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# Effects of Constant and Gradual Exposure to Sodium Chloride Stress on DNA, RNA, Protein and Certain Protein-Amino Acids in two varieties of Barley

By

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Summary

The changes in nucleic acids, protein, and certain protein-amino acids of roots were studied in two varieties of barley (*Hordeum vulgare* L.), Giza-118 and Sahrawy, under the influence of constant and gradual exposure to NaCl stress in solution culture. Under the lower constant NaCl levels Sahrawy survived more, relative to Giza, but both failed to survive under the higher levels. Under gradual exposure to NaCl increments up to 0.2 M both varieties were able to survive and to reach the flowering and the fruiting stages.

Under exposure to constant NaCl levels RNA content decreased in Giza but remained unchanged and DNA increased in Sahrawy. With gradual exposure DNA decreased and RNA increased with 4 NaCl increments, decreased in Sahrawy but remained unchanged in Giza with 1 and 2 increments. The protein/DNA and RNA/DNA ratios in shoots increased in Sahrawy with 3 and 4 gradual NaCl increments and in Giza with 4 increments, in contrast to the corresponding constant NaCl levels. In both varieties the root-protein content increased and the level of each protein-amino acid increased under gradual, in contrast to constant exposure of NaCl, and the capacity was greater for Giza.

Zusammenfassung

Wirkungen konstantgehaltener und schrittweise ansteigender NaCl-Belastung auf DNA, RNA, Proteine und einige proteinogene Aminosäuren bei zwei Hafer-Varietäten

An zwei Varietäten von Hafer (*Hordeum vulgare* L.), Giza-118 und Sahrawy, wurden die Veränderungen des Gehaltes an Nukleinsäuren, Protein und an einigen proteinogenen Aminosäuren unter konstant gehaltener bzw. schrittweise ansteigender NaCl-Belastung untersucht. Konstant gehaltene niedere

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NaCl-Konzentrationen wurden von der Sorte Sahrawy etwas besser ertragen als von Giza, in höheren (über 0,1 M NaCl) gingen jedoch beide Sorten zugrunde. Bei schrittweiser Erhöhung der Salzkonzentration vermochten beide Sorten bis 0,2 M zu überleben und das Blüh- und Fruchtstadium zu erreichen.

Unter konstant gehaltener NaCl-Belastung nahm der RNA-Gehalt in Giza-Hafer ab, in Sahrawy blieb er unter gleichzeitiger Zunahme des DNA-Gehaltes gleich. Bei schrittweiser Erhöhung der Salzkonzentration über 4 Konzentrationsstufen (zu je 0,05 M NaCl) nahm der DNA-Gehalt ab und der RNA-Gehalt zu; bei Erhöhung um 1—2 Stufen nahm er in Sahrawy ab, in Giza blieb er unverändert, nicht jedoch bei konstant gehaltenen Salzkonzentrationen. Im Gegensatz zu konstanter NaCl-Belastung stieg der Gehalt an Protein in der Wurzel und der Gehalt an proteinogenen Aminosäuren bei schrittweiser Erhöhung der Salzgabe in beiden Varietäten an, wobei sich Giza als leistungsfähiger erwies.

(Vom Editor übersetzt und ergänzt; Ergänzungen in Klammern.)

## Introduction

Chloride salinity suppressed cell enlargement and cell division proportionally in leaves and that DNA and RNA levels decreased per leaf (Nieman 1965). It was suggested that the sites most inhibited under chloride salinity would be protein and nucleic acid synthesis (Morozovskii & Kabanov 1968). Uptake and incorporation of amino acids into proteins was shown to be reduced in roots affected by either NaCl or $Na_2SO_4$, and that different proteins were synthesized in roots grown in both types of salinity (Kahane & Poljakoff-Mayber 1968). A more rapid protein decay was detected in intact leaves from plants subjected to 4 bars of added NaCl than intact leaves from control (Prisco & O'Leary 1972) and that the capacity for protein synthesis and amino acid incorporation was reduced in salt-stressed tissue (Aliza *et al.* 1967).

