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## GROWTH RESPONSE OF *VICIA FABA MINOR* TO NaCl SALINITY

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### Abstract

Plants of *Vicia faba minor* var. Diana were grown for 30 d in Hoagland's solution. The media were salinized with NaCl concentrations ranging from 0 to 150 mM. The growth of the plants was stimulated by NaCl upto a concentration of 25mM. Salt induced growth inhibition occurred at salinities higher than 40mM. However, the inhibition was only about 20% of control at 50mM NaCl<sub>ext</sub>. Growth inhibition was associated with an increase in Na<sup>+</sup> content in the tissue along with a concomitant decrease of K<sup>+</sup> accumulation only when the endogenous K<sub>+</sub>/Na<sup>+</sup> ratio fell below a value of 10. This critical value was reached at a slower rate in the younger parts of the aerial organs than in the roots. The presence of low concentrations of NaCl in the growth medium induced significant increase in the K<sup>+</sup> content of the young leaves.

### Introduction

Salinity has a negative effect on the growth of most of the glycophytes, and the leguminous plants of agro-alimentary importance are no exception to it. Although *Medicago sativa* tolerates to some extent the salinity (Bernstein and Ogata 1966, Subba Rao *et al.* 1972, Sauvage 1982), yet, it is known that the degree of growth inhibition of legumes depends not only on the species and its varieties but also on the type of nitrogen nutrition practised (Bernstein and Ogata 1966, Wilson 1970, Balasubramanian and Sinha 1976, Huq and Larher 1983a, Huq 1984). Thus, the nodulated plants are known to be more susceptible to salinity due to the fact that inhibitory effects of salt does not act only on the nodulation process itself but on the nitrogen fixing activity of the nodules as well (Hua 1981, Huq and Larher 1983a). If the nodules are functional at moderate salinity, then Na<sup>+</sup> penetrates into the nodulated-root cytosol and into

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the bacteroids thereby inducing an efflux of  $K^+$  (Sauvage *et al.* 1983). There is a good correlation between the capability of the  $N_2$ -fixing bacteroids to control the  $K^+$  efflux and the resistance of the symbiotic system to NaCl salinity. In this context *Glycine max* appears to be hypersensitive to salt (Sauvage *et al.* 1983).

Concerning the behaviour of French bean (*Vicia faba minor* L.) towards NaCl, reference could be made to the recent works of Youssef and Sprent (1983) where they demonstrated the inhibitory effect of salinity on the growth and nitrogen content of *V. faba* irrespective of whether the plants were inoculated with *Rhizobium leguminosarum* or fed with ammonium nitrate. These results can not *a priori* be extrapolated to *V. faba minor*. The observations made by the authors are from experiments with salinities starting from 50mM NaCl<sub>ext</sub> which is already known to be excessive for glycophytes and especially for legumes (Greenway and Munns 1980). Moreover, the salt treatments were applied to 4 week-old plants grown on media without salt. This leads to osmotic shock rather than its acclimatization to salinity (Huq 1984). The results presented here show the diverse responses of non-nodulated French beans to salt concentrations ranging from 0 to 150 mM NaCl<sub>ext</sub> applied from the onset of seed imbibition. Particular attention has been given to the changes in the  $Na^+$  and  $K^+$  contents occurring in the different organs in order to establish ratio of the two cations in these organs.

### Materials and Methods

*Plant material and culture* : Seeds of *V. faba minor* var. Diana were germinated directly on vermiculite placed in 7l rectangular plastic pots perforated at the bottom. The vermiculite was soaked with a half strength solution of Hoalgand and Arnon (1938). The  $K^+$  content of the solution was 6 m e.  $l^{-1}$ . For the salt treatment, NaCl at concentrations of 10,25,50,100 or 150 mM was applied to the vermiculite alongwith the nutrient solution. NaCl applied to the growth medium is designated as NaCl<sub>ext</sub> in the text. The salt stress was applied from the very onset of seed imbibition and continued until the end of the experiment.

The plants were grown in a controlled environment growth chamber, the details of which are described elsewhere (Huq and Larher 1983b). Distilled water was added daily to the growth medium to maintain a constant moisture content in the root zone. Both nutrient and NaCl solutions were renewed weekly. Plants were sampled at 30 days after germination. At harvest, the root free space was unloaded by three 1 min washings in 1l distilled water.

