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Plant Growth, Metabolism and Adaptation in Relation to Stress Conditions
IX. Endogenous Levels of Hormones, Minerals and Organic Solutes in *Pisum sativum* Plants as Affected by Salinity

By

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With 3 Figures

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Summary

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Salinity treatments altered the balance of hormonal levels in growing *Pisum sativum* plants. Thus −0.3 and −0.6 MPa salinity induced a significant increase in growth promoter levels concurrently with a decrease in abscisic acid (ABA) at all stages. A reverse situation was observed with higher levels of salinity.

At each developmental stage, the various salinity levels induced the accumulation of Na^+ and Ca^{2+} that was associated with a decrease in K^+. For Mn^{2+} content, either an increase in shoots or a decrease in roots was induced by the low levels of salinity. An opposite situation was observed with the higher levels of salinity. Phosphorus content was, in general, increased in shoots with −0.6, −0.9 and −1.2 MPa, whereas an opposite situation was recorded in roots.

Depending upon the level of salinity applied and the stage of development, marked changes were recorded for α-keto, citric and oxalic acid contents in both

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roots and shoots. Proline content appeared either to increase (with −0.3 and −0.6 MPa) or to decrease (with −0.9 and −1.2 MPa) in pea shoots at all developmental stages, whereas the accumulation of proline in pea roots was found to be directly proportional to the salinity level used.

The present results showing marked changes in a wide array of compounds are discussed in relation to the action of salinity.

Zusammenfassung

ABO-HAMED S. A., YOUNIS M. E., EL-SHAHABY. O. A. & HAROUN S. A. 1990. Wachstum, Stoffwechsel und Adaptation von Pflanzen an Streßbedingungen. IX. Innerer Hormonspiegel, Mineralstoffe und gelöste organische Substanzen in *Pisum sativum* unter Salzstreß. – Phyton (Horn, Austria) 30 (1): 187–199, 3 Figuren. – Englisch mit deutscher Zusammenfassung.

Unter Salzeinwirkung änderte sich die hormonale Balance in wachsenden *Pisum sativum*-Pflanzen. −0,3 und −0,6 MPa Salz erhöhte in allen Stadien signifikant die Menge fördernden Wuchsstoffe, während ABA abnahm. Bei höherem Salzspiegel kehrten sich die Verhältnisse um. NaCl förderte in jedem Entwicklungsstadium die Akkumulation von Na^+- und Ca^{2+} bei gleichzeitiger Abnahme von K^+. Durch niedrige Salzgaben wurde der Mn^{2+}-Gehalt entweder im Sproß erhöht oder in den Wurzeln erniedrigt; bei höherer Salinität wurde das umgekehrte Verhalten beobachtet. Der P^{3+}-Gehalt stieg bei −0,6 bis −1,2 MPa durchwegs an, die Wurzeln verhielten sich umgekehrt. Abhängig vom Grad der Salzgaben und dem Entwicklungsstadium wurden deutliche Veränderungen im Gehalt von α-Keto-Zitronen- und Oxalsäure in Wurzeln und Sproß beobachtet. Prolin stieg in den Sprossen in −0,3 und −0,6 MPa Salz an und nimmt in −0,9 und −1,2 MPa ab, während er in den Wurzeln proportional mit der Salzgabe anstieg. Die reiche Palette von Veränderungen wird im Hinblick auf die Salzwirkung diskutiert.

1. Introduction

It is now well documented that water stress impairs numerous metabolic and physiological processes in plants (see GREENWAY & MUNNS 1980, HANSON & HITZ 1982 for review). Endogenous hormonal levels of many plants were reported to undergo various and rapid changes under water stress (ITAI & VAADIA 1971, DAVENPORT & al. 1980, ZEEVAART 1983).

Salinity as one of the major external factors which affect mineral metabolism has been given much attention. A typical response of many plants to saline environments, particularly halophytes, is to accumulate high intracellular concentrations of Na^+ and Cl^- (WYN-JONES 1981). Furthermore, profound changes in the total amount and in the relative composition of the ion pool in plant tissues were recorded by various authors (IMAMUL-HUQ & LARHER 1983, HASANEEN & al. 1987, 1989).

