

Dynamics of Na⁺, K⁺, and Proline Accumulation in Salt-treated *Vigna sinensis* (L) and *Phaseolus aureus* (L)

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Abstract

The dynamics of Na⁺, K⁺, and proline accumulation in various organs of non nodulated *Vigna sinensis* and *Phaseolus aureus* was followed during their acclimation to two levels of salinities for a period of 35 days and was correlated to the vegetative growth of the two species. The rate of Na⁺ and K⁺ absorption is at a maximum during the first 15 to 20 days of culture. K⁺ absorption is not completely inhibited even at 100 mM NaCl although the endogenous Na⁺ largely surpasses that of K⁺ in certain organs. Low salinity rather accelerates K⁺ absorption in both species. The relative growth rates (RGR) correlate with the rate of Na⁺ and K⁺ accumulation. At low salinity (10 mM NaCl), the RGR of *V. sinensis* is greater than that of *P. aureus*. However, at high salinity (100 mM NaCl) the RGR is the same for both species. The growth of the younger parts of the two species is not arrested by salt treatment. Very high accumulation of Na⁺ is avoided in organs with less vacuolated tissues. At no time does the endogenous K:Na ratio in these organs fall below 1.0. Certain organs, especially the roots, hypocotyls, and the lower parts of the stems are capable of storing large quantities of Na⁺. In *V. sinensis*, the accumulated Na⁺ and K⁺ are evenly distributed among the various organs while in *P. aureus* they are rather concentrated in the roots. External salinity creates water deficiency in the younger plant parts and as a consequence, proline accumulates especially in the youngest aerial organs – more in *P. aureus* than in *V. sinensis*. The accumulation of this amino acid in both the species is dependent on time and correlates directly, not only with the water deficit, but also with the K⁺ contents. In contrast, it does not seem to depend directly on the endogenous Na⁺ content. The relative salt tolerance of the two species and the possible role of K⁺, Na⁺ and proline in the osmotic adjustments of the two species under saline conditions are discussed.

Key words: *Vigna unguiculata* syn. *sinensis*, *Phaseolus aureus*, salt stress, Na⁺, K⁺, Proline, relative growth, ion absorption rate, osmotic adjustment.

Introduction

In recent works on the salt tolerance of *V. sinensis* and *P. aureus* it was observed that the growth of the plants was either favored or negligibly affected by low salinity while higher salinity caused significant growth reduction in both the species, more in the latter than in the former species (Imamul Huq and Larher, 1983 a, 1983 b, 1984).

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Abbreviations: RGR = relative growth rate; NaCl_{ext} = NaCl solution applied to the growth medium.

S. M. IMAMUL HUQ and F. LARHER

The cause of an overall growth reduction was attributed to the «ion excess» effect that caused a lowering of the internal K:Na ratios to values considered to be critical for several metabolic functions (Greenway and Munns, 1983). These observations were, however, based on the results obtained from the plants collected after a definite period of growth and the interpretation made on the analyses of the composition of the aerial and underground parts without taking into considerations either the dynamics of the accumulation of Na⁺ and K⁺ and its ultimate effect on the relative growth promotion or inhibition of the two species or the preferential accumulation of one or both of these ions in any particular organ of the two species. Different plant parts are known to behave differently while adjusting to external salinity (Rains, 1972). A differential accumulation of Na⁺ and K⁺ in different plant parts as well as varied rates of absorption of these ions might not only indicate the susceptibility of certain plant parts towards Na⁺ toxicity at different times of their growth but also of the mechanism by which certain organs avoid a high accumulation of this non-essential toxic element to maintain survival. During their acclimation to salinity the plants need to adjust osmotically to the decreased water potential and as a result higher plants are known to synthesize and accumulate proline among other organic compatible solutes (Hsiao, 1973; Hellebust, 1976; Stewart and Larher, 1980) although the exact role played by proline in salt-stressed higher plants is still ill defined.

So far no work has been done on the dynamics of the Na⁺ and K⁺ accumulation and its ultimate consequence on proline accumulation in various plant parts of salt-acclimated plants except that of Goas et al. (1982) with the halophyte *Aster tripolium* subjected to a short term saline shock.

In the present work we studied the evolution of Na⁺ and K⁺ accumulation in various plant parts of non-nodulated *V. sinensis* and *P. aureus* acclimated to two levels of salinity and the influence of their absorption on the relative growths of the two species. The time course of proline accumulation in various organs were followed. Correlations were made with this amino acid and some endogenous physiological factors known to affect growth during the acclimation of the plants to changed environmental conditions (Greenway and Munns, 1983).

Materials and Methods

Plant material and culture

Seeds of cowpea [*Vigna unguiculata* (L.) Walp. Syn *Vigna sinensis* (L.) Savi ex Hassk] and mung bean (*Phaseolus aureus* Roxb.) were germinated directly on vermiculite and allowed to grow under controlled conditions with mineral N in the presence of 10 and 100 mM NaCl (NaCl_{ext}). The source of the seeds, composition of the nutrient solution (K = 2 mM) and growing conditions are the same as described elsewhere (Imamul Huq and Larher, 1983 a, 1983 b).