It is believed that sudden development of salt-stress causes more injury than gradual development (Lunin *et al.* 1961, Meiri & Poljakoff-Mayber 1970, Meiri *et al.* 1970, and El-Shourbagy & Ahmed 1975). Subjection to salt-stress merited further knowledge of metabolic responses of plants.

The present study is an additional contribution to the understanding of the effect of constant and gradual exposure to NaCl on the metabolism of the plant. Two varieties of barley (*Hordeum vulgare* L.); Giza-118 and Sahrawy with different sensitivities to salinity were compared with respect to the content of DNA, RNA, protein, and certain protein-amino acids of roots under constant and gradual exposure ot NaCl in the solution culture.

## Materials and Methods

Seeds of two varieties of barley (*Hordeum vulgare* L.); Giza-118 and Sahrawy were germinated in pure sand and irrigated with distilled water until emergence, then with a base nutrient solution similar to one-half

concentration of that used by HOAGLAND & ARNON (1950) and composed of: $5\times10^{-4}$M $KH_2PO_4$, $2.5\times10^{-3}$M $KNO_2$, $2.5\times10^{-3}$M $Ca(NO_3)_2.4H_2O$, $10^{-3}$M $MgSO_4.7H_2O$, $4.6\times10^{-5}$M $H_2BO_4$, $9\times10^{-6}$M $MnCl_2.4H_2O$, $7\times10^{-7}$M $ZnSO_4.7H_2O$, $3.2\times10^{-7}$M $CuSO_4.5H_2O$, $1.6\times10^{-7}$M $(NH_4)_6Mo_7O_{24}.4H_2O$, and $1.6\times10^{-4}$M iron citrate. Two week-old seedlings of homogeneous growth dimensions were transferred to solution cultures containing the above nutrients. In each cylindrical glazed pot of 20 cm height and 4-liter capacity, plants were fastened in place with bored cork stoppers inserted in 4 holes in a wooden board. The board, which served as a cover for each pot allowed to hold 10 plants in each hole with roots completely submerged in the solution. The solution was aerated regularly by means of an automatic air-compressor through a penetrating capillary tube. In one series (I), plants were exposed constantly to different NaCl concentrations in addition to the control which contained the base nutrient solution for a period of 10 weeks. The NaCl. concentrations used were 0.05, 0.1, 0.15, and 0.2 M, added to the required volume of the base nutrient solution. Every two weeks each solution was replaced by a fresh one having the same NaCl concentration. In a second series (II), plants were exposed gradually to NaCl increments (0.05 M) in the solution culture every two weeks for a 12-week culture period. Two weeks from the transfer of seedlings to the base nutrient solution, plants were transferred to another culture solution containing 0.05 M NaCl, whereas a lot of plants (A) remained in the base nutrient solution. After another 2 weeks, plants which had been grown in the culture solution containing 0.05 M NaCl were transferred to another solution containing 0.1 M NaCl, while a lot of plants (B) remained in the 0.05 M NaCl-solution. The same steps were followed until the 0.2 M NaCl-culture solution was reached. This was attained 10 weeks from the begining of culture experiment. At each step of transfer, each solution was replaced by a fresh one having the same concentration. At the end of the experiment 5 groups were designated in the series, group (A) the control, while groups (B), (C), (D), and (E) had been exposed periodically to NaCl increments during the growth period, each representing 20% of pots used in the series. The two experimental series (I) and (II) were conducted during the winter season under the glasshouse conditions, and the pH of the culture solution was maintained at 6.5.

Every 2 weeks throughout the culture period, plants of each treatment in each series were removed from culture, separated into shoots and roots and weighed immediately. Fresh samples were used for DNA, RNA and protein estimation, while a portion was used for the determination of shoot and root moisture content and for the estimation of certain protein-amino acids by drying in an aerated-oven at 70° C until constant weight.

