

**OSMOREGULATION IN HIGHER PLANTS:  
EFFECTS OF NaCl SALINITY ON  
NON-NODULATED *PHASEOLUS AUREUS* L.  
I. GROWTH AND MINERAL CONTENT**

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SUMMARY

The adaptation, growth and changes in mineral composition of *Phaseolus aureus* L. were studied at six levels of NaCl, ranging from 10 to 200 mM. Plants tolerated up to 150 mM NaCl in the growth medium although their growth and mineral composition were adversely affected. Accumulation of Na<sup>+</sup> in plant tissue increased with increasing NaCl<sub>ext</sub> reaching toxic concentrations and inhibited transport of different inorganic ions. K<sup>+</sup> constituted only about 10% of the total mineral content of plants grown in 150 mM NaCl as against 55% in the controls. The principal balancing anion to counter Na<sup>+</sup> accumulation was Cl<sup>-</sup>. 'Ion excess' in the plant is suggested to be the cause of reduced growth. The behaviour of *P. aureus* under condition of salt stress is discussed.

INTRODUCTION

High levels of NaCl in the external medium are supposed to adversely affect the growth and inorganic and organic solute composition of glycophytic higher plants. Leguminous plants occupy a special position in that any imbalance in the environment is likely to impair N-fixation. This has been shown by several authors including Balasubramanian and Sinha (1976), Sprent (1973), Imbamba (1973), Subba Rao *et al.* (1972), Helal and Mengal (1981). Despite these studies, information is lacking as to the adaptation of grain legumes to relatively high salinity and subsequent effects on growth and accumulation of different organic and inorganic solutes. It is believed that non-nodulated legumes are likely to be more resistant to salt toxicity than the nodulated ones (Wilson, 1970). In the present study we examine the influence of different levels of external NaCl (NaCl<sub>ext</sub>) on the growth and mineral composition of the grain legume *Phaseolus aureus* L., typically known as Mung bean.

MATERIALS AND METHODS

*Plant material*

Seeds of *P. aureus* were germinated directly on vermiculite placed in perforated (at the bottom) rectangular plastic pots of about 7 l capacity. The vermiculite was soaked with a nutrient solution containing NO<sub>3</sub><sup>-</sup>, 4 mM; NH<sub>4</sub><sup>+</sup>, 2 mM; P, 0.8 mM; S, 0.6 mM; K<sup>+</sup>, 2 mM; Ca<sup>+2</sup>, 1.5 mM; Mg<sup>+2</sup>, 0.2 mM; Fe, 4 μM; B, 32 μM; Mn, 11 μM; Zn, 1.2 μM; Mo, 0.93 μM; Cu, 0.5 μM; Co, 0.34 μM; Cl, 2.0 mM. NaCl at concentrations of 0, 10, 25, 50, 100, 150 or 200 mM was added to the vermiculite along with the nutrient solution. These treatments are designated as control, S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, S<sub>5</sub> and S<sub>6</sub> respectively. Seeds started to germinate from

## S. M. IMAMUL HUQ AND F. LARHER

the second day of sowing and seedlings emerged on the fourth day. Seeds failed to germinate in the  $S_6$  treatment. The plants were allowed to grow for 4 weeks in a controlled environment growth chamber at a daylight intensity of  $30 \text{ W m}^{-2}$  at leaf level, and day and night temperatures of  $26 \pm 1 \text{ }^\circ\text{C}$  and  $22 \pm 1 \text{ }^\circ\text{C}$  respectively. The corresponding relative humidities were 70 and 80 % under a 14 h day and 10 h night.

Distilled water was added daily to maintain a constant moisture content in the root medium. Both nutrient and NaCl solutions were renewed weekly. Plants were harvested 28 days after germination. The root free space was unloaded by three 1 min washings in distilled water. Lengths and fresh weights of above ground shoots and roots were measured. Dry matter was determined by drying at  $85 \text{ }^\circ\text{C}$  for 48 h. Organic matter was estimated by deducting total mineral content from total dry matter.

