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Effect of Amino Acids on Growth and Protein Content of Salt-Stressed Barley Seedlings

By

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Summary

Seeds of two cultivars of barley (*Hordeum vulgare* L.), Giza-118 and Sahrawy, of different sensitivities to salinity were germinated at different temperature and NaCl levels. The most suitable temperature was 25° C, and the decrease in germination percentage with salinity was smaller in Sahrawy than in Giza. A mixture of six amino acids; arginine, aspartic acid, cystine, glutamic acid, lysine, and tryptophan induced an increase in the shoot-dry weight, but did not affect the root-dry weight of the O. 1 M NaCl-treated seedlings of Sahrawy. Addition of the amino acid mixture resulted in an increase in the protein content of both shoot and root of Sahrawy seedlings grown in media either with or without NaCl, and in the protein content of Giza roots from seedlings grown without salt. In the latter cultivar no effect was demonstrated in shoots of either control or salt-treated seedlings and in roots of salt-treated seedlings.

Zusammenfassung

Samen zweier Sorten von Hafer (*Hordeum vulgare* L.) mit verschiedener Salzempfindlichkeit (Giza 118 und Sahrawy) wurden bei verschiedenen Temperaturen (Optimum 25° C) und abgestuften NaCl-Konzentrationen keimen gelassen. Die Keimfähigkeit von Sahrawy wurde durch NaCl weniger beeinträchtigt als die von Giza. Zusatz einer Lösung von sechs Aminosäuren (je 10^{-5} M Arginin, Asparagin- und Glutaminsäure, Cystin, Lysin und Tryptophan) führte nach 10 Tagen bei den mit 0,1 M NaCl be-

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handelten Sahrawy-Pflanzen zu einem höheren Trockengewicht der Sprosse, nicht aber der Wurzeln, der Proteingehalt stieg dabei bei NaCl-Pflanzen wie bei den salzfreien Kontrollen in Sproß und Wurzel an. Bei Giza erhöhte Zusatz der Aminosäuren nur den Proteingehalt salzfrei gezogener Pflanzen, in den Wurzeln der mit NaCl-gezogenen Pflanzen sowie in den Sprossen blieb er unbeeinflußt. (Editor)

Introduction

Germinating seedlings not only provide reserve materials for the growing embryo, but are also capable of a concurrent synthesis of certain enzymes. The maximum rate of growth of the germinating seedlings coincides with the maximum rate of hydrolysis of storage proteins (Varner 1965). It is possible that under salt stress osmotic effects develop which result in decreased hydration of proteins including enzymes (Chen *et al.* 1964), and thereby produce changes in rates of metabolic processes. El-Shourbagy (1964) was able to induce partial salt tolerance of roots from salt-intolerant tomato (*Lycopersicum esculentum* L.) plant by supplying them with certain amino acids, and suggested that either the enzyme system involved in the synthesis of these amino acids, or other enzyme containing these amino acids, or both, are inhibited under saline conditions.

The present study represent an attempt to provide a biochemical basis for the adaptation of seedlings from two cultivars of barley (*Hordeum vulgare* L.), Giza-118 and Sahrawy to salt stress conditions.

Methods

Seeds (obtained from the Agricultural Experimental Station at Giza, Egypt) were surface-sterilized, then germinated in darkness on filter papers in sterilized plastic dishes of 15 cm diameter $\times$ 5 cm depth containing 10 ml distilled water. Each treatment was represented by 10 dishes of 20 seeds each.

At first, five temperature levels; 5°, 15°, 25°, 35°, and 45° C were used. At each temperature level three concentrations of NaCl solution 0.05, 0.1, and 0.15 M were used in addition to the control in which seeds were soaked in distilled water. The time of incubation was 10 days and the percentage of germination was recorded daily by counting seeds having emerged roots.

In a second experiment, seeds were germinated in 10 ml distilled water (control) as well as in 0.1 M NaCl solution in the presence or absence of an amino acid mixture which contained L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, L-lysine, and L- tryptophan, each at 10^{-5} M. The pH was fixed at 6 and each treatment was incubated at 25° C for 10 days. Thereafter, the fresh weights, dry weights, and the protein contents of shoot and root were determined. After fresh weight determinations, samples were dried in an incubator with fan-driven hot air at 70° C until constant weight, this being termed as the crude dry weight.