DNA and RNA Extraction and Estimation:

12 g of the fresh detached shoots were homogenated with 60 ml ethanol-

NaCl mixture (100 ml 95% ethanol + 25 ml 10% NaCl solution) in an electric blender for 5 min. Another 60 ml ethanol-NaCl mixture was added to the homogenate, then centrifuged at 27 000 g for 30 min at 0° C, by means of a Sorvall Super-Speed Refrigerated Centrifuge Model-RC2. The subsequent steps of salt extraction were followed according to GUINN (1966) until RNA was separated from DNA. Several dilutions for both RNA and DNA were tested for the suitable readings within the range of the UV-spectrophotometer used (Unikam Model SP 500), read at 260 nm against 0.2 N perchloric acid (PCA). The wave length was selected after constructing the spectral curve for each of RNA and DNA, by reading diluted solutions of their standards (Nutritional Biochemicals Corp, USA). RNA and DNA concentrations were calculated through determination of optical densities at 260 nm according to OGUR & ROSEN (1950). Values were expressed on the crude dry weight bases.

Protein Extraction and Estimation:

5 g of the fresh sample was homogenated with 25 ml absolute ethanol in an electric blender for 5 min. The homogenate was then transferred to a polypropylene 50 ml tube, then centrifuged at 27 000 g for 15 min at 0° C by means of the Sorvall centrifuge. After centrifugation, the supernatant was discarded and the residue was treated with 80% acetone, then centrifuged again. The subsequent steps of extraction were followed according to LOWRY *et al.* (1961) until the protein precipitate was obtained, dissolved in 10 ml 1NNaOH, then heated at 100° C for 10 min. Protein was estimated colorimetrically using egg albumen as standard and values were expressed on the crude dry weight bases.

Protein-Amino Acid Determination:

1 g dry root sample was transferred to a soxhlet apparatus for lipid and pigment extraction, by using an equivalent mixture of petroleum ether (b. p. 40—60° C) and diethyl ether for 24 hrs. The remaining residue was dissolved in 50 ml 80% ethanol in a 250 ml flask, fitted with a reflux condenser and heated on a water-bath for 2 hrs, then filtered. The remaining residue was then hydrolyzed for 4 hrs with 30 ml 1N HCl in a flask fitted with a reflux condenser on a hot plate, then filtered. The residue was subjected to another hydrolysis with 6N HCl for 24 hrs. Excess HCl was removed under vacuum until a residue was left, which was dissolved in 10 ml 10% isopropanol for protein-amino acid analysis. Amino acids were separated by the ascending two-dimensional method of paper chromatography using Whatman No. 4 paper. The first solvent system used was n-butanol: acetic acid: water 12 : 3 : 5, and the second was phenol: water : ammonia 160 : 40 : 1 (SMITH 1962).

Chromatographic separation was carried out in a stainless steel chamber of 12-sheet capacity with 3 applications conducted for each sample under

the same conditions. The time required to attain complete separation (about 30 cm beyond the point of application) was about 18 hrs for the first and 20 hrs for the second solvent at room temperature. The amino acid spots were made visible by treating the chromatograms with ninhydrin reagent (SMITH 1962), dried at room temperature, then in an oven at 105° C for 3 min. The amino acids of the extract were identified by comparison with standards in an artificial amino acid mixture developed under the same conditions. Analysis of root protein-amino acids showed that in most cases 16 different amino acids could be identified visually. Seven of them overlapped one with another in 3 distinct groups; leucine-isoleucine-phenylalanine, glycine-serine and valine-methionine. These 3 groups were always present in each variety and in each treatment throughout the study. Each of the other 9 amino acid spots; aspartic acid, glutamic acid, arginine, lysine, cystine, threonine, alanine, tyrosine and proline was separated individually. The spot-area of each was estimated and its concentration was calculated according to equation devised by ÅKERFELDT (1954) relating the spot area with concentration: $^{10}\log 100\ C/M = A\ F_p$, where C = spot concentration of amino acid in μg, M = molecular weight of amino acid, A = spot area in $cm^2$, and $F_p$ = molecular spot-area constant. The $F_p$ value for each amino acid was practically constant within the range 10 to 50 μg of spot concentration used.