Both nutrient solutions and salts were renewed weekly. Distilled water was added daily to the vermiculite to maintain a constant moisture content in the growing medium. Plant samples were collected every 5 days for 35 days after seedling emergence. At harvest the root free space was unloaded with three 1 min washings in 1 l distilled water. The plants were separated into

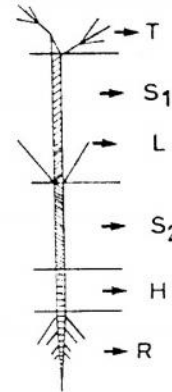
Dynamics of Na^+ , K^+ , and proline accumulation in legumes

Fig. 1: A schematic representation of the different plant parts retained for the study. T = trifoliates; S₁ = upper part of the stems; L = primary leaves; S₂ = lower part of the stems; H = hypocotyls; R = roots.

trifoliates (T), tendrils and uppermost part of the stems (S₁), primary leaves (L), lower parts of the stems (S₂), hypocotyls (H), and roots (R) according to the stage of development of each of the organs (Fig. 1). For the first sampling (5 days after seedling emergence) the hypocotyls and the stems were grouped together as they could not be distinctly separated. The fresh and dry weights of each of the plant organs were determined (Imamul Huq and Larher, 1984) and the water contents calculated. The results presented are the averages of two individual replicates of 5 plants in each. The standard errors were between 5 and 10%.

Analyses

Na^+ , K^+ , and proline contents in each of the organs were determined respectively by flame photometry and colorimetry as described in Imamul Huq and Larher (1984) and the results are presented in terms of dry matter.

Rate of Na^+ and K^+ absorption was calculated according the equation used by Jarvis (1982). The relative growth rates (RGR) of the whole plant were calculated according to Kingsbury et al. (1984). In both the cases the calculations were made on the dry matter. Simple regression analyses, correlations and dependence ('t') tests (Dixon and Messey, 1969) were performed on the results obtained to study the relationships between the proline concentrations and other physiological factors of the plants likely to influence its accumulation in the two species.

Results

Na⁺ and K⁺ contents in different plant parts

The content of Na^+ and that of K^+ was different in the two species according to the concentration of Na in the external media (Fig. 2). In *V. sinensis*, at 10 mM NaCl_{ext} there was an appreciable accumulation of Na^+ in the lower parts (S₂, H, R) of the plants without any significant perturbations in the K^+ content of these organs. The entry of Na^+ into the youngest parts (T, S₁) was quite slow. Na^+ started to accumulate in the aerial parts only after 3 weeks of culture. At no time did the endogenous Na^+ content surpass that of K^+ content in any of the organs. On the other hand, in *P. aureus* at the same salinity level, there was a very high accumulation of Na^+ in the roots which was attained at the expense of K^+ accumulation. The K^+ level was much lower than that of Na^+ in the roots during the first 15 days of acclimation. An uneven distribution of Na^+ among the various plant parts was