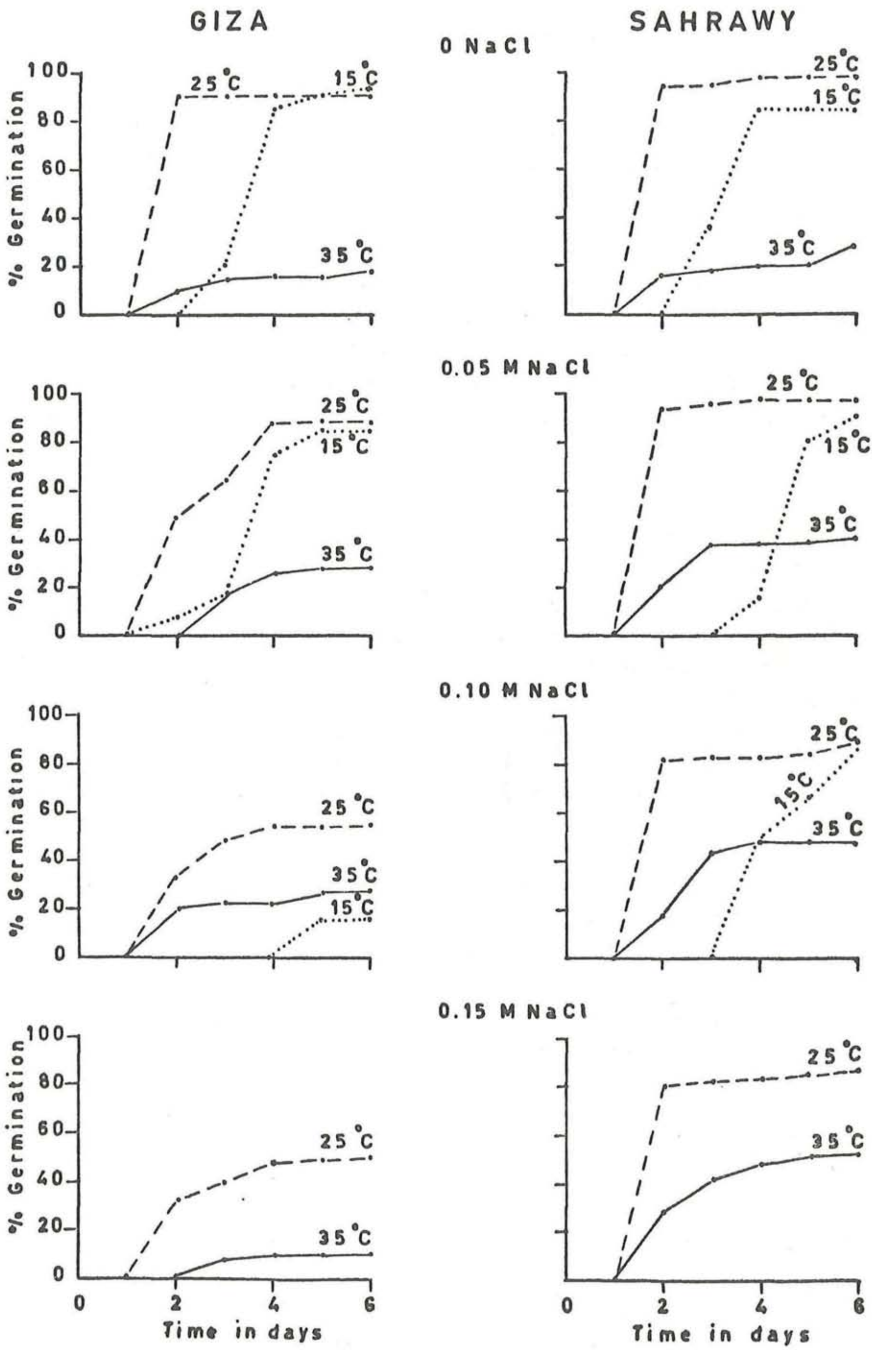

Fig. 1. Germination percentage of Giza and Sahrawy seeds under different NaCl and temperature levels.

Protein extraction and estimation:

5 g of fresh sample was homogenated with 35 ml absolute ethanol in an electric blender for 5 min. The homogenate was then transferred to a polypropylene 50 ml centrifuge tube and subjected to centrifugation at 27,000 g for 15 min at 0° C by means of a Sorval Super Speed Refrigerated centrifuge (Model-RC-2). 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.* (1951) until the protein precipitate was obtained, dissolved in 10 ml 1 N NaOH, then heated at 100° C for 10 min.

Protein was estimated colorimetrically according to LOWRY *et al.* (1951) using egg albumin as standard. Duplicate estimates for each sample were averaged and were expressed on a dry weight basis.