Values obtained from the control and each of the two lower NaCl treatments from the experimental series I and II represent means of more than three samples and were statistically analyzed using the KRUSKAL-WALLIS test (CONOVER 1971, DANIEL 1974).

## Results

### Growth and Survival:

In series I the growth rate of each variety decreased with NaCl concentration. Two weeks from salt treatment a few plants of Giza tolerated the 0.2 M NaCl level, while none of Sahrawy was able to survive for more than one week. Two weeks later, the 0.2 M NaCl treatment was lethal for the remaining Giza plants. After 6 weeks of salt treatment both varieties failed to survive the 0.15 M NaCl level and Sahrawy was unable to tolerate even the 0.1 M NaCl. By the end of the experimental period Giza was unable to survive any NaCl level, while Sahrawy succeeded to survive the 0.05 M NaCl and were able to form spikes with dry seeds.

In series II the growth rate of each variety decreased with the application of the gradual NaCl increments. However, all plants from each variety were able to survive and to form spikes with all NaCl increments until the termination of the experimental period. In Sahrawy initiation of flower buds started 4 weeks earlier than Giza variety. In both varieties, plants which had reached the 0.2 M NaCl level were the last to flower.

DNA Content of Shoots:

Although DNA in Giza was almost constant with constant exposure to different NaCl levels (series I), except with 0.15 M, it showed a gradual increase in Sahrawy, indicating a NaCl-activated cell division over enlargement (Table 1). In contrast, when each variety was exposed gradually (series II) to one (B), and 2 (C) NaCl increments DNA content was similar to that of the control, but declined with 3 (D) and 4 (E) NaCl increments, indicating an enlargement over division.

Table 1

Concentration of DNA, RNA, and protein (mg/g dry weight) in the shoots of two varieties of barley subjected to constant and gradual NaCl stress

| | Constant subjection | | | | | Gradual subjection | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0.15 | 0.2 | 0 | 1 | 2 | 3 | 4 |
| | M NaCl | | | | | 0.05 M NaCl increments | | | | |
| | | | | | Giza | | | | | |
| DNA | 2.0 | 2.1 | 2.0 | 1.3 | 2.2 | 1.8 | 1.7 | 1.9 | 1.5 | 1.3 |
| RNA | 8.8 | 8.1 | 7.9 | 6.0 | 5.2 | 7.0 | 6.5 | 6.6 | 4.4 | 9.2 |
| Protein | 54 | 57 | 44 | 43 | 43 | 42 | 45 | 38 | 44 | 42 |
| RNA/DNA | 4.4 | 3.9 | 4.0 | 4.6 | 2.4 | 3.9 | 3.8 | 3.5 | 2.9 | 7.1 |
| Protein/DNA | 27.0 | 27.1 | 22.0 | 33.1 | 19.6 | 23.3 | 26.5 | 20.0 | 29.3 | 32.3 |
| | | | | | Sahrawy | | | | | |
| DNA | 1.4 | 2.0 | 2.7[1] | 2.8 | | 1.4 | 1.2 | 1.3 | 0.9 | 0.8 |
| RNA | 5.7 | 5.7 | 7.4[1] | 5.6 | | 4.8 | 3.5[2] | 2.9[2] | 3.6 | 5.2 |
| Protein | 50 | 37 | 46 | 33 | | 38 | 37 | 29 | 45 | 42 |
| RNA/DNA | 4.1 | 2.9 | 2.7 | 2.0 | | 3.4 | 2.9 | 2.2 | 4.0 | 6.5 |
| Protein/DNA | 35.7 | 18.5 | 17.0 | 11.8 | | 27.1 | 30.8 | 22.3 | 50.0 | 52.5 |

[1]) Significant difference between control and NaCl treatment at $P < 0.1$.
[2]) Significant difference between control and NaCl treatment at $P < 0.05$.