Saline conditions have been shown to affect the pattern of organic acid metabolism as well as reducing the total concentration (WIGNARAJAH & al. 1975, RAO & RAO 1978, HASANEEN & al. 1987). Of interest also, keto and amino acids; in particular proline, appear to play a protective role in saline habitats in addition to the metabolic functions (STROGONOV, 1970). These

protective functions consist in binding the excess ions absorbed by the plant and in maintaining the electrical neutrality of the cells and finally neutralizing the basic compounds (STROGONOV 1970, STEWART & LEE 1974).

The main objective of the present investigation was to confirm and correlate the changes in endogenous hormonal levels, ionic composition and in organic solutes, and to obtain useful information regarding the significance of these changes in salinized *Pisum sativum* plants at various stages of growth and development.

2. Materials and Methods

2.1 Plant material, culture and experimental design

Essentially as in EL-SHAHABY & al. 1990, *Pisum sativum* L. (var. Little Marvel) seeds were surface sterilized, thoroughly washed and then allowed to germinate in the dark at 25° C. Uniform 7-day-old seedlings were then cultured in ⅕-strength Pfeffer's nutrient solution for another period of 7 days after which the seedlings were divided into 3 groups that were treated with different concentrations of NaCl at the vegetative (stage A, 14-day-old), flowering (stage B, 26-day-old) and fruiting (stage C, 36-day-old) stages as in EL-SHAHABY & al. 1990. An additional group of seedlings was allowed to grow on nutrient medium alone to serve as control. The plants were allowed to grow indoor at laboratory conditions and at 4 days after each treatment triplicate samples were taken for analyses. The data obtained were statistically analysed using the least significant difference (L.S.D.) at the 1% and 5% probability levels.

2.2 Analytical methods

2.2.1 Estimation of growth regulating substances

The method adopted for extraction and separation of plant growth substances in shoots of control & treated plants was that described by SHINDY & SMITH 1975. For bioassay of auxins, the straight-growth test of *Hordeum* coleoptile sections as in YOUNIS & EL-TIGANI 1970 was used. For measurement of gibberellin-like substances, the lettuce hypocotyl bioassay adopted by FRANKLAND & WAREING 1960 was followed. The technique used to assay the activity of cytokinins was as in ESASHI & LEOPOLD 1969; cotyledons of *Xanthium brasilium* seeds were used as the test specimen. ABA was bioassayed by the straight-growth test of *Triticum* coleoptile segments as recommended by WRIGHT 1969.

2.2.2 Estimation of elements

Determination of Na, K, Ca and Mn. At the time of sampling, plant roots were rinsed in distilled water to remove culture solution, lightly blotted and then plants were separated into shoots and roots. A known dry weight was digested in concentrated HNO_3 and made up to volume with distilled water. K^+ and Na^+ concentrations were measured by flame emission spectrophotometry and Ca^{2+} and Mn^{2+} concentrations were measured by atomic absorption spectrophotometer (Unicam SP 90 A Series 2 Atomic Absorption Spectrophotometer).

Determination of phosphorus. The procedure adopted for extraction of phosphorus was essentially that described by BARKER & MAPSON 1964. For quantitative determination of phosphorus in the extracts the method of KUTTNER & LICHTENSTEIN 1932, was adopted in the present investigation.

2.2.3 Determination of acid components

The method adopted by FREIDMAN & HAUGEN 1943 was used to determine total α-keto acids (in terms of α-keto-glutaric acid) in this study. For estimation of oxalic and citric acids, the methods used were essentially those described by HASANEEN & al. 1987.

2.2.4 Determination of proline

The content of proline was determined by the method of BATES & al. 1973.

3. Results

3.1 Changes in growth regulating substances (Fig. 1)

Total auxins and gibberellins were significantly increased in response to −0.3 and −0.6 MPa salinity levels. On the other hand, −0.9 and −1.2 MPa resulted in a significant decrease in total auxins and gibberellins below the control levels at all developmental stages.

At −0.3 MPa, salinity resulted in a highly significant increase in cytokinin levels, at stages A, B and C, whereas at −0.6 MPa cytokinins appeared to increase non-significantly at stage A and significantly at stages B and C. On the other hand, −0.9 and −1.2 MPa NaCl induced the cytokinin levels to decrease significantly at stages A, B and C below the control levels.