*Analyses*

The dried plant material was ground in a ball mill and passed through a 0.5 mm sieve. Total P was determined colorimetrically with molybdate–vanadate solution after digesting in a ternary acid mixture (IRRI, 1976) and is expressed as  $\text{PO}_4^{-3}$ .  $\text{K}^+$  and  $\text{Na}^+$  were determined by flame photometer, and  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$  by atomic absorption spectrophotometry, after extraction for 24 h in 1 N HCl (IRRI, 1976).  $\text{NO}_3^-$  was determined colorimetrically following the method of Cataldo *et al.* (1975) and  $\text{Cl}^-$  by conventional titration with  $\text{AgNO}_3$ , after extraction in water at  $45 \text{ }^\circ\text{C}$  for 1 h.

## RESULTS

*Growth*

Germination started 1 day after sowing in all pots except for  $S_6$ , but appeared greater in the control,  $S_1$ ,  $S_2$  and  $S_3$  treatments. The seedlings appeared healthier in the treatments up to  $S_3$ . The rate of leaf development was retarded above  $S_2$ . The leaves of the plants grown in 50 mM NaCl and higher were darker in colour than the rest. Over the first 2 weeks of growth, the plants of  $S_3$  to  $S_5$  treatments showed very poor root development; during this period secondary roots did not appear in  $S_5$  plants. The effects of increasing salinity on the different growth parameters are shown in Figure 1. It is clear that all parameters decreased with increasing  $\text{NaCl}_{\text{ext}}$ , the correlation coefficients between these parameters and  $\text{NaCl}_{\text{ext}}$  concentration all being significant at the 1 % level. Shoot fresh weight, however, increased up to  $S_2$ . Plant height decreased by more than 15 % at  $S_1$  and 74 % at  $S_5$ . The total fresh weight showed only a slight decrease (0.5 %) at  $S_2$  while at  $S_5$ , the decrease was more than 78 %. The decrease in dry matter weight was even more spectacular, amounting to more than 87 % for both shoots and roots at  $S_5$ . The decrease in fresh and dry weights was greater for roots than shoots. The shoot/root ratio (fresh weight) varied between 1.8 for control and 2.14 at  $S_4$  and in dry matter, between 2.83 for control and 5.74 at  $S_4$ . The decrease in organic matter below control was 90 and 95 % respectively for shoots and roots of  $S_5$  plants.

*Mineral solutes*

Changes in the level of  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{NO}_3^-$ ,  $\text{Cl}^-$  and  $\text{PO}_4^{-3}$  in the tissues as affected by different concentrations of  $\text{NaCl}_{\text{ext}}$  are shown in Figure 2.