S. M. IMAMUL HUQ and F. LARHER

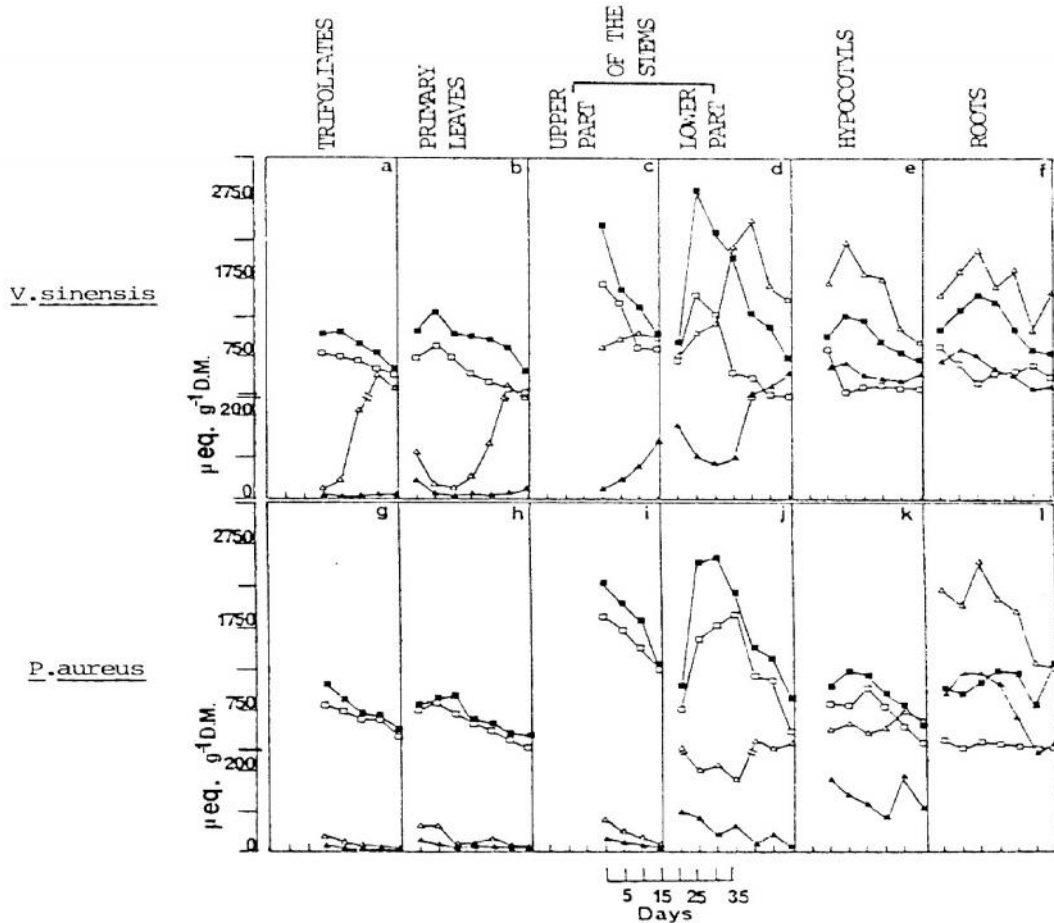


Fig. 2: Na^+ and K^+ content in various parts of *V. sinensis* (a, b, c, d, e, f) and *P. aureus* (g, h, i, j, k, l) at different stages of their acclimation to salinity. (■) K^+ at 10 mM NaCl_{ext} ; (□) K^+ at 100 mM NaCl_{ext} ; (▲) Na^+ at 10 mM NaCl_{ext} ; (△) Na^+ at 100 mM NaCl_{ext} .

observed in this species. The diminution of the content of the cations in different plant parts at later stages of acclimation might be related to a dilution effect.

At 100 mM NaCl_{ext} , the Na^+ was accumulated very rapidly in the roots of both the species during the first 15 days. In *V. sinensis*, the Na^+ contents largely exceeded that of K^+ in the roots (R) and the hypocotyls (H) and the situation remained as such up to the end of the experiment. Na^+ contents in the lower parts of the stems (S_2) and the primary leaves (L) of this species exceeded that of K^+ during the later stages of growth whereas such a situation never occurred in the youngest aerial parts (T & S_1).

Dynamics of Na⁺, K⁺, and proline accumulation in legumes

Enrichment of the organs by Na⁺ was always accompanied by a depletion of K⁺, more in the older parts than in the younger ones. The accumulated Na⁺ and K⁺ appeared to be distributed among the different organs of this species. In *P. aureus*, the Na⁺ content in the roots was 5 to 6 times higher than that of K⁺. It appears that this species avoids accumulation of high Na⁺ in the aerial organs although noticeable amounts of the cation could be detected in the lower parts of the stems (S₂) and the hypocotyls (H).

Na⁺ and K⁺ absorption rates

Table 1 shows the calculated rates (Jarvis, 1982) of Na⁺ and K⁺ absorption by the roots of *V. sinensis* and *P. aureus* at two salinity levels. At 10 mM NaCl_{ext}, *V. sinensis* absorbed Na⁺ at a lower rate than *P. aureus* up to the first 10 days of culture after which the rate of Na⁺ absorption became greater in the former species. The average rate of Na⁺ absorption for the whole period of study (5 to 35 days) was practically the same for the two species. The rate of K⁺ absorption on the other hand, was greater in *P. aureus* during the first 10 days. *V. sinensis* absorbed K⁺ at a higher rate than *P. aureus* at the low salinity up to 20 days of culture. It seems that the absorption rate of K⁺ in both the species is favored by the presence of low concentrations of Na⁺ in the growing media – more in *V. sinensis* than in *P. aureus*. The maximum rate of K⁺ absorption coincides with that of Na⁺ indicating some kind of synergism between the two ions. Although the average rates of Na⁺ absorption at the low salinity remained almost identical for the two species, the rate of K⁺ absorption however, was more than 25 % lower in *P. aureus*.

At 100 mM NaCl_{ext}, *V. sinensis* absorbed Na⁺ at a significantly higher rate than *P. aureus* throughout the period of acclimation, the maximum rate being between 15 to 20 days. For *P. aureus*, the maximum rate of Na⁺ absorption occurred between 10 to 15 days. The absorption maxima for Na⁺ absorption were delayed by 5 days in both the species at high salinity. As to the K⁺ absorption at this salinity, *P. aureus*

Table 1: Rate of Na⁺ and K⁺ absorption by the roots of *V. sinensis* and *P. aureus* (μeq · g⁻¹ d.m. of roots · d⁻¹) at different stages of growth and acclimated to 10(1) and 100(2) mM NaCl_{ext}.

| Days after emergence | <i>V. sinensis</i> | | | | <i>P. aureus</i> | | | |
|----------------------|--------------------|------|----------------|------|------------------|-------|----------------|------|
| | Na ⁺ | | K ⁺ | | Na ⁺ | | K ⁺ | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 5 to 10 | 88 | 456 | 830 | 462 | 210 | 82 | 1033 | 306 |
| 10 to 15 | 233 | 482 | 2083 | 52 | 54 | 697 | 795 | 639 |
| 15 to 20 | 74 | 1188 | 1235 | 1438 | 41 | – | 652 | 374 |
| 20 to 25 | 147 | 739 | 467 | 280 | 58 | 381 | 586 | 541 |
| 25 to 30 | 58 | 159 | – | – | – | 54 | – | 201 |
| 30 to 35 | – | 73 | 181 | 1 | 38 | 104*) | 339 | 60*) |
| 5 to 35 | 24 | 432 | 462 | 234 | 25 | 176 | 346 | 247 |

*) between 25 and 35 days; (–) no absorption.