The content of ABA showed a significant decrease with −0.3 MPa salinity, while with −0.9 and −1.2 MPa an opposite response was apparent throughout the experimental period. At − 0.6 MPa, salinity induced either a significant increase (at stage A) or a significant decrease (at stages B and C) in the ABA content in relation to control levels.

3.2 Changes in mineral content

3.2.1 In shoots (Fig. 2):

The different levels of salinity caused a significant increase in Na^+ and Ca^{2+} contents above the control values, except at −0.3 MPa where Ca^{2+} content was non-significantly increased at stages A and B. An opposite situation was, however, observed for K^+ content at all stages.

Manganous content was, in general, significantly increased with salinity at −0.3 and −0.6 MPa at stages A, B and C. On the other hand, −0.9 and −1.2 MPa salinity, in general, decreased the Mn^{2+} content significantly throughout plant development.

Phosphorus content was increased throughout the experimental period with all salinity levels. Progressively greater increases were observed with an increase in salinity concentration.

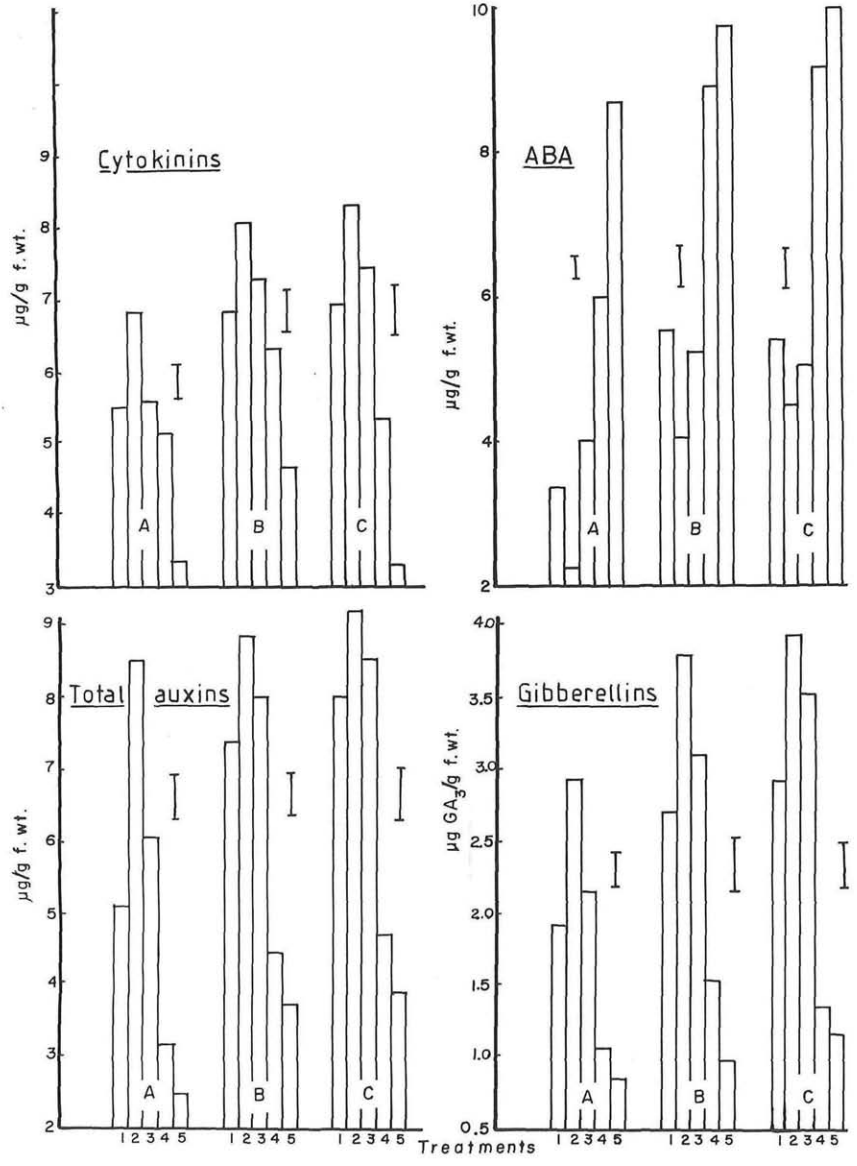


Fig. 1. Changes in growth regulators of *Pisum sativum* shoots stressed with increasing concentrations of NaCl at different stages of growth and development.
1 = Control (nutrient solution); 2 = nutrient solution + NaCl at −0.3 MPa; 3 = nutrient solution + NaCl at −0.6 MPa; 4 = nutrient solution + NaCl at −0.9 MPa; 5 = nutrient solution + NaCl at −1.2 MPa. The vertical bars represent the respective least significant differences (L. S. D.) at 1% level.

