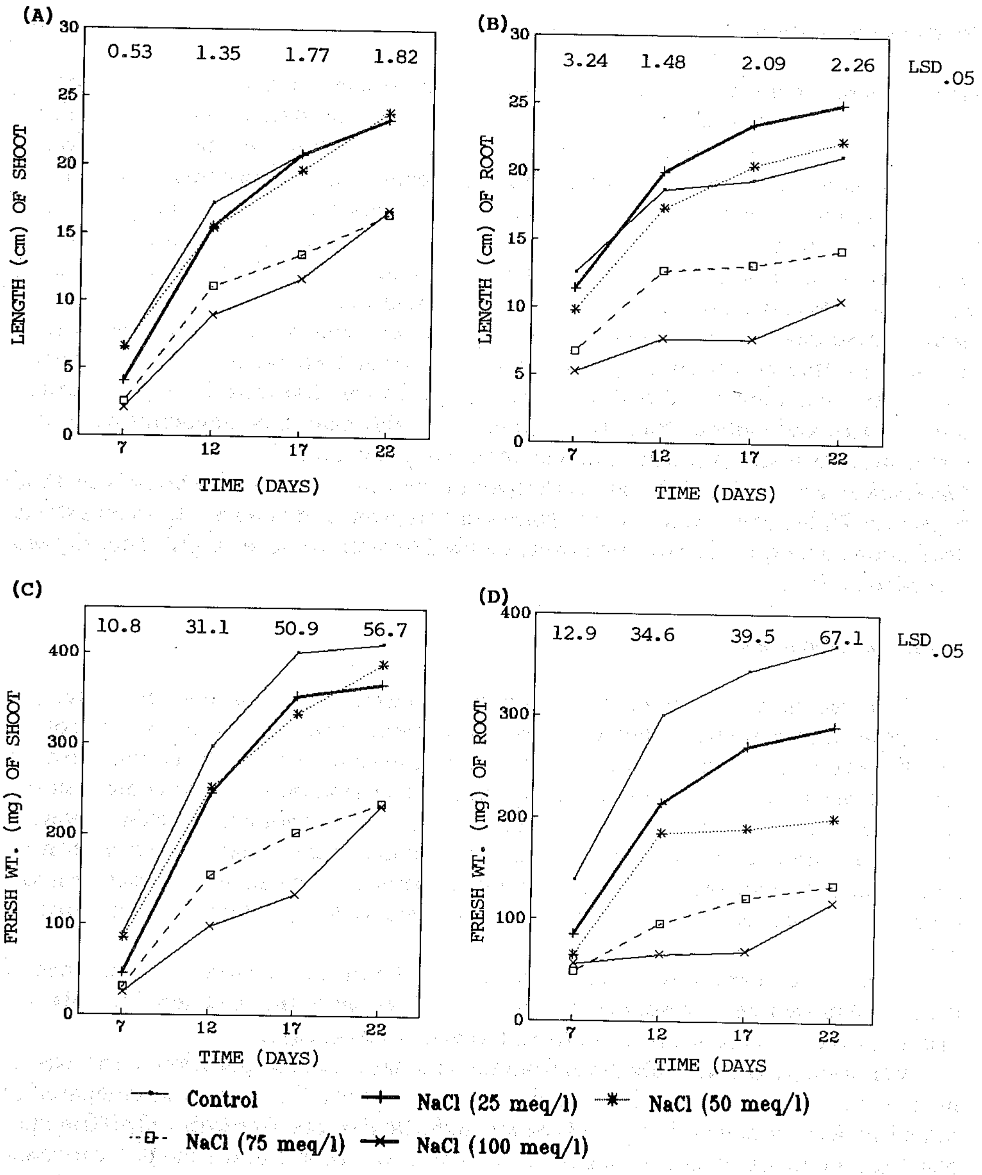

Fig.3. Length of (a) shoot & (b) root and their (c & d) fresh weights in gram seedlings exposed to different levels of salinity.

Materials and Methods

Germination: Seeds of a local variety of chick pea (*Cicer arietinum* L.) were surface sterilized with 0.1% mercuric chloride for few minutes, rinsed thoroughly with distilled water and soaked in distilled water for four hours for imbibition. Fifty seeds were placed over a nylon-net plate in such a manner that the seeds remained in contact with 500 ml of half strength Hoagland's solution kept in plastic pot underneath. The seeds were allowed to germinate in growth chamber at 10 h photoperiod under 6 Klux cool white flourescent light. Day and night temperature was maintained at 28±1°C and 25±1°C, respectively. Relative humidity during the experiment ranged from 65-70%.
Salinity Treatments: Germinated seeds on nylon net plate were transferred in pots containing different concentrations of NaCl in half strength Hoagland's solution. Salinity levels were maintained at 0 (Control) and 25, 50, 75 and 100 meq. l^{-1} NaCl. Nutrient solution with and without NaCl were changed on alternate days. Seedlings were harvested after 48 hours of sowing and then after 5 days interval.
Biochemical Estimations: Total carbohydrate was analyzed by Anthrone's method (Yemm & Willis, 1956),. whereas, total nitrogen was estimated by micro- kjeldahl's steam distillation technique after reaction with modified Nessler's reagent. Optical density was recorded at 550 nm.