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the shoots than in the roots. The growth stimulation at 25mM  $\text{NaCl}_{\text{ext}}$  amounted to 12% more in terms of dry matter production. This stimulation was relatively higher for shoots than for roots.

*K<sup>+</sup> and Na<sup>+</sup> accumulation rate in different organs of V. faba minor as affected by salinity:* Contents of  $\text{K}^+$  and  $\text{Na}^+$  in different organs of the plants acclimatized to different  $\text{NaCl}_{\text{ext}}$  concentrations are presented in Fig. 1. For the roots (Fig. 1c) the rapid influx of  $\text{Na}^+$  due to the presence of even a small amount of the element in the growth medium, brings about a decrease in the  $\text{K}^+$  accumulation rate. The quantity of the two ionic species in the tissue attained equality very rapidly. The  $\text{NaCl}_{\text{ext}}$  concentration at which this was attained was found to be 20mM by extrapolation. In the roots, the fall in the  $\text{K}^+/\text{Na}^+$  ratio is then due essentially to a rapid influx of  $\text{Na}^+$ . The net accumulation of  $\text{Na}^+$  in the roots continued at high salinity. The rate of accumulation however, was slowed beyond  $\text{NaCl}_{\text{ext}}$  concentration of 25mM. The intracellular  $\text{K}^+$  content decreased in a parallel manner though the uptake was quite appreciable at high salinities.

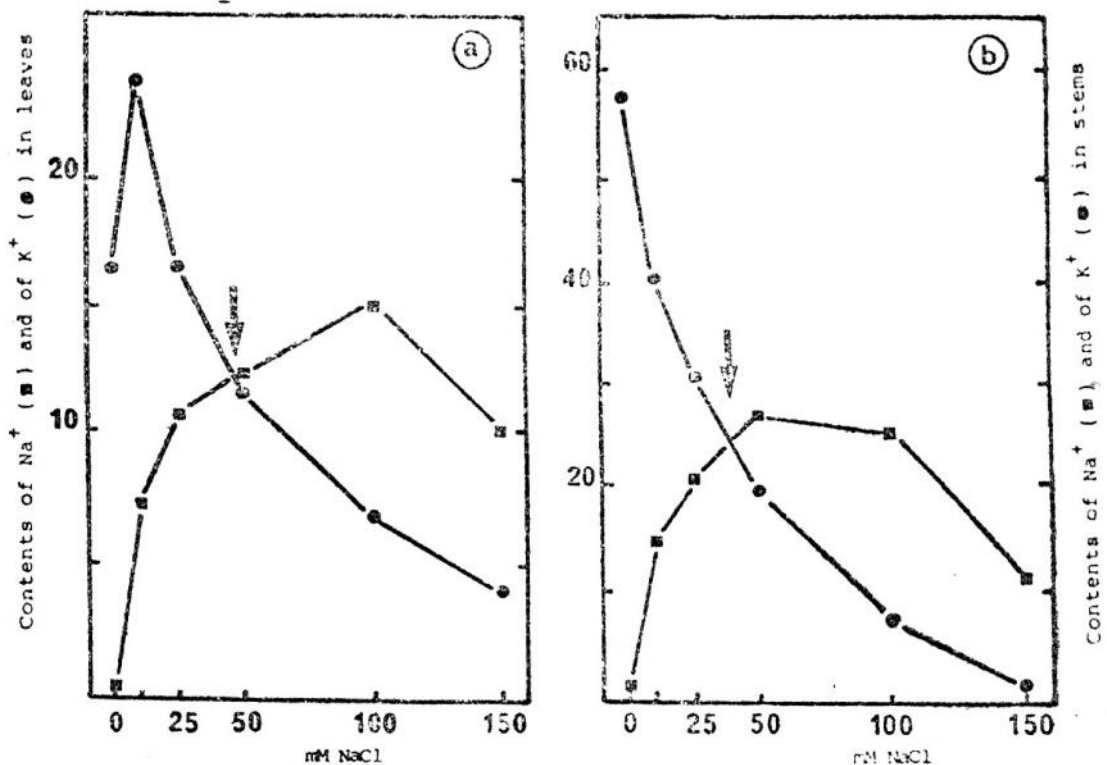
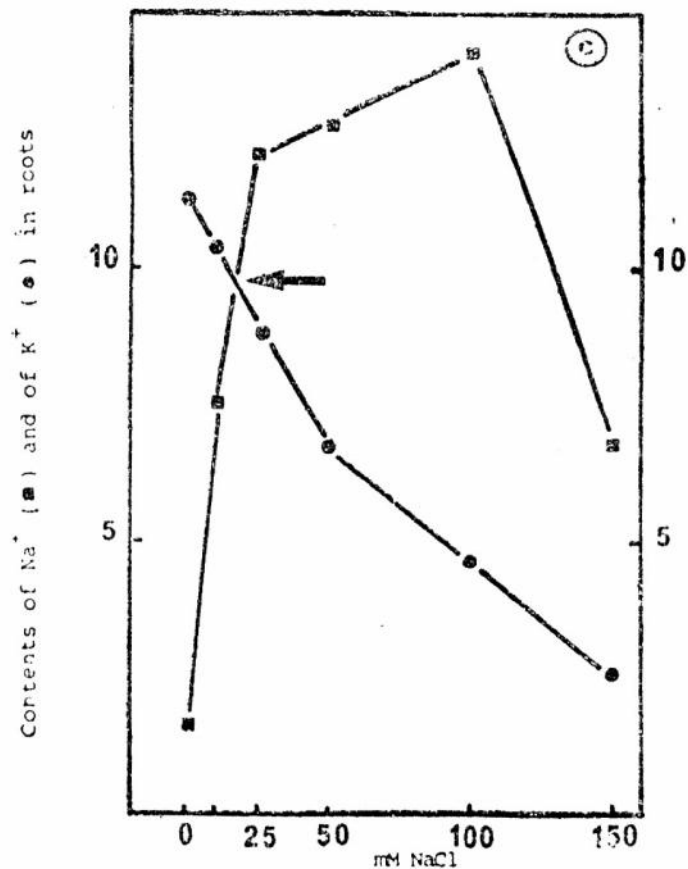