## Osmoregulation in higher plants I

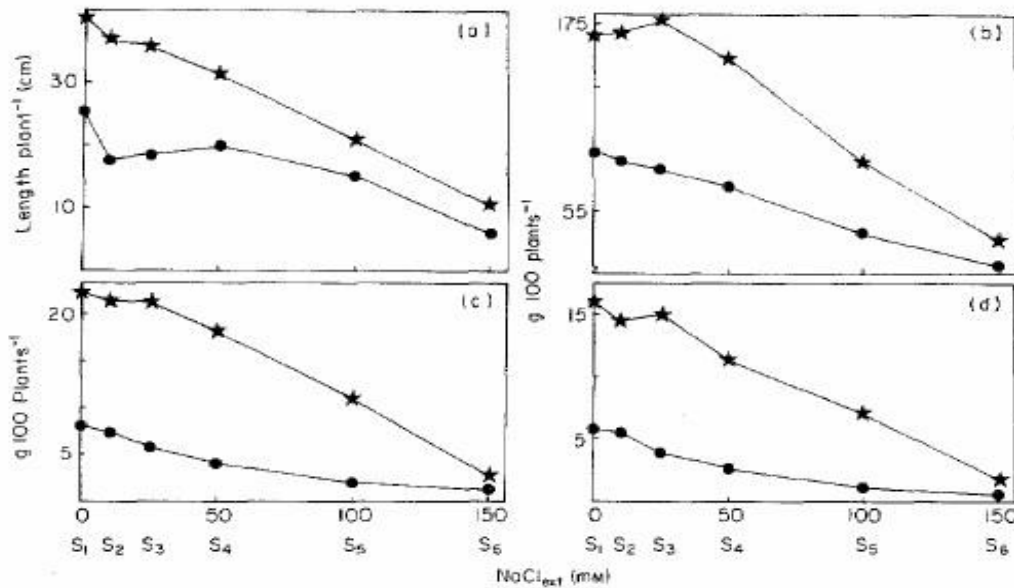


Fig. 1. Effect of  $\text{NaCl}_{\text{ext}}$  on various growth parameters of *P. aureus*. (a) shoot and root length; (b) fresh weights; (c) dry weights and (d) organic matter production. ★, Shoots; ●, roots.

**Cations.**  $\text{K}^+$  and  $\text{Ca}^{+2}$  concentrations in both shoots and roots decreased with increasing salinity, while  $\text{Na}^+$  and  $\text{Mg}^{+2}$  increased. The decrease in  $\text{K}^+$  and increase in  $\text{Na}^+$  in both plant parts were significantly correlated with salinity,  $r$  being  $-0.9286^{**}$ ,  $-0.8572^*$  and  $0.8934^{**}$  and  $0.9475^{**}$  for shoots and roots for  $\text{K}^+$  and  $\text{Na}^+$  respectively.  $\text{K}^+$  accumulation in both shoots and roots decreased with increasing  $\text{NaCl}_{\text{ext}}$ ,  $\text{K}^+$  concentration decreasing more in roots than in shoots; the same was also true for  $\text{Ca}^{+2}$ , except at  $S_5$  where the decrease was greater in shoots than in roots. The  $\text{K}^+$  concentration in the roots of  $S_5$  plants decreased more than 72% as against 9% at  $S_1$ .  $\text{Ca}^{+2}$  showed a decrease of more than 65% from control in the shoots of  $S_5$  plants. While  $\text{K}^+$  decreased, there was a 17-fold increase in  $\text{Na}^+$  concentration in the roots of  $S_5$  plants.  $\text{Mg}^{+2}$  content was higher in shoots than in roots for all treatments except  $S_2$  where a decrease of about 40% below control was noted.

**Anion.** The  $\text{NO}_3^-$  concentration in the shoots [Fig. 2(c)] decreased significantly ( $r = -0.9972^{***}$ ) with increasing salinity while in the roots it increased up to 100 mM  $\text{NaCl}_{\text{ext}}$ , but showed a 28% decrease in the  $S_6$  plants. The shoots of  $S_5$  plants contained 78% less  $\text{NO}_3^-$ . While  $\text{NO}_3^-$  concentration decreased, both  $\text{PO}_4^{-3}$  and  $\text{Cl}^-$  concentrations increased (in both organs) with increasing  $\text{NaCl}_{\text{ext}}$ . Accumulation of  $\text{PO}_4^{-3}$  was higher in shoots than in roots except at  $S_5$  where it was higher in the roots. The increase in  $\text{PO}_4^{-3}$  accumulation was greater in roots than in shoots and showed a gradual rise up to  $S_5$  when it rose abruptly. The shoot/root ratio of  $\text{PO}_4^{-3}$  varied between 2.1 for control and 0.42 at  $S_5$ . The  $\text{Cl}^-$  content of both organs increased significantly with increasing salinity ( $r = 0.8324^*$  for shoots and  $0.9854^{***}$  for roots), and was higher in the shoots except at  $S_4$  and  $S_5$  when it was higher in the roots. However, in the control plants, the  $\text{Cl}^-$  concentrations were the same for both organs. The shoot/root ratio of  $\text{Cl}^-$  varied