Results and Discussion

The plants did not exhibit any significant changes in fresh and dry weight of cotyledons under different salinity levels (Figs.1a and b) from 12 to 22 days of growth. Control plants exhibited greater decrease in fresh and dry weight as compared to salinized plants from the 2nd day after sowing. The amount of water content in the cotyledons decreased significantly with increase in salinity with control plants showing more water content. Decrease in seedling growth and increase in moisture content have been reported by Sheoran & Garg (1979) in mung bean when exposed to different salt salinities. The reduction in growth under chloride (NaCl) salinity is mainly due to accumulation of toxicions.

Relative decrease in dry weight and increased moisture content gives an index of the rate of movement of reserve food from the cotyledons to the seedlings. The rate was faster at lower salinity levels as compared to high levels of salts.

Variation in total carbohydrate from the 2nd day till the termination of the experiment at 22 days was greater in control and 25 meq.l^{-1} NaCl treatment as compared to other salinity treatments (Fig.2a). However, at the final stages of growth, only 100 meq.l^{-1} NaCl exhibited significant changes as compared to control and salinity treatments. Decrease in carbohydrate resources indicates that metabolite production at high salt stress is significantly affected, either due to hindrance in water uptake (osmotic effect) or toxic effect of NaCl (Waisel, 1972). Activity of α - amylase under NaCl stress have been reported to effect carbohydrate production in wheat (Khan & Patel, 1972; Ansari *et al*., 1977).

There was no significant changes in total nitrogen of cotyledons at any salinity levels and days of germination (Fig.2b), with the exception of a sharp decline after 2nd day, indicating that the resevered nitrogen sources are exhausted during early stages of