Figure 1. Changes of sodium and potassium contents in the various organs of *Vicia faba minor* as modified by applied  $\text{NaCl}$  (m.e. 100 plants <sup>-1</sup>) (a) leaves; (b) stems; (c) roots (See p. 57).

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The influx of  $\text{Na}^+$  in the stems (Fig. 1b) was more pronounced than that in the roots and it was accompanied by a drastic fall in the accumulation rate of  $\text{K}^+$ . The two cations were present in equal quantities in the stems at  $\text{NaCl}_{\text{ext}}$  40mM which was twofold that for roots. Beyond  $\text{NaCl}_{\text{ext}}$  50mM, there was no net accumulation of  $\text{Na}^+$  in the stems of the plants whose growth was severely retarded.

The influx of  $\text{Na}^+$  in the leaves (Fig. 1a) was slow at low  $\text{NaCl}_{\text{ext}}$  concentration. For 10 mM  $\text{NaCl}_{\text{ext}}$ , there was a concomitant influx of  $\text{Na}^+$  and  $\text{K}^+$ ; it appeared that the presence of low  $\text{NaCl}_{\text{ext}}$  concentration exerted a beneficial



effect on the absorption and accumulation of  $\text{K}^+$ . Beyond  $\text{NaCl}_{\text{ext}}$  10 mM, the  $\text{Na}^+$  influx in the leaves continued while  $\text{K}^+$  accumulation rate was slowed down. However, this decrease was much slower than that observed for the stems. The equality of the two cations in the leaves was attained around 50mM  $\text{NaCl}_{\text{ext}}$ .

The control plants had a high  $K^+$  content, amounting to 6.5% of the dry matter. With increase in salinity upto 25mM, the plants became enriched with  $Na^+$  while  $K^+$  contents decreased. At 25mM  $NaCl_{ext}$  growth was found to be optimum and the amount of  $K^+$  lost from 100 plants was equivalent to that of  $Na^+$  gained (ca.1lg of each element). So, the increase in dry matter at this salinity was not due simply to an increased accumulation of  $Na^+$  in the tissues. Above  $NaCl_{ext}$  25mM where growth was severely inhibited, the plants continued to accumulate  $Na^+$ . However, the rate of accumulation was relatively slower than that observed at low salinity. At the same time, the  $K^+$  content continued to decrease with increasing salinity and at 150mM  $NaCl_{ext}$ , the  $K^+$  content was 10-fold less than that in the control plants.