S. M. IMAMUL HUQ AND F. LARHER

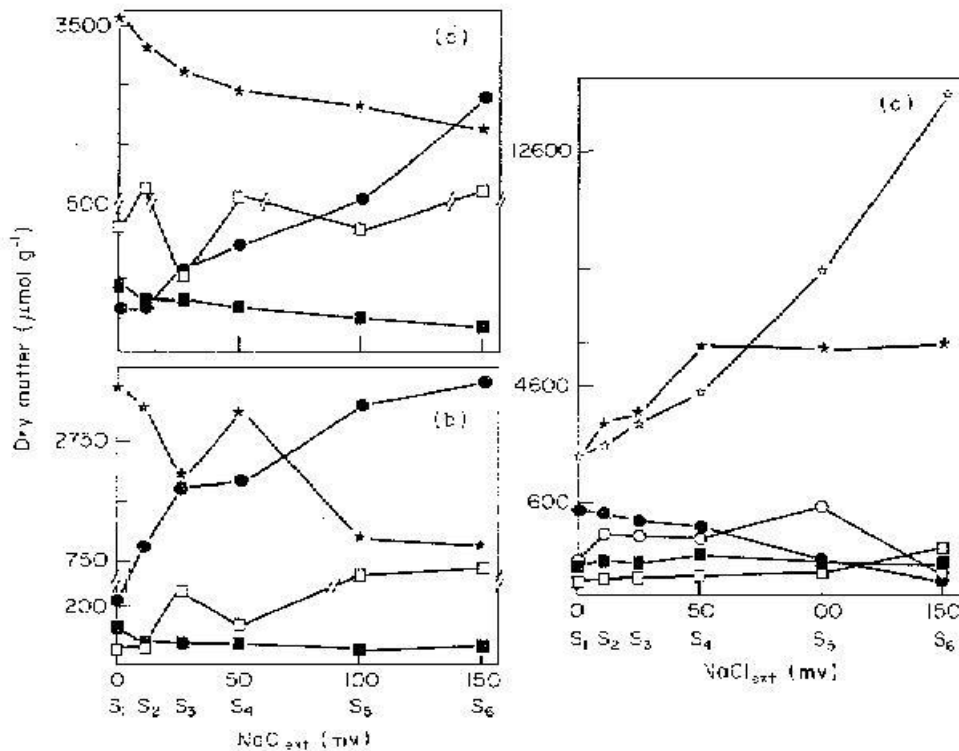


Fig. 2. Concentration of various cations and anions in *P. aureus* as affected by  $\text{NaCl}_{\text{ext}}$ . Results expressed as  $\mu\text{mol ion g}^{-1}$  dry matter. (a) Cations in shoots, (b) cations in roots, (c) anions in shoots and roots. (a) Cations in shoots: ★,  $\text{K}^+$ ; ●,  $\text{Na}^+$ ; □,  $\text{Mg}^{2+}$ ; ■,  $\text{Ca}^{2+}$ . (b) Cations in roots: symbols as in (a). (c) Anions in shoots and roots: ★,  $\text{Cl}^-$  shoot; ☆,  $\text{Cl}^-$  root; ●,  $\text{NO}_3^-$  shoot; ○,  $\text{NO}_3^-$  root; ■,  $\text{PO}_4^{3-}$  shoot; □,  $\text{PO}_4^{3-}$  root.

between 1.3 at  $S_1$  and 0.4 at  $S_6$ . The  $\text{Cl}^-$  concentration in the roots of  $S_5$  plants was more than seven times that in the controls.