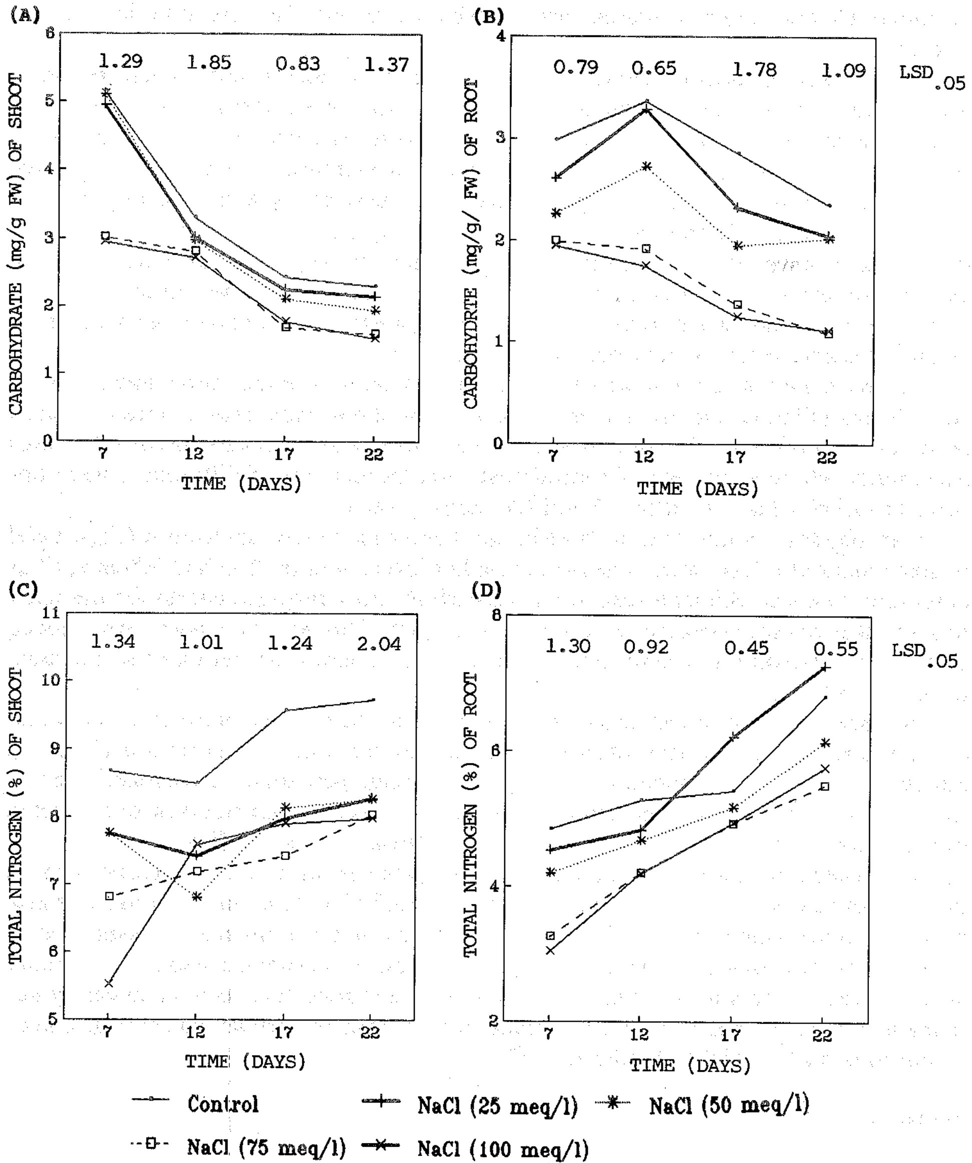

Fig.4. Carbohydrate content in (a) shoot and (b) root of gram seedlings exposed to different salinity levels. Figures (c) and (d) shows the nitrogen content in shoot and root, respectively.

germination by the embryo. Similar results have been reported in case of barley (Folkes & Yemm, 1958).

After 7 days of growth, significant changes in shoot length was evident between control and 25 meq.l^{-1}NaCl treatment as compared to other NaCl treatments where with an increase in NaCl levels, shoot length decreased (Fig. 3a). However, root length in 25 and 50 meq.l^{-1} NaCl treatment showed a significant increase as compared to control after 12 days of growth (Fig. 3b). Other treatments, depicted a decrease, however, the trend in all the treatments were similar. In general, plants that show tendency for salt-tolerance have the ability to adapt gradually under stress conditions. This effect was more pronounced in roots as compared to shoots since the roots are directly exposed to the stress medium where they not only have to withstand the stress but also transport metabolites and water to other parts of the plants.

Fresh weights of root and shoot showed similar pattern of reduction under various NaCl levels (Figs.3c and d). However, in case of shoot, very high differences were observed in control and 25 and 50 meq.l^{-1} NaCl with that of 75 and 100 meq.l^{-1} NaCl treatments, whereas, in case of root almost same percentage of differences were observed in all NaCl levels except 75 and 100 meq.l^{-1} NaCl.

Carbohydrate concentration, both in shoot and root varied significantly (Figs. a and b) since marked effects were observed at higher levels of salts of 75 and 100 meq.l^{-1} as compared to lower salinity levels. Since the carbohydrate provides energy for the plant to grow, it is readily converted into starch and lipids. This activity is more pronounced under stress conditions where more carbohydrate reserves are required to maintain normal growth rates.

Nitrogen content in the shoots showed marked differences between control and NaCl treatments, after 7 days interval with no differences during later period. (Fig. 4 c). Excepting, control, the concentration of nitrogen remained almost unchanged from 12 to 22 days of growth, indicating either over-utilization of nitrogen reserves, or restricted mobilization from root, resulting in increased amount in the roots (Fig.4 d).

Dry weight of shoot which increased with growth period showed a decrease with an increase in salinity levels of 75 and 100 meq.l^{-1} NaCl (Fig. 5a). Similar trend was also observed in dry weight of roots (Fig. 5 d), but the dry matter remained unchanged after twelve days and onwards, with significant differences between treatments. Under salinity stress, fresh and dry weights of both shoot and root decreases significantly due to maintenace of turgor in the plants. Similar loss of fresh and dry weights have also been reported in barley (Mehta & Bharti, 1983).

References

Ansari, R., S.M. Naqvi and A.R. Azmi. 1977. Effect of salinity on germination, seedling growth and ∝ - amylase activity in wheat. *Pak. J. Bot.*, 9: 163-166.

Folkes, B.M. and E.W. Yemm. 1958. The respiration of barley plants. X. Respiration and the metabolism of amino acids and proteins in germinating grain. *New Phytol.*, 57: 106-131.

Khan, M.I. and Z. Patel. 1972. Effect of sodium chloride on germination and enzymes of two varieties of wheat seeds. *Sind Univ. Res. Jour.*, 6: 7-14.

Luttge, U. and J.A. Smith. 1984. Structural, biophysical and biochemical aspects of the role of leaves in plant adaptation to salinity and water stress. In: *Salinity Tolerance in Plants* : Strategies for Crop Improvement (Eds.) R.C. Staples and G.H. Toenniessen, John Wiley & Sons Inc., pp. 125-150.