The endogenous  $K^+/Na^+$  ratio was 2.5 for the plants at 10mM and 1.2 for those at 25mM  $NaCl_{ext}$ . The  $K^+/Na^+$  ratio was less than 1.0 in the plants whose growth was severely inhibited by excess salinity in the growth media.

*Relative  $Na^+$  and  $K^+$  concentrations in the different parts of the aerial organs of V. faba minor as affected by salinity:*  $Na^+$  concentrations in the oldest part of the stems ( $S_3$ ) increased with increasing salinity and attained a value of more than 2 m.e.g<sup>-1</sup> of dry matter at 150mM  $NaCl_{ext}$  which was about 4.6% of the dry matter (Table 2). Almost a similar situation was found for the middle internodes ( $S_2$ ). For  $S_3$ , the massive influx of  $Na^+$  was associated with a drastic fall in the  $K^+$  concentration. At 150mM  $NaCl_{ext}$ , the  $K^+$  concentration in  $S_3$  was 10 times less than that observed in the control plants. On the

Table 2.  $Na^+$  and  $K^+$  concentrations in different parts of the stems of *V. faba minor* affected by salinity m.e.g<sup>-1</sup> d.w.)

Treatment (mM $NaCl_{ext}$ )	Part of stem					
	$S_1$		$S_2$		$S_3$	
	$Na^+$	$K^+$	$Na^+$	$K^+$	$Na^+$	$K^+$
0	0.03	1.39	0.03	1.40	0.07	3.52
10	0.18	1.30	0.58	1.64	0.67	1.80
25	0.63	2.30	0.92	1.43	0.67	0.86
50	0.60	1.28	1.07	0.89	2.10	1.04
100	0.25	1.52	2.14	0.85	1.76	0.26
150	n.d.	n.d.	2.00	0.39	2.54	0.34

n.d. = not determined;  $S_1$  = last two internodes ;  $S_2$  = three intermediate internodes ;  $S_3$  = epicotyls and first two internodes

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contrary, for  $S_2$ , the  $K^+$  concentration was higher at 10mM  $NaCl_{ext}$  relative to that for the control plants and this trend was maintained upto 50mM  $NaCl_{ext}$  where it showed a lower value than for  $Na^+$  concentration, the latter being increased to some extent.

The relative  $Na^+$  concentration in the primary leaves ( $L_3$ ) increased with increasing  $NaCl_{ext}$  while  $K^+$  concentration decreased (Table 3). However, this decrease was relatively less marked than that observed for the stems. The median leaves ( $L_2$ ) also accumulated appreciable amount of  $Na^+$  though the  $K^+$  concentration was found to be increased upto  $NaCl_{ext}$  10mM. This however decreased to more than 5% at 50mM  $NaCl_{ext}$  and above. In the developing leaves i.e., the trifoliate ( $L_1$ ), the  $Na^+$  concentration did not exceed a value of 0.43m. e.g.<sup>-1</sup> dry matter corresponding to 45mM (calculated on the basis of total tissue water content of these organs). In this part of the plant, the relative  $K^+$  concentration showed a net increase and it became less than that of the control plants only at 50mM  $NaCl_{ext}$ . There was a mere 30% decrease in the  $K^+$  concentration at 100mM  $NaCl_{ext}$ . It seems that the acclimatization of *V. faba minor* to  $NaCl_{ext}$  proceeds with a preferential accumulation of  $Na^+$  in the older organs (stems and leaves) which permits avoidance of an excess accumulation of this ion in the developing organ of the plants. Along with such an unbalanced repartition of  $Na^+$ , the distribution of  $K^+$  is found to be considerably modified: its concentration being higher in the younger growing organs and reduced in the older organs.

Table 3.  $Na^+$  and  $K^+$  concentrations in the different leaves of *Vicia faba minor* as affected by salinity (m.e.g.<sup>-1</sup> d.w.)