Results

Effect of NaCl Concentration on Germination at Different Temperature Levels:

NaCl, at whatever concentration retarded and reduced germination of Giza barley more than Sahrawy barley. Temperature of 25° C was the most suitable, and was the temperature chosen for the next experiment. Above and below this temperature germination was reduced or nil, particularly at high NaCl concentrations (Fig. 1). Statistical analysis indicated that the effects of salinity, cultivar, and their interaction were highly significant (to 1% level) on the germination percentage for each temperature level.

Effect of Amino Acid Mixture on Growth and Protein Content of NaCl-Treated Seedlings:

In this experiment the 0.1 M NaCl level was chosen because of its intermediate effect on germination as compared with the other levels. Most of the amino acids used in the mixture were found most effective in counteracting NaCl effects (EL-SHOURBAGY 1964).

The shoot-fresh weight of the control as well as the 0.1 M NaCl treatment without the amino acid mixture was higher in Sahrawy than in Giza (Fig. 2). In contrast, the root-fresh weight of the control and the 0.1 M NaCl treatment without the amino acid mixture was higher in Giza than in Sahrawy. The amino acid mixture induced a slight increase in the shoot-fresh weight of Giza, and in the root-fresh weight of Sahrawy. In fact, the shoot-fresh weight of the latter cultivar decreased by the amino acid mixture.

In the control, the shoot-dry weight was higher and the root-dry weight was lower in Sahrawy than in Giza, while in the 0.1 M NaCl treatment the shoot-dry weight was lower in Sahrawy than in Giza. In Giza, the amino acid mixture induced a slight increase in the shoot-dry weight of either the control or the 0.1 M NaCl treatment and in the root-dry weight of the control. In Sahrawy, however, it induced an appreciable increase in the shoot-dry weight, reaching more than twice that value obtained by the salt treatment, although no effect was indicated in the root-dry weight.

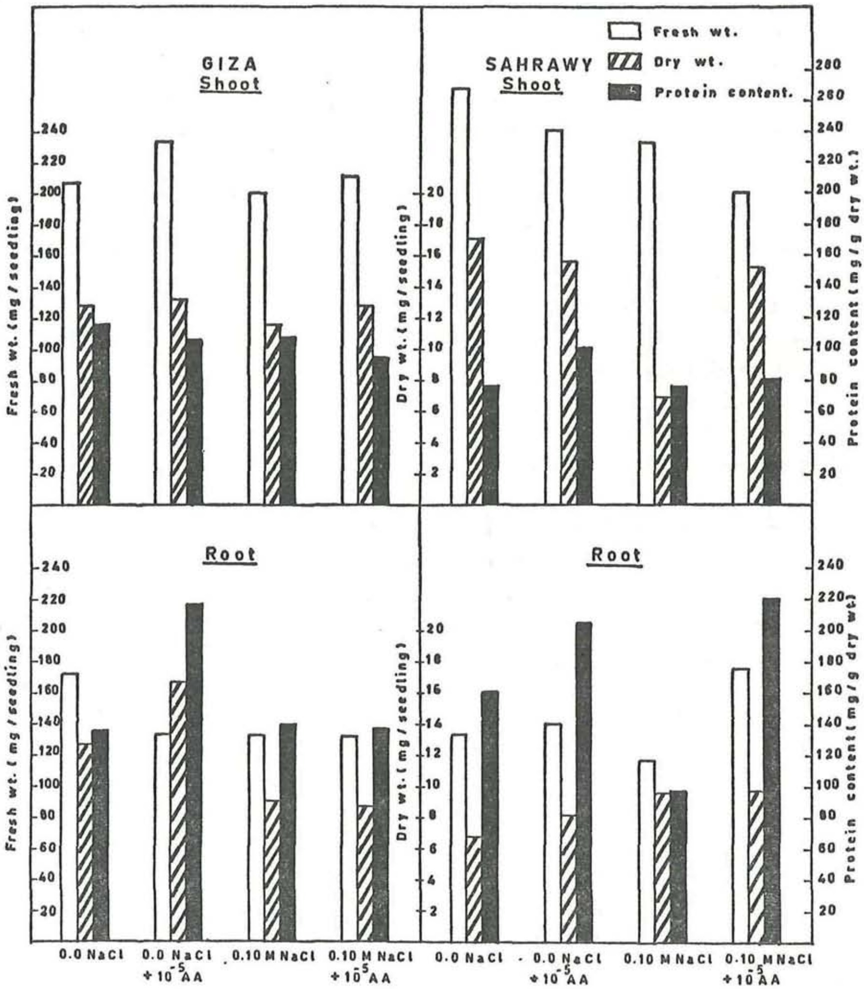

Fig. 2. Growth and protein content of shoot and root of Giza and Sahrawy seedlings as influenced by amino acid mixture (AA). The AA mixture contained arginine, aspartic acid, cystine, glutamic acid, lysine and tryptophan, each at 10^{-5} M.

