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Alleviation of changes in protein metabolism in NaCl-stressed wheat seedlings by thiamine

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Abstract

NaCl-stress induced a pronounced suppression in growth of wheat seedlings. The most abundant amino acids (cysteine, arginine, methionine) constituting about 55 % of total free amino acid content in control wheat were reduced in 100 mM NaCl-treated plants. However, valine, isoleucine, aspartic acid and proline accumulated in response to NaCl stress and NaCl-treated wheat seedlings showed 1.6 fold increase in total free amino acids compared to the control. Addition of 2 μM thiamine alleviated the effects of NaCl on the amino acid composition and the amount of total free amino acids decreased to that in the control. Content of 26 kDa protein increased in NaCl-treated plants, stimulation was more pronounced in roots than in shoots. In contrast, the contents of 13 and 20 kDa proteins decreased. After addition of thiamine, the 24 kDa protein, which disappeared with NaCl treatment, has been initiated again. Moreover, thiamine treatment stimulated the accumulation of the 20 kDa protein.

Additional