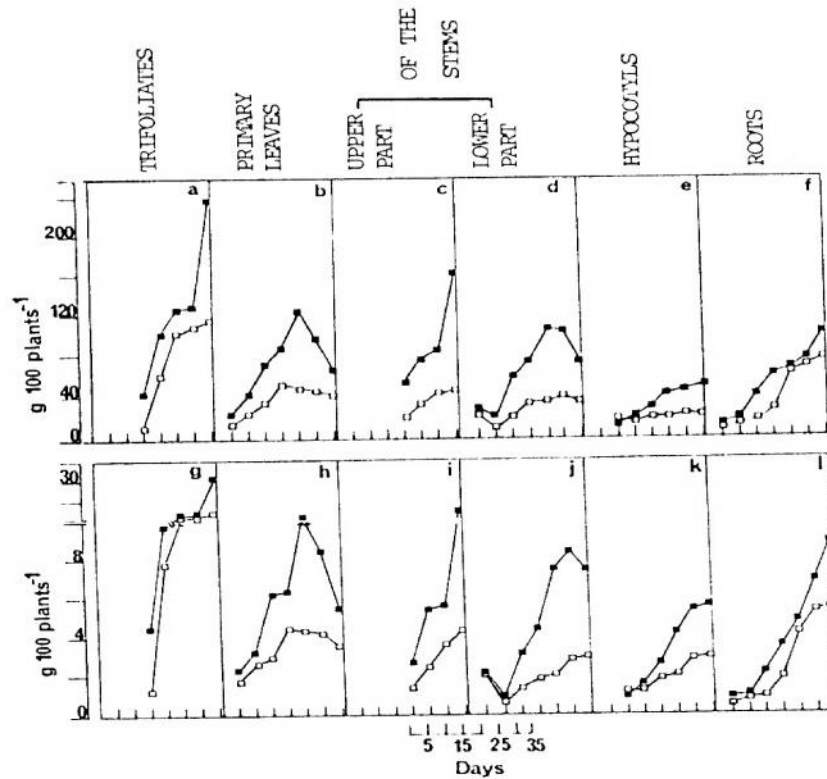


Fig. 3: Fresh (a, b, c, d, e, f) and dry (g, h, i, j, k, l) matter production in the various parts of *V. sinensis* acclimated to 10 mM (■) and 100 mM (□) NaCl_{ext} .

maintained a fairly constant diminishing rhythm while it fluctuated in *V. sinensis*. However, the maximum absorption rates of K^+ in both the species corresponded to that of Na^+ absorption. The average rate of Na^+ absorption for the entire period of acclimation (5 to 35 days) for *V. sinensis* was significantly higher (ca. 46%) than that for *P. aureus* although the K^+ absorption remained almost identical in both the species at 100 mM NaCl_{ext} .

Growth of the different plant parts

The growth of the different parts of the two species are expressed as mass of the fresh and dry matter of 100 plants and are presented in figures 3 and 4 respectively for *V. Sinensis* and *P. aureus*.

Although appreciable, the vegetative growth of the two species (fresh and dry matter production) at 100 mM NaCl_{ext} was markedly lower than that at 10 mM

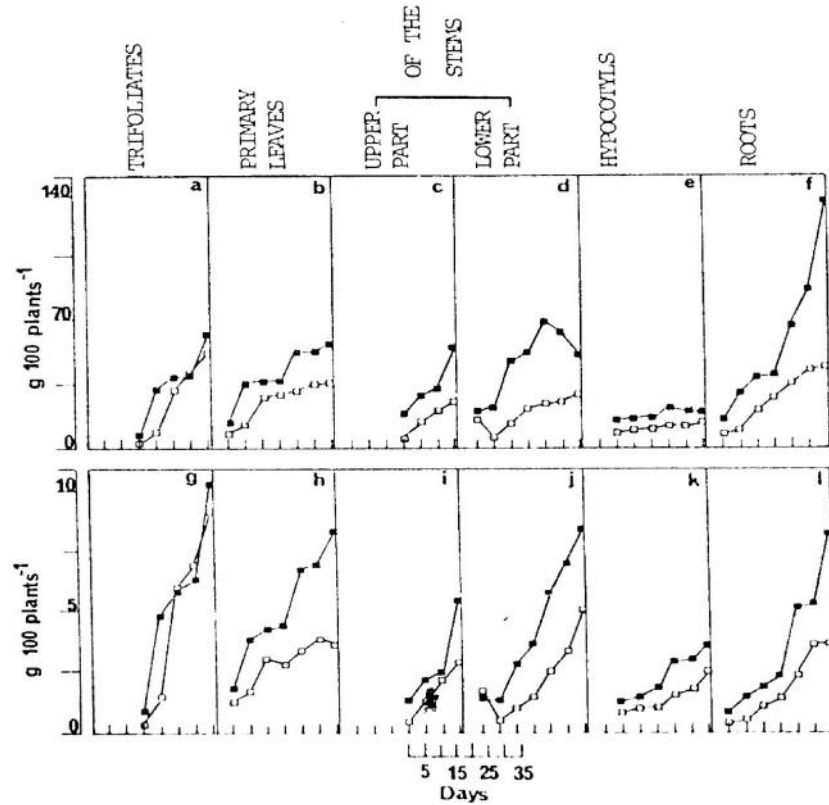
Dynamics of Na^+ , K^+ , and proline accumulation in legumes