3.2.2 In roots (Fig. 2):

In contrast with the significant decreases in K^+ contents, Ca^{2+} and Na^+ contents in pea roots were significantly increased above the control levels in response to the different salinity treatments during all stages of plant development.

Mn^{2+} content was significantly decreased in response to −0.3 MPa salinity at stages A, B and C, whereas −0.6 MPa induced a non-significant effect. At −0.9 and −1.2 MPa, Mn^{2+} content was found to increase significantly above the control values.

The content of phosphorus appeared either to increase significantly (with −0.3 and −0.6 MPa) or to decrease significantly (with −0.9 and −1.2 MPa) below the control levels at all stages of plant development.

3.3 Changes in keto acids, organic acids and proline contents

3.3.1 In shoots (Fig. 3):

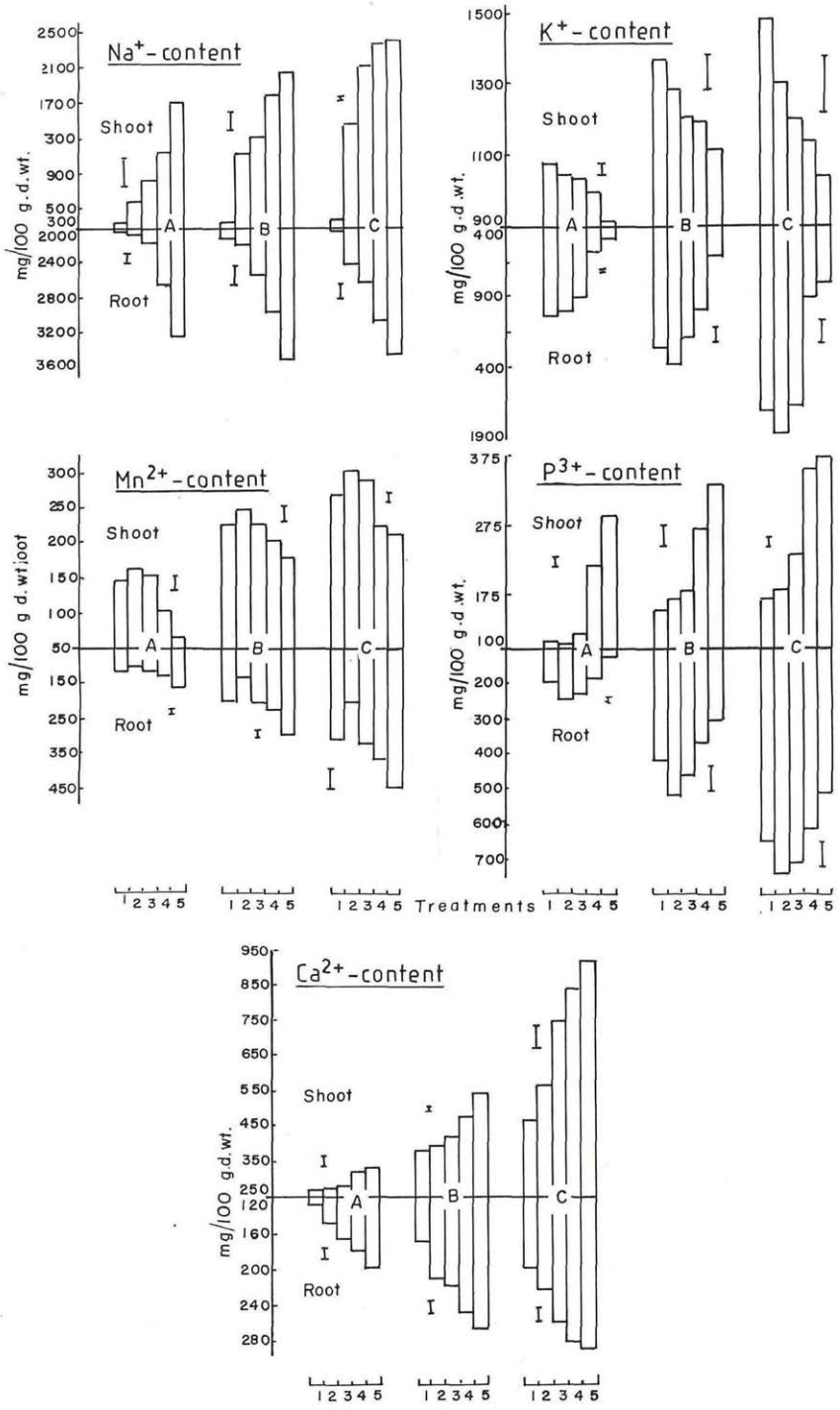
3.3.1.1 Keto acids: Treatment with different levels of salinity, at all stages of plant development, induced a highly significant decrease in keto acid contents below the control levels, except for salinity at −0.3 MPa when a non-significant decrease and a significant increase were observed in keto acid contents at stages B and C respectively and for salinity treatment at −0.6 MPa which non-significantly affected these metabolites at stage C.