RNA Content of Shoots:

With all constant or gradual NaCl exposures Giza contained higher RNA than Sahrawy. With constant exposure to NaCl RNA content decreased in Giza from the control until the 0.2 M NaCl level, but in Sahrawy it was higher in the 0.1 M and did not differ in the 0.05 and 0.15 M NaCl treatment from the control. With gradual exposure to NaCl increments Giza contained higher RNA than Sahrawy with different treatments. Still in Giza a remarkably higher level was detected with 4 gradual NaCl increments, which exceeded that of the control and was even more than twice that of the 3 NaCl increments. In Sahrawy RNA content decreased from the control with exposure to one or 2 NaCl increments but increased again with 4 increments.

Protein Content of Shoots:

Protein content was generally higher in Giza than Sahrawy. Constant exposure to NaCl resulted in a decrease in the protein content of Giza with 0.1 M, which remained almost unchanged until 0.2 M, but in Sahrawy the protein level changed with NaCl exposures. In contrast, despite the drop that took place with 2 NaCl increments, gradual exposure to 3 and 4 NaCl increments resulted in a maintenance of higher protein content in both varieties.

RNA/DNA Ratio:

With constant exposure to different NaCl levels up to 0.15 M, the ratio decreased progressively in Sahrawy, but did not change appreciably in Giza, which had experienced a drop with 0.2 M. On the other hand, gradual exposure to one and 2 NaCl increments decreased the ratio in Sahrawy, but that of Giza decreased with 3 increments. Both varieties showed a remarkable increase with exposure to 4 increments.

Protein/DNA Ratio:

Sahrawy showed relatively higher ratios with gradual than constant exposure to different NaCl levels, but did not differ in Giza appreciably, except at the highest NaCl level. With constant exposure to NaCl up to 0.15 M, the ratio decreased progressively in Sahrawy. In both varieties, the ratio increased with gradual exposure to 3 or 4 NaCl increments, and the increase was greater in Sahrawy.

Protein Content of Roots:

In both varieties of barley the protein content of roots showed a progressive decrease with constant exposure to increased NaCl levels up to 0.15 M (Table 2). In contrast, gradual exposure to NaCl resulted in a progressive increase in the root-protein of Giza until 4 NaCl increments, and was higher in Sahrawy with one, 2, and 4 increments than the control. By the termination of exposure to gradual increments both varieties were similar in protein content at the highest NaCl level.

Concentration of Nine Protein-Amino Acids in Roots:

In most cases, the concentration of each amino acid was appreciably higher with gradual than with constant exposure to NaCl. Under constant exposure to different NaCl levels Sahrawy possessed higher arginine, lysine, tyrosine and proline concentrations than Giza. In contrast, with gradual exposure to NaCl Giza attained relatively higher lysine, cystine, alanine, threonine and proline levels. In Sahrawy, glutamic acid content decreased in plants exposed constantly to 0.05 and 0.15 M NaCl, while maintained almost the same level with gradual exposure to different NaCl increments.