Fig. 4: Fresh (a, b, c, d, e, f) and dry (g, h, i, j, k, l) matter production in the various parts of *P. aureus* acclimated to 10 mM (■) and 100 mM (□) NaCl_{ext} .

NaCl_{ext} indicating a global inhibitory effect of a strong salinity. The differences in growth of the different plant parts of the two species at the two salinities indicated the capability or susceptibility of different organs to cope with excess Na^+ . Thus, for *V. sinensis*, the root growth at 100 mM NaCl_{ext} was very little affected except at the end of culture, particularly concerning the dry matter production. On the other hand, the growth of the hypocotyls (H) and the stems (S_1 & S_2) was significantly inhibited at the higher salinity. The growth of the primary leaves (L) continued to increase up to 20 days of culture after which it was drastically inhibited. The growth of the trifoliates (T) appeared to be little affected by salinity except at the end of culture. The dry matter production was almost the same at the two salinities up to 30 days. The growth of all the plant parts except the primary leaves (L) and the lower parts of the stem (S_2) was maintained up to the end of culture.

In *P. aureus*, the dynamics of the root growth were similar at the two salinities up to

S. M. IMAMUL HUQ and F. LARHER

the 20th day of culture after which there was an acceleration of growth in the plants at 10 mM NaCl_{ext}. However, the root growth of the plants with both the treatments continued up to the end of culture. Similar phenomena were observed for the primary leaves (L) and the stems (S₁ & S₂). The hypocotyls (H) of this species had identical growth evolution with both the salinities. Like that of *V. sinensis* the growth of the trifoliates (T) of *P. aureus* was also identical at the two salinities. A decrease in the production of fresh matter of the lower parts of the stem (S₂) and a concomitant increase in the dry matter production in it may be related to a decrease in the water content in this organ.

Salinity affected the water status of different organs differently according to the concentration of NaCl in the growing media and the species. The water contents were lower at the high salinity – the deficit being higher in *P. aureus* than in *V. sinensis* – throughout the acclimation period. The water content in various organs decreased from 90% on an average at 5 days to about 87% at the end of culture. The trifoliates (T), lower parts of the stem (S₂), and the hypocotyls had the minimum water content in both the species.

Relative Growth Rates (RGR)

The calculated RGR of the two species at two salinity levels at different times of growth are presented in Table 2. The calculated RGR values for the whole plant showed that at low salinity *V. sinensis* had on an average a relatively higher growth rate than that observed for *P. aureus*. At higher salinity, both the species had however, similar average RGR values although the RGR at different times of sampling differed widely between the two species indicating the differences in the potentiality of the two species to their acclimation to high salinity.

Table 2: Relative growth rates (RGR) of the plants of *V. sinensis* and *P. aureus* acclimated to two salinities (g · g⁻¹ d.m. d⁻¹).

| Days after emergence | <i>V. sinensis</i> | | <i>P. aureus</i> | |
|----------------------|--------------------|----------------|------------------|----------------|
| | 10 mM NaCl | 100 mM NaCl | 10 mM NaCl | 100 mM NaCl |
| 10 | 0.024 (0.001) | 0.032 (0.002) | 0.122 (0.006) | 0.011 (0.0005) |
| 15 | 0.215 (0.015) | 0.074 (0.005) | 0.073 (0.003) | 0.128 (0.010) |
| 20 | 0.101 (0.006) | 0.194 (0.013) | 0.095 (0.007) | 0.040 (0.002) |
| 25 | 0.089 (0.004) | 0.062 (0.003) | 0.079 (0.005) | 0.142 (0.011) |
| 30 | 0.009 (0.0004) | 0.038 (0.003) | 0.022 (0.0007) | 0.051 (0.003) |
| 35 | 0.086 (0.007) | 0.014 (0.0006) | 0.080 (0.006) | 0.043 (0.002) |
| Average | 0.087 | 0.069 | 0.078 | 0.069 |

Values in parentheses are the standard errors.