Nieman, R.H. and E.V.Maas. 1978. 6th Inter. Biophys. Cong. Abst., pp. 121.

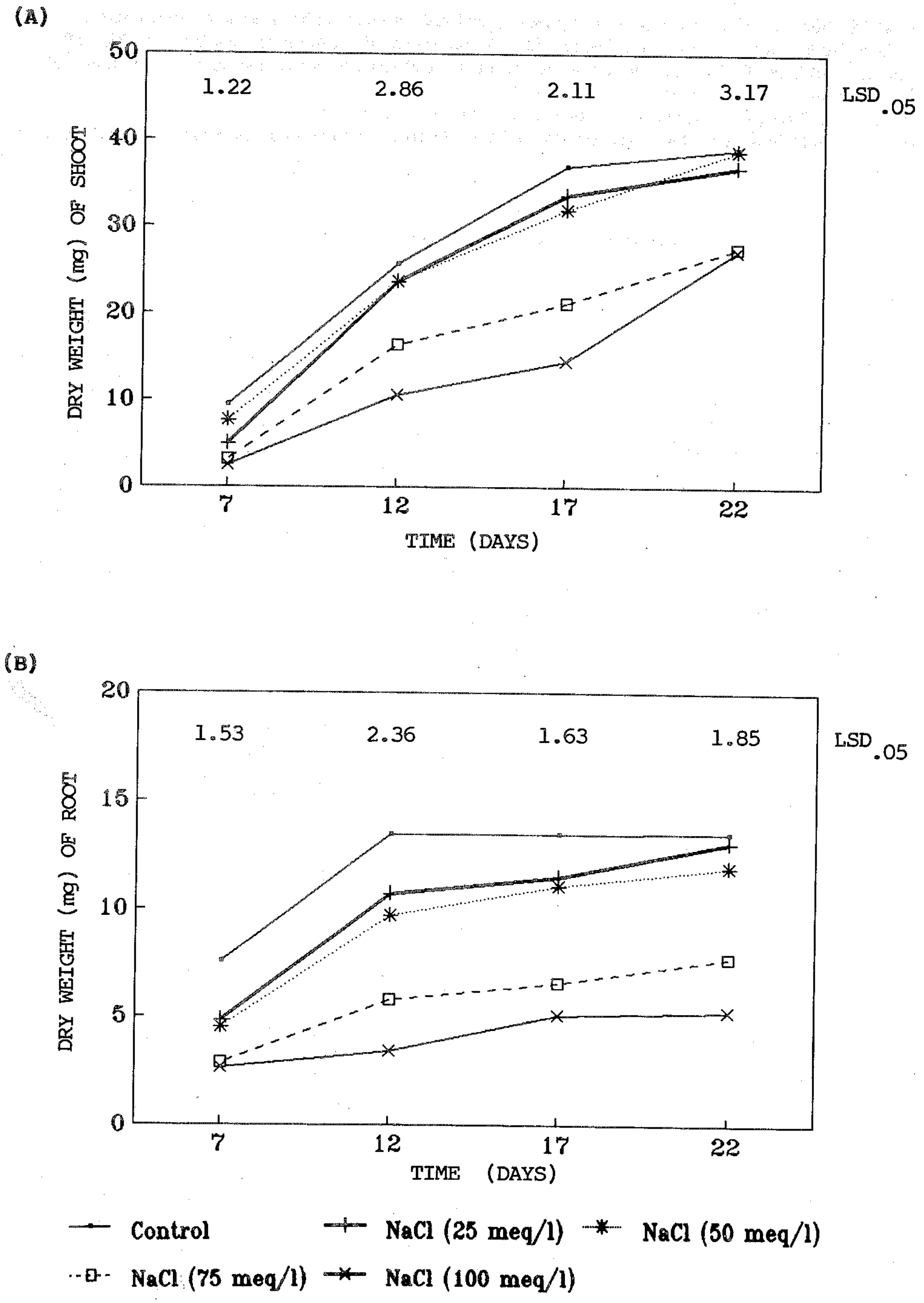

Fig.5. Dry weight of (a) shoot and (b) root of gram seedlings exposed to various salinity levels.

Poljakoff-Mayber, A. 1982. Biochemical and physiological responses of higher plants to salinity stress. In: *Biosaline Research*: A look to the future (Eds.) A. San Pietro, Plenum Press, New York, pp 245-269.

Sheoran, I.S. and Garg, O.P. 1979. Qualitative and quantitative changes in peroxidase during germination of mung bean under salt stress. *Physiol. Plant.*, 46 : 147-150.

Waisel, Y. 1972. Biology of halophytes, Academic Press Inc., New York, 395 pp.

Yemm, E.W., and Willis, A.J. 1956. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. Jour., 57 : 508-511.*

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