3.3.1.2 Oxalic acid. As compared with controls, treatment with salinity at −0.3 MPa decreased the oxalic acid content non-significantly at stages A and B and significantly at stage C. Also treatment with −0.6 MPa salinity decreased the levels of oxalic acid significantly at stages A, B and C, whereas highly significant decreases in oxalic acid contents at stages A, B and C were elicited by the higher levels of salinity (−0.9 and −1.2 MPa).

3.3.1.3 Citric acid. Throughout the experimental stages, the lowest concentration of salinity (−0.3 MPa) increased citric acid significantly, whereas with −0.6 MPa salinity a non-significant increase (at stage A) and significant increases (at stages B and C) were observed. In contrast, the higher salinity levels (−0.9 and −1.2 MPa) significantly decreased citric acid contents.

3.3.1.4 Proline. Proline content in shoots was either significantly increased (with −0.3 and −0.6 MPa) or significantly decreased (with −0.9 and −1.2 MPa) below the control levels at all stages of plant growth and development.

Fig. 2. Changes in mineral content in shoots and roots of *Pisum sativum* plants stressed with increasing concentrations of NaCl at different stages of growth and development. For description of treatments 1 to 5 see Fig. 1.



3.3.2 In roots (Fig. 3):

3.3.2.1 Keto acids. Throughout plant development, the low concentrations of salinity (−0.3 and −0.6 MPa) increased the keto acids content significantly. In contrast, the high levels of salinity used (−0.9 and −1.2