The maximum RGR values for both the species at the two salinities corresponded to the maximum rates of K⁺ and Na⁺ absorption by the roots. Although the RGR values were the same for the two species at 100 mM NaCl_{ext}, the growth inhibition

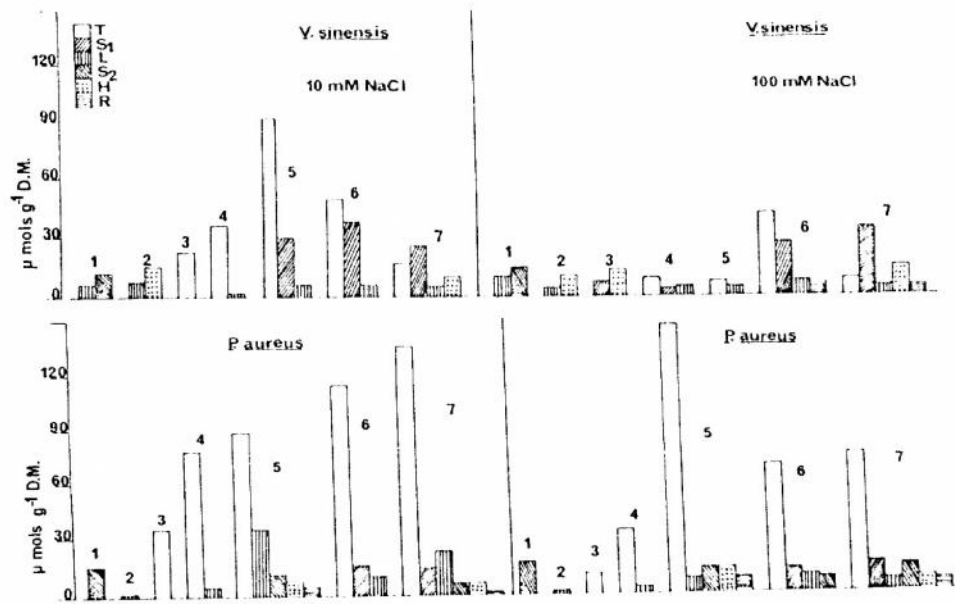
Dynamics of Na^+ , K^+ , and proline accumulation in legumes

Fig. 5: Time course of proline content in the various plant parts of *V. sinensis* and *P. aureus* acclimated to 10 mM and 100 mM NaCl_{ext} . The times of sampling are represented by the superscripts (1 = 5d, 2 = 10d, 3 = 15d, 4 = 20d, 5 = 25d, 6 = 30d, 7 = 35d). The standard errors are within the limits of 10%.

(expressed as total dry matter production) at salinity over 10 mM NaCl_{ext} was greater for *P. aureus*, about 9% higher than that observed for *V. sinensis*.

The two species differed in the trends of RGR in the course of time. *V. sinensis* showed a great increase in RGR during the 15th and 20th day at both the salinities while *P. aureus* had the two increases at 100 mM NaCl_{ext} only, one during the 15th and the other during the 25th day of culture. The differences in the RGR values for the two species might be related to the different maturation times of the two species.

Proline contents in different plant parts

The content of proline in different parts of the two plants acclimated to the two salinities are presented in figure 5 and the proline production per plant of the two species in figure 6.

The accumulation of proline in response to external salinity was different in the two species. In general, *P. aureus* had higher proline contents at both the salinities. Both accumulation and production of the amino acid were higher at the low salinity in both the species. The appearance and accumulation of proline appeared to be time-dependent and selective to the organs. It was found that at low salinity proline accumulated preferentially in the youngest aerial parts (T, S₁) of both the species. The

S. M. IMAMUL HUQ and F. LARHER

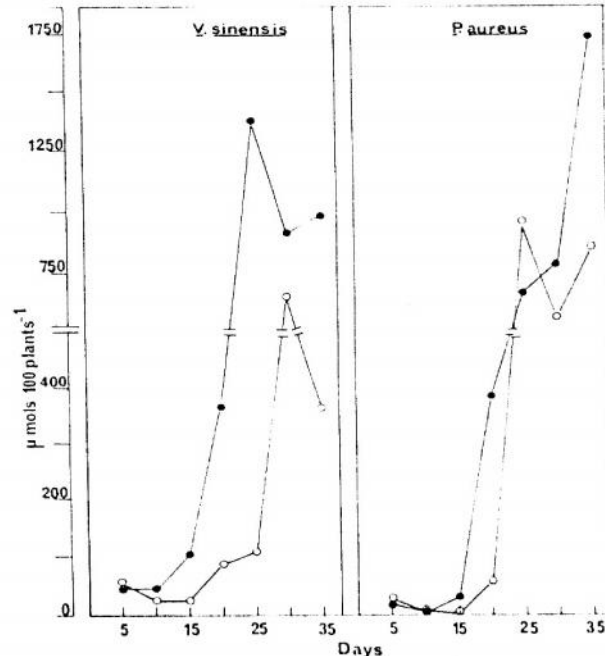


Fig. 6: Proline production by *V. sinensis* and *P. aureus* acclimated to 10 mM (●) and 100 mM (○) NaCl_{ext}. The standard errors are within the limits of 10%.

level of this amino acid became appreciable after 15 days of acclimation in *P. aureus* at 10 mM NaCl_{ext}. It continued to increase up to the end of acclimation of 35 days while it decreased in *V. sinensis* after attaining the maximum on the 25th day of cultured. At 100 mM NaCl_{ext}, *P. aureus* had a relatively higher proline content in all the organs than *V. sinensis*. Moreover, the plant part that accumulated the maximum quantity of the amino acid was the trifoliolate in the former species but in the latter species it was rather the upper part of the stem that accumulated an appreciable quantity of proline. At this salinity, the maximum accumulation was observed on the 25th day in *P. aureus* and on the 30th day in *V. sinensis*.