Treatment (mM $NaCl_{ext}$ )	Leaves					
	$L_1$		$L_2$		$L_3$	
	$Na^+$	$K^+$	$Na^+$	$K^+$	$Na^+$	$K^+$
0	0.00	0.85	0.02	0.75	0.04	0.85
10	0.14	1.07	0.40	1.01	0.60	0.74
25	0.43	0.90	0.51	0.59	0.85	0.45
50	0.36	0.87	1.03	0.59	1.14	0.38
100	0.38	0.71	1.50	0.43	1.47	0.27
150	1.16	0.59	2.17	0.28	n.d.	n.d.

n.d. = not determined;  $L_1$  = last trifoliate and apex;  $L_2$  = three median leaves;  $L_3$ —two primary leaves

## Discussion

The experiments on the evaluation of the salt tolerance of legumes are difficultly comparable. In the present study an attempt was made to examine the potentialities of non-nodulated (mineral-N fed) *Vicia faba minor* to acclimatize to external salinities ranging from 0 to 150 mM NaCl.

In good agreement with the classification of Greenway and Munns (1980) it was observed that the maximum concentration of NaCl (150mM) used in this experiment significantly inhibited the growth of *V. faba minor*. Actually, the growth inhibition became apparent only at  $\text{NaCl}_{\text{ext}}$  between 40 and 50mM. As noted by Winter and Laüchli (1982) in *Trifolium*, the inhibitory effect of salt in the present case also was more pronounced in the leaves and stems than in the roots. Below the  $\text{NaCl}_{\text{ext}}$  concentration, which is also high for glycophytes, the growth of *V. faba* was found to be stimulated, the optimum being at 25mM  $\text{NaCl}_{\text{ext}}$ . This stimulation of growth could be attributed to an increased organic matter production as the  $\text{Na}^+$  accumulation was compensated (weight per weight) by an efflux of  $\text{K}^+$ . The beneficial effect of  $\text{Na}^+$  and  $\text{Cl}^-$  remains to be explained because the essentiality of  $\text{Na}^+$  has so far not been established except for some  $\text{C}_4$  type plants (Clarkson and Hanson 1980). Such an observation (growth stimulation) was not made either by Helal and Mengel (1981) or by Youssef and Sprent (1983) with *V. faba*, as in both cases, the  $\text{NaCl}_{\text{ext}}$  concentration started from 50mM. Such a growth stimulation of legumes by  $\text{NaCl}_{\text{ext}}$  at moderate concentration has recently been reported for *Vigna sinensis* (Huq and Larher 1983a, 1984). On the contrary, *Phaseolus aureus* and *Glycine max* have been reported to be hypersensitive to  $\text{NaCl}_{\text{ext}}$  (Bernstein and Ogata 1966, Huq and Larher 1983b, Huq *et al.* 1984). Similar observations are also in report for *Trifolium* subjected to salinity after their initial developmental stages (Helal and Mengel 1981, Winter and Laüchli 1982).

The growth stimulation of *V. faba* is accompanied by an enrichment in the young developing tissues (younger leaves and apical zones of the stems) by potassium suggesting an accelerated mobilization of this element in these organs at the expense of older organs where the  $\text{K}^+$  content shows regression in response to a  $\text{Na}^+$  influx. The growth rate observed at higher  $\text{NaCl}_{\text{ext}}$  concentration indicates that  $\text{Na}^+$  might act as a replacing ion for  $\text{K}^+$  in *V. faba minor*. However, the growth could be maintained at an appreciable level if the cellular  $\text{K}^+/\text{Na}^+$  ratio does not fall below 1.0. Similar observations were made recently by Huq and Larher (1985) with *V. sinensis* and *P. aureus*. The critical  $\text{NaCl}_{\text{ext}}$  concentration that creates such a situation was found to

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be 45 mM for *V. faba*. This concentration of NaCl appeared to be the limiting value for resistance to salinity of this plant. This behaviour also reveals the aptitude of the species to a preferential repartition of the excess Na<sup>+</sup> in the stems and older leaves allowing at least, a partial protection to the less vacuolated younger tissues, partially confirming the recent hypothesis of Greenway *et. al.* (1983). This microhalophilic nature of *V. faba* could be taken advantage of. The plants could be used in the culture of moderate salinity at least under mineral-N nutrition. For its salt tolerance under nodulated conditions in association with *R. leguminosarum*, it needs however, further research on the salt tolerance of the bacteria because preliminary study has shown that this bacterium is much more sensitive to salinity than its host.

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