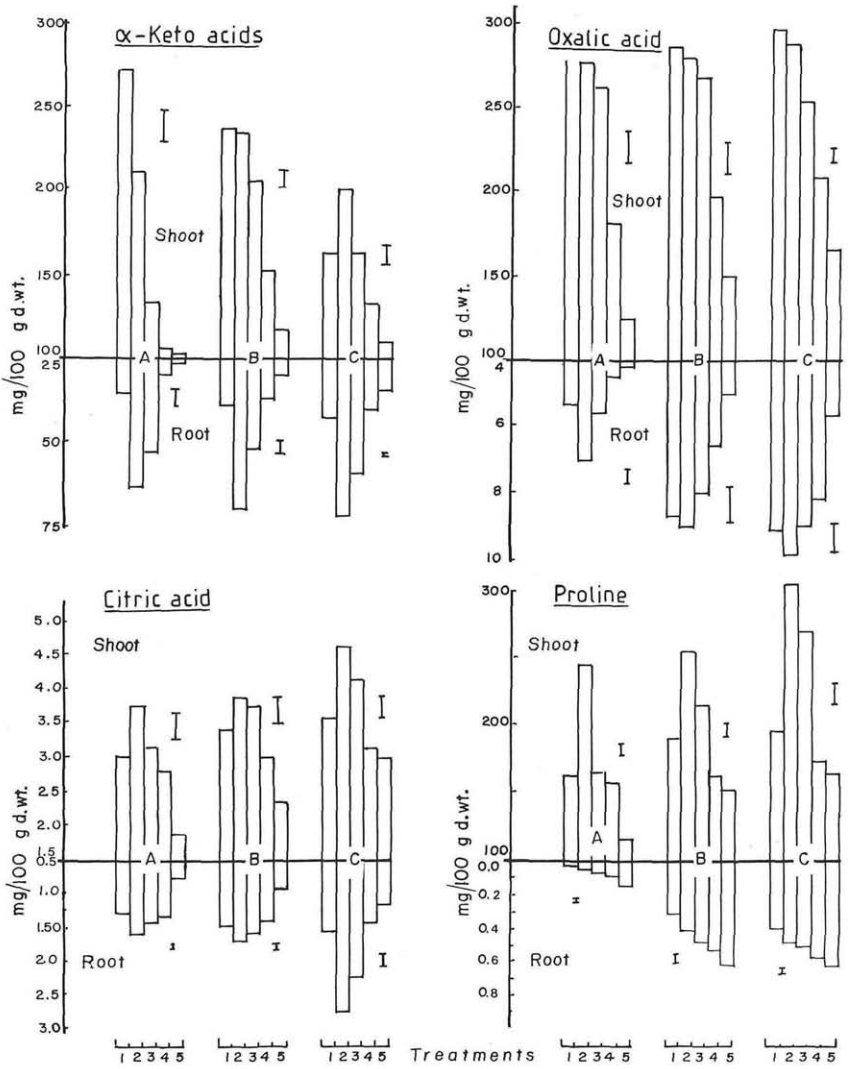


Fig. 3. Changes in contents of α-keto acids, citric, oxalic and proline in shoots and roots of *Pisum sativum* plants stressed with increasing concentrations of NaCl at different stages of growth and development. For description of treatments 1 to 5 see Fig. 1.

MPa), in general, appeared to decrease these metabolites significantly in relation to control levels.

3.3.2.2 Oxalic acid. In relation to control levels, salinity at −0.3 MPa increased oxalic acid contents significantly at stages A and C and non-significantly at stage B, whereas with salinity at −0.6 MPa a significant increase, a significant decrease and a non-significant decrease in oxalic acid contents were induced at stages A, B and C respectively. On the other hand, highly significant decreases were observed in response to the highest concentrations of salinity (−0.9 and −1.2 MPa) throughout the experimental period.

3.3.2.3 Citric acid: As compared with controls salinity at −0.3 and −0.6 MPa, in general, increased the citric acid contents significantly at stages A, B and C, whereas a significant increase (at stage A) and a decline (at stages B and C) were elicited in response to salinity at −0.9 MPa. Moreover, a highly significant decrease was induced by the highest level of salinity (−1.2 MPa) at all stages of plant development.

3.3.2.4 Proline. All salinity treatments included in this study appeared to increase the proline contents significantly in pea roots throughout the experimental period; progressively greater increases being observed with an increase in NaCl concentration.

4. Discussion

In the present study, the observed decreases in auxins, gibberellins and cytokinins that occurred concurrently with an increase in ABA, at all stages of plant development, in response to the higher levels of salinity used, are in accord with the results reported by other workers (ITAI & VAADIA 1971, AHARONI & RICHMOND 1978, DAVENPORT & al. 1980, ZEEVAART 1983).

Endogenous growth regulators have been shown to be critical determinators of growth and differentiation (STREET & COCKBURN 1970) and YOUNIS & EL-TIGANI 1970 stated that growth may depend on the ratios rather than on the absolute levels of these growth substances in the plant. Thus, the promotion of growth of pea plants observed by EL-SHAHABY & al. 1990 under low salinity levels seems to be correlated with increased levels of auxins, gibberellins and cytokinins that were associated with decreased ABA content (Fig. 1). Furthermore, the inhibition of growth of plants treated with high salinity levels appears to coincide with the decline in growth promotery substances and accumulation of ABA. In support of these correlations, YOUNIS & al. 1990 and unpubl. observed that seed presoaking in GA_3 or IAA was in most cases capable of partially or completely counteracting the varied harmful effects maintained in pea plants by the different levels of salinity used. This indicates some tolerance through the inductive changes in the endogenous levels of growth regulators (YOUNIS & al. 1989b).