DISCUSSION

The present work was designed to study the growth and mineral status of *P. aureus* under saline conditions ranging from 0 to 150 mM. It was found that in the given experimental conditions, the seeds of *P. aureus* germinated in  $\text{NaCl}_{\text{ext}}$  concentrations as high as 150 mM, although germination is thought to be affected by salt concentration in the growth medium. Young plants exhibited a significant ability to adapt osmotically to a high  $\text{NaCl}_{\text{ext}}$  concentration since full turgidity was maintained in all sets. However, all measured growth parameters were adversely affected beyond 50 mM  $\text{NaCl}_{\text{ext}}$ . Our results are in good general agreement with those observed for leguminous plants by others like Balasubramanian and Sinha (1976), Imbamba (1973), Subba Rao *et al.* (1972), Wilson (1970). However, in all these cases,  $\text{NaCl}$  stress was applied several days after germination so that strict comparisons cannot be made.

At high salinities, roots of *P. aureus* developed very poorly as was evident from the decreased organic matter yield; this was accompanied by an increased mineral accumulation, notably of  $\text{Na}^+$ . Shoot development was affected less drastically and

### *Osmoregulation in higher plants I*

it was found that the chlorophyll content of the leaves of plants treated with 50 mM NaCl and higher was greater than in other plants (data not shown). Similar observation concerning chlorophyll in NaCl-treated pasture legumes (Wilson, 1970) and *Lavatera arborea* (Okusanya, 1980) have also been reported. It should be noted that in the present study, enhanced chlorophyll content was associated with higher  $Mg^{+2}$ .

Of special interest is the observation that NaCl in the growth medium within the range 10 to 20 mM may be favourable for this species since there was no significant decrease at these  $NaCl_{ext}$  levels. This finding suggests some kind of stimulative effect of  $Na^+$ , presumably *via* an osmoregulatory role.

The well-known effect of  $Na^+_{ext}$  on  $K^+_{int}$  content of plant tissue was also confirmed by our results. With increasing  $Na^+_{ext}$ ,  $Na^+_{int}$  increased and in consequence,  $K^+_{int}$  decreased.  $K^+$  deficiency could probably be created in such a situation since  $Na^+_{int}$  in plants treated with 50 mM NaCl or higher surpassed  $K^+_{int}$ . However, in tissues invaded by NaCl, osmotic adjustment cannot be achieved in the same manner as with  $K^+$  since  $K^+$  is usually prominent in the cytosol while high amounts of  $Na^+$  are not generally tolerated in this compartment (Flowers, Troke and Yeo, 1977).  $Na^+$  is presumably stored in the vacuoles and as a consequence, organic solutes such as sugars and amino acids (Hsiao, 1973) and amides (Stewart and Larher, 1980) have to be accumulated in the cytoplasm.

The decrease in  $Ca^{+2}$  content in plant treated with NaCl is also well known and the ability of plant organs to maintain a satisfactory level of  $Ca^{+2}$  when  $Na^+$  is present in the external medium can be considered as a measure of salt resistance (Epstein, 1961). In our case, it should be noted that the plants maintained an appreciable level of  $Ca^{+2}_{int}$  which clearly indicates some kind of salt resistance of this species.

NaCl treated plants also accumulated quite high amounts of  $NO_3^-$  in their roots. This suggests the possibility that  $NO_3^-$  uptake is maintained even in the presence of high  $NaCl_{ext}$  in order to maintain osmoregulation since the major part of this anion is stored in the vacuoles (Martinoia, Heck and Wiemken, 1981). On the other hand the transport of this ion was inhibited by  $Cl^-$  as is evident from the relative lower  $NO_3^-$  in the shoots. Similar observations have been reported for a number of halophytic species (Stewart *et al.*, 1979).

Whatever the specific behaviour of the various ions, it is clear that plants grown with 150 mM  $NaCl_{ext}$  had only 44% organic matter as against 74% for the controls. It is therefore believed that the reduced growth of *P. aureus* observed in the present study was due to 'ion excess' (Greenway and Munns, 1980) in the tissues caused principally by enhanced  $Na^+$  uptake. As a result of this 'ion excess' the osmotic balance of the cells was disrupted, and to maintain turgidity, various compatible solutes had to be produced.

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## S. M. IMAMUL HUQ AND F. LARHER

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