Table 2

Concentration of protein and nine protein-amino acids (mg/g dry weight) in the roots of two varieties of barley subjected to constant and gradual NaCl stress

| | Constant subjection | | | | | Gradual subjection | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.05 | 0.1 | 0.15 | 2.0 | 0 | 1 | 2 | 3 | 4 |
| | M NaCl | | | | | 0.05 M NaCl increments | | | | |
| | | | | | Giza | | | | | |
| Protein | 75 | 60 | 46[1] | 29 | 37 | 32 | 33 | 43 | 49 | 51 |
| Asp. acid | 0.7 | 0.6 | 0.8 | 0.2 | 0.3 | 0.6 | 0.9 | 1.2 | 1.3 | 0.8 |
| Glu. acid | 1.0 | 1.5 | 1.5 | 1.2 | 0.8 | 1.9 | 1.9 | 2.2 | 1.4 | 1.9 |
| Arginine | 0.6 | 0.7 | 0.5 | 0.4 | 0.6 | 1.0 | 0.7 | 1.1 | 1.0 | 1.0 |
| Lysine | 1.7 | 1.6 | 1.1 | 0.6 | 1.2 | 2.0 | 3.3 | 3.2 | 3.0 | 1.9 |
| Cystine | 1.0 | 0[1] | 0.8 | 0 | 0 | 0.9 | 1.7[1] | 2.6 | 2.0 | 2.0 |
| Threonine | 0.3 | 0.1 | 0.4 | 0 | 0 | 0.9 | 1.0 | 1.0 | 0.7 | 1.2 |
| Alanine | 0.6 | 0.6 | 1.1 | 0.4 | 1.3 | 1.5 | 2.2 | 2.3 | 1.2 | 2.8 |
| Tyrosine | 0.4 | 0.2 | 0.4 | 0 | 0.4 | 0.6 | 0.5 | 1.3 | 0.4 | 0 |
| Proline | 0.7 | 1.7 | 1.0 | 0.4 | 0 | 4.7 | 14.2 | 12.5 | 5.5 | 0 |
| Amino acids/ protein | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.4 | 0.8 | 0.6 | 0.3 | 0.2 |
| | | | | | Sahrawy | | | | | |
| Protein | 52 | 62 | 41 | 40 | | 24 | 39[2] | 34 | 25 | 55 |
| Asp. acid | 0.6 | 0.8 | 0.6 | 0.4 | | 0.6 | 1.0 | 0.9 | 0.8 | 0.9 |
| Glu. acid | 2.0 | 0.9[2] | 1.8 | 0.8 | | 1.6 | 1.9 | 1.8 | 1.8 | 2.2 |
| Arginine | 1.0 | 1.0 | 1.0 | 0.7 | | 0.8 | 0.9 | 1.2 | 0.3 | 0.9 |
| Lysine | 2.3 | 2.1 | 1.8 | 0.6 | | 1.1 | 1.8 | 2.5 | 1.1 | 1.4 |
| Cystine | 0.3 | 0.4 | 0.5 | 0.6 | | 1.0 | 3.0 | 1.8 | 0.4 | 1.8 |
| Threonine | 0.4 | 0.3 | 0.3 | 0.3 | | 0.2 | 0.5 | 0.8 | 0.5 | 0.9 |
| Alanine | 2.3 | 1.7 | 1.1 | 0.5 | | 1.4 | 1.8 | 1.2 | 0.8 | 1.7 |
| Tyrosine | 0.7 | 1.1 | 0.7 | 0.3 | | 0.4 | 0.4 | 0.7 | 0.5 | 0 |
| Proline | 0.9 | 1.4 | 3.3 | 1.3 | | 1.3 | 5.3 | 6.3[2] | 3.3 | 22.5 |
| Amino acids/ protein | 0.2 | 0.2 | 0.3 | 0.1 | | 0.4 | 0.4 | 0.5 | 0.4 | 0.6 |

[1]) Significant difference between control and NaCl treatment at $P < 0.1$.
[2]) Significant difference between control and NaCl treatment at $P < 0.05$.

### Nine Protein-Amino Acids/Protein Ratio:

Sahrawy possessed higher amino acid/protein ratio than Giza with constant exposure to different NaCl levels. In both varieties, however, gradual exposure to different NaCl increments resulted in an appreciable increase in the ratio. With one and 2 increments the ratio was higher in Giza than Sahrawy, but the reverse was true with 4 increments.