It is evident that salinity induced an accumulation of Na^+ and Ca^{2+} in both shoots and roots at all stages of plant development and this was associated with decreases in K^+ content. In connection with these results, several workers have demonstrated an increase in Na^+ levels and a decline in that of K^+ with a concomitant decline in K^+/Na^+ ratio in plants treated with NaCl (IMAMUL-HUQ & LARHER 1983, HASANEEN & al. 1987, 1989). Thus the well known effect of Na^+_{ext} on K^+_{int} content of plant tissues was further substantiated by our results. Furthermore, the increase in calcium content with rise in salinity is in agreement with the findings of ASHRAF & al. 1986 and EPSTEIN 1961 stated that the maintainance of appreciable levels of Ca_{int} clearly indicates some kind of salt resistance of the plant.

The low levels of salinity (−0.3 and −0.6 MPa) which increased Mn^{2+} content in shoots caused a decline of that content in roots. With −0.9 and −1.2 MPa, Mn^{2+} content was either significantly increased in roots or significantly decreased in shoots. These differential changes in Mn^{2+} content under salinity stress are in conformity with the results of MEHROTRA 1971 and HEIKAL & al. 1980. A tentative explanation to the present changes could be based on ion compartmentation which is considered as a component of salt adaptation of glycophyte cells (BINZEL & al. 1988).

Furthermore, phosphorus uptake and translocation from root to shoot was increased rather than inhibited by high salinity levels. In this respect, WILSON & al. 1970 and EL-SHAHABY 1981 reported that, whatever may be the salinizing agent and the duration of salinization, the increase in phosphorus content in the plant seems to be a dominant effect of salinity.

The changes herein reported for the contents of organic and α-keto acids under salinity appeared to be dependent mainly on the salinity level, the stage of the treated plants and/or the plant organ. In general, there appeared to be decreases in these acids in shoots and roots with the relatively higher concentrations of salinity and an opposite situation was apparent with the relatively lower levels. The decreased contents of these metabolites in shoots and roots in response to certain salinity levels may be due to the stimulatory effect of salinity on the respiratory oxidation of these acids. This is to be expected in part for supply of energy for the observed ion uptake and accumulation and in part for maintaining metabolic functions.

Of interest in this connection, DIVATE & PANDY 1981 and YOUNIS & al. 1987 stated that salinity stimulated the rate of respiration of grape leaves and germinating seeds and the greatest stimulation was observed with the highest levels of salinity. Also WIGNARAJAH & al. 1975 and HASANEEN & al. 1987 showed a greater reduction of organic acids especially malic, oxalic and citric acids in salinized primary trifoliate leaves of *Phaseolus vulgaris* and in germinating broad bean seeds. Inconsistent results were, however, obtained for succinic acid.

The close parallelism between the decrease in α-keto acids and the increase in amino-N content (EL-SHAHABY & al. 1990) in pea plants in

response to certain salinity levels points to a stimulatory effect of these levels on the transamination reactions. In this connection, RAO & RAO 1978 recorded decreases of α-keto acids concomitantly with increases in glutamic acid, alanine and proline.

The present changes in proline content in addition to the observations of YOUNIS & al. (unpubl.) who found that presoaking of pea seeds in either GA_3 or IAA appeared either to augment the observed increase in proline in roots and shoots or to nullify the observed decreases in shoots at −0.9 and −1.2 MPa, are of interest. The connection between these observations and the recovery in the growth of pea plants under these hormonal treatments (YOUNIS & al. 1990), led us to suggest that proline accumulation can be used as an indicator in selection for withstanding saline stress through the involvement in osmoregulation.

In conclusion, NaCl at −0.3 MPa appeared to be favourable for the growth of *Pisum sativum* plants. Higher concentrations (−0.6 and −0.9 MPa) can be tolerated, whereas −1.2 MPa appeared harmful to the plants.

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Recensio

Progress in Botany 50, 1989. Fortschritte der Botanik 50, Struktur, Physiologie, Genetik, Systematik, Geobotanik, hgg. von BEHNKE D., ESSER K., KUBITZKI K., RUNGE M. & ZIEGLER H. – Gr.-8°, XXI + 386 S. mit 31 Abbildungen, hart gebunden. – Springer Verlag Berlin, Heidelberg, New York, London, Paris, Tokyo. – DM 258,–. – ISBN 3-540-50289-0.

Seit dem Jahre 1931 sind die ‚Fortschritte' den wissenschaftlich arbeitenden Botaniker sensu latissimo ein treuer, jährlich erscheinender Führer durch die ständig