The amino acid mixture brought about an appreciable increase in the protein content of both shoot and root of Sahrawy seedlings, grown either with or without salt. However, in Giza, the root-protein of the control only increased with the addition of the amino acid mixture, while only a slight reduction in the protein content of the shoot was observed either in the control or in salt treatment. The amino acid mixture raised the average protein concentration of the control seedlings of both Giza and Sahrawy. With that mixture, however, the average protein content of the 0.1 M NaCl-treated Sahrawy seedlings increased more than that of the control (0.0 NaCl), to an extent approaching twice the value with salt only. In contrast, the amino acid mixture was without effect, or even slightly reduced, the protein concentration of the salt-treated Giza seedlings.

Discussion

There was significant cultivar difference in the salt tolerance of barley when germinated in NaCl solutions ranging up to 0.15 M. The most suitable temperature for germination was 25° C and the decrease in the germination percentage with salinity was smaller in the Sahrawy than in the Giza cultivar. OTA and YASUE (1957) demonstrated significant cultivar differences in the salt tolerance of wheat (*Triticum aestivum* L.) when germinated in NaCl solutions ranging up to 1%. UNGAR (1967) indicated that there was an interaction between the effect of temperature and salinity on seed germination. *Salicornia europaea* the most salt tolerant species he studied was stimulated at high temperature, whereas germination of *Medicago sativa* and *Spergularia marina* was inhibited by the highest temperature (32° C).

Germinating barley seedlings showed cultivar differences in decreased growth rate with NaCl, which is a fairly common plant response to salt (HAYWARD and BERNSTEIN 1958, BERNSTEIN 1961 and GREENWAY 1963). When a mixture containing a number of amino acids; arginine, aspartic acid, cystine, glutamic acid, lysine, and tryptophan, each at 10^{-5} M, was added to culture media containing inhibitory concentrations of NaCl it was shown that this mixture induced an increase in the shoot-dry weight of salt affected seedlings of both cultivars, but the effect was more remarkable in Sahrawy. In this regard, EL-SHOURBAGY (1964) was able to restore a significant proportion of root growth inhibited by 0.12 M NaCl when tomato roots, cultured *in vitro*, were treated with a mixture of amino acids. He suggested that the enzyme systems involved in the synthesis of these amino acids or other enzymes containing these amino acids, or both, were inhibited under saline conditions. HIATT and EVANS (1960a) suggested that Cl^- has the capacity to form a hydrogen bond which compete with the intramolecular hydrogen bonding of the malic dehydrogenase enzyme protein from spinach (*Spinacia oleracea* L.) leaves, resulting in some physical changes. They indicated also (1960b) that acetic thiokinase was inhibited by sodium.

In the present study, addition of the amino acid mixture brought about an appreciable increase in the protein concentration of both shoots and roots of Sahrawy seedlings grown in media either with or without salt. In Sahrawy roots, the effect was greater in media containing salt than those having no salt. When germinating Giza seedlings were treated with the amino acid mixture, on the other hand, the effect of this mixture on the protein concentration was detected only in roots of the control seedlings, whereas no effect was demonstrated in shoots of either control or salt-treated seedlings or in roots of salt-treated seedlings. In this regard, Ben-Zioni *et al.* (1967) studied the protein synthesis of tobacco (*Nicotiana tabacum* L.) leaves under water and salt stresses and found that the incorporation of L-leucine in stressed leaves was lower than that of the control. Barnett and Naylor (1966) studied the effect of water stress on leaves and turn-over of both free and protein-bound amino acids, using ^{14}C labelling, and detected differences between common and coastal varieties of Bermuda grass in their nitrogen metabolism. During water stress, free proline accumulated in coastal shoots, but under well-watered conditions, common shoots contained large amounts of free asparagine. Chen *et al.* (1964) reported successive increase, decrease, and a seasonal increase in protein levels with increasing drought stress in citrus seedlings.

It seems possible that the two cultivars of barley used differ in their general response to salt stress and that differences also exist in their response to amino acid mixture supplied to counteract the salt-induced loss of protein. Some of these amino acids, such as arginine and lysine have been found in basic nuclear and ribosomal proteins (Bonner 1965). This could mean the increase in the shoot-dry weight and in the protein concentration of Sahrawy, the more salt-tolerant, involving the use of particular amino acids to synthesize proteins that have some enzymatic or osmotic function under salt stress.

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