Although the former species had a less luxuriant growth than the latter, the proline production in *P. aureus* remained higher at both the salinities, especially at the end of the acclimating period.

As proline is supposed to be accumulated in plants subjected to environmental stresses (Hsiao, 1973; Greenway and Munns, 1983; Stewart and Larher, 1980) an attempt was made to correlate the presence of this amino acid with other endogenous physiological factors that are subject to changes in the plants acclimating to excess NaCl. Thus, simple linear regression equations, correlation coefficients, and dependency ('t') tests were performed on the endogenous proline concentrations and

Dynamics of Na⁺, K⁺, and proline accumulation in legumesTable 3: Relationships between concentrations of endogenous Na⁺ and K⁺, K:Na ratios and water content and that of proline concentrations in salt acclimated *V. sinensis* and *P. aureus*.

| | <i>V. sinensis</i> | <i>P. aureus</i> |
|--------------------|---|---|
| mM K ⁺ | mM Pro = 0.21 + 0.02 mM K ⁺ 'r' = 0.2381 't' = 1.4505 | mM Pro = 0.21 + 0.05 mM K ⁺ 'r' = 0.2474 't' = 7.9152 |
| mM Na ⁺ | mM Pro = 1.89 + 0.0004 mM Na ⁺ 'r' = 0.0087 't' = 0.0515 | mM Pro = 6.61 - 0.06 mM Na ⁺ 'r' = -0.2793 't' = -1.6959 |
| K/Na | mM Pro = 1.29 + 0.02 K/Na 'r' = 0.4186 't' = 2.7267 | mM Pro = 1.44 + 0.08 K/Na 'r' = 0.4233 't' = 2.7244 |
| % H ₂ O | mM Pro = 14.51 - 0.18 H ₂ O 'r' = -0.2299 't' = -1.3967 | mM Pro = 150.57 - 1.64 H ₂ O 'r' = -0.6468 't' = -4.9451 |

N = 37; significance levels: 'r': 0.2766 (r_{0.95}); 0.4002 (r_{0.99});
't': 1.3051 (t_{0.90}); 2.4332 (t_{0.95}); 2.7178 (t_{0.995}).

the corresponding Na⁺, K⁺ concentrations, K/Na ratios, and water content of the two plants (Table 3).

It appeared that the proline concentrations in the plants were directly related to the K⁺ concentrations, more in *P. aureus* than in *V. sinensis*. Contrarily, the accumulation of the amino acid was inversely related to the Na⁺ concentration in *P. aureus*. The correlation in *V. sinensis*, though positive, was very insignificant indicating the fact that the appearance or accumulation of proline in the two species was in no way related to a direct consequence of Na⁺ accumulation in excess. The organs accumulating higher Na⁺ was found to contain less proline (e.g. T of *P. aureus* $y = 11.68 - 2.018 x$, $r = -0.5325$). Proline concentrations were also found to be significantly related directly to the endogenous K/Na ratios in both the species and appeared to be significantly dependent on the maintenance of a high K/Na ratio. As to the water content and the proline concentrations, it was observed that the accumulation of the amino acid in the two salt acclimated legumes was significantly related to the conditions of water deficit created by salinity in the growing media, the lower the water content in the tissues the higher was the proline concentration in them. The influence of a situation of water deficit on the proline accumulation was more pronounced for *P. aureus* in which case the 't' test revealed a significant dependence of the presence of the amino acid on the decreased water content in the organs created as a result of decreased water potential in the external media by an excess of NaCl.

Discussion

The present study revealed that the accumulation of Na⁺ and in consequence that of K⁺ in the two legumes acclimated to salinity differed in the plant parts in both the

S. M. IMAMUL HUQ and F. LARHER

species, the youngest organs accumulating the least Na^+ . These observations corroborate the recent hypothesis proposed by Greenway and Wolfe (1983) where they advocated that the less expanded tissues are capable of avoiding high accumulation of Na^+ which is not favorable for the maintenance of their osmotic adjustments under conditions of saline stress and as a result certain organic solutes are needed to adjust the deficit.

The distribution of the absorbed Na^+ and K^+ among the different plant parts varied in the two species. A better salt tolerance of *V. sinensis* than that of *P. aureus* (Imamul Huq and Larher, 1983 b) might be due not only to its halophilic character (Imamul Huq and Larher, 1984) but also to its capability for an even distribution of the ions among its different parts, even at high salinity. In *P. aureus* on the other hand, most of the Na^+ absorbed was mainly localised in the underground parts at both the salinities. This might have created a condition of ion supersaturation thereby slowing down the absorption of other cations and rendering the species more susceptible to external salinity. The study also revealed that under the present experimental conditions, the entry of Na^+ into the plants is not maintained at a uniform rate throughout the entire period of acclimation, the maximum absorption of the ion occurring during the early 15 to 20 days of growth. Although the rate of K^+ absorption is slowed down due to an absorption of Na^+ at a higher rate (at 100 mM NaCl_{ext}) the absorption of the former ion however was not completely inhibited. This of course caused a lowering of the K/Na ratio of the tissues of certain organs of the plants acclimating at the high salinity. At low salinity however, a synergistic effect of Na^+ on the K^+ absorption was observed. At the high salinity, a diminished rate of K^+ absorption is compensated to a large extent by an increased absorption of Na^+ in *V. sinensis* while in *P. aureus*, the imbalance created by a slowed entry of K^+ is not filled in by Na^+ which is supposed to create an ionic deficit to counteract the excess Cl^- and other anions that are known to occur in plants acclimated to NaCl salinity (Imamul Huq and Larher, 1983 a, 1984; Winter and Läuchli, 1982). This might contribute to a better salt tolerance of *V. sinensis*.