## Discussion

Results obtained with the two investigated varieties of barley showed differences in tolerance to NaCl. Under constant exposure Sahrawy could survive at least the lower salt level, while Giza was respresented only by the control with the termination of the experimental period. The fact that plants of both varieties were able to survive when exposed gradually to NaCl increments up to 0.2 M suggests that gradual rather than abrupt changes in the cell osmotic pressure were not inhibitory for synthetic processes essential for growth (El-Shourbagy & Ahmed 1975). Tal (1977) found that although nucleic acids and protein are influenced by water stress the polyploid tomato plants resembled the diploids in their metabolic adaption to salt stress induced by high salinity. In the present study, it is attempted to find out if the level of cell constituents represented by DNA, RNA, and protein in barley are less affected by gradual than by constant exposure to different NaCl levels, and to what extent such effects differ between Sahrawy and Giza varieties.

Under constant exposure to increased NaCl levels RNA content decreased in Giza, but remained unchanged and DNA content increased in Sahrawy, relative to control. On the other hand, gradual exposure to different NaCl increments maintained a lower and less variable DNA content in both varieties. With 1 or 2 NaCl gradual increments RNA content decreased significantly in Sahrawy, but remained unchanged in Giza and increased in both varieties with 4 increments. The maintenance of lower, but balanced, contents of DNA, besides increased capacity for RNA synthesis with 4 NaCl increments must be related to survival of both varieties. The decreased content of DNA per unit tissue dry weight of barley shoots under gradual, relative to constant exposure to salt stress, where the decrease being greater in Sahrawy, may be attributed to lower number of cells per unit weight. Gradual exposure to 3 and 4 NaCl increments compared to 0.15 and 0.2 M under constant exposure resulted in a maintenance of a more stable shoot-protein content in both varieties and increased protein/DNA and RNA/DNA ratios in Sahrawy. Both ratios increased in Giza only with 4 gradual increments.

In both varieties the level of each of the 9 investigated protein-bound amino acids increased in roots with gradual relative to constant exposure to salt stress. In Giza root-protein decreased with constant, but increased with gradual exposure to NaCl stress, together with increased formation of the protein-bound lysine, cystine, threonine, alanine and proline. In Sahrawy protein content increased significantly with one gradual increment and that of proline with 2 increments.

Results indicate that the ratio of 9 amino acids/protein increased in both varieties with gradual exposure and the increase was greater in Giza. Under constant exposure to NaCl Sahrawy roots were rich in arginine,

lysine, tyrosine, and proline, but with gradual exposure almost all salt treatments of each variety were superior than the control. This could be attributed to stimulation of synthesis of new type protein that was not being made with constant exposure to salt stress, through activation of the ribosome-messenger system or by the formation of functional polysomes (Morcus *et al.* 1966). Possible differences in the formation of adaptive protein type in both varieties could exist with gradual exposure to NaCl. Although both showed increased formation of the 9 protein-bound amino acids, the capacity was greater in Giza for lysine and alanine. The synthesis of protein types rich in certain amino acids could be the key to survival of each variety under gradual exposure to NaCl stress.

The fact that RNA/DNA and protein/DNA ratios in shoots increased in Sahrawy with gradual exposure to 3 and 4 NaCl increments and in Giza with 4 increments, besides the increased content of root-protein rich in certain amino acids suggests that lethal effects due to losses of synthetic processes were diminished with gradual exposure to salt stress. Shah & Loomis (1965) proposed that changes in growth caused by moisture stress may be related to changes in RNA and protein metabolism and Todd & Basler (1965) observed that lethal effects in leaves of some varieties of wheat due to moisture stress were due to breakdown of various subcellular components and losses in synthetic machinery. Similar evidence has been reported by Levitt (1972), including nucleic acids, protein, hormones, protein synthesis and RNase activity.

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