Although the growth of some of the plant parts was retarded after a certain period of salt acclimation, the overall growth of the two species, was not completely stopped, especially at the higher salinity. The maintenance of the rhythm of growth of the root system even in the presence of 100 mM NaCl_{ext} indicates some kind of morphogenetic adaptation of the roots of the two legumes at high salinities.

A relatively higher growth rate (RGR) of *V. sinensis* at a low salinity further substantiates the halophilic nature of the species observed in previous studies (Imamul Huq and Larher, 1983 b, 1984). These values indicate that salinity affected the growth of the two legumes although its action is different on the various plant parts. Although the RGR at the high salinity was found to be the same for both the species, yet the salt sensitive species, *P. aureus* (Imamul Huq and Larher, 1983 b) had a greater growth inhibition at the high salinity. This may be related to a better production potential of *V. sinensis* at the low salinity. The maintenance of growth at

Dynamics of Na^+ , K^+ , and proline accumulation in legumes

a more or less uniform rate of the younger aerial parts that had the minimum water content indicates that the inhibition of cell expansion is not due only to the water status of the tissue. It seems that the developmental process is not significantly affected by salinity until either the photosynthetic activity (Helal and Mengel, 1981), hormonal balance (Prisco and O'Leary, 1973), or some enzymatic activities (Stewart and Ahmad, 1983) are drastically affected by an appreciable fall of the K/Na ratio of the tissue (Greenway and Munns, 1983).

A direct relationship observed between «the maximum» absorption rates of Na^+ and K^+ and the high RGR of the plants suggest an enhanced accumulation of the ions in the plants when the growth activity is at its maximum. The growth inhibition by excess salinity is rather a secondary phenomenon of an excess of Na^+ in the tissues.

Although proline is known to be accumulated in glycophyte acclimated to saline environments (Weimberg et al., 1982; Dix and Pearce, 1981) the real significance or consequence of such an accumulation in salt acclimated legumes is still obscure. It became apparent from the present study that the appearance or accumulation of this amino acid in the two legumes is gradual and confined to the organs where excess Na^+ does not bring in a lowering of the K/Na ratio of the tissues. Moreover, the accumulation of the amino acids is in no way related to the relative salt tolerance of the species. The species with less proline content acclimates better to salinity. A relatively lower proline content in the plants acclimating to a higher salinity renders the role of this amino acid in the cytoplasmic osmotic adjustments of the two legumes doubtful. A possible involvement of Na^+ in this phenomenon has been envisaged (Imamul Huq, 1984). Mozafar and Oertli (1984) have recently shown that 4.4% of the tissue's total Na^+ can be present in the cytoplasm of cotton leaf cells. Actually, after Flowers et al. (1977) cytoplasmic Na^+ concentrations can reach as high as 150 mM without causing any adverse effect on cytoplasmic enzymes. Even if proline is involved in the osmotic adjustment, its contribution is negligible compared to that of free sugars in *V. sinensis* (Imamul Huq and Larher, 1984) and other nitrogenous solutes in *P. aureus* (Imamul Huq and Larher, 1983 c). In both the cases K^+ and NO_3^- are the principal osmotica even in the presence of high salinity (Imamul Huq and Larher, 1983 c, 1984). As to the site of proline synthesis, it is obvious that the younger plant parts are the principal sites of accumulation and a transfer might occur to other organs some time during the growth of the plants. This observation has been further substantiated by experiments with isolated plant parts of *P. aureus* cultured *in vitro* under aseptic conditions (Imamul Huq, unpublished). The existence of an inverse relationship between the proline content and that of Na^+ and conversely of a direct correlation with the water deficit substantiates the hypothesis (Hellebust, 1976) that proline accumulation is primarily associated with water deficit. Similar observations have also been made in our laboratory (Imamul Huq, unpublished) with isolated organs of *P. aureus* and *Sorghum bicolor* and with barley (Aspinall and Paleg, 1981). So, proline accumulation in the two legumes acclimated to salinity may be considered either as symptomatic of a salt induced

S. M. IMAMUL HUQ and F. LARHER

water deficit or as a temporary deviation of the metabolic pathway of NH_4 assimilation at the level of glutamic acid. The proline oxidation is perhaps slowed down to conserve some of the metabolic energy for the maintenance of a better growth when the stress conditions are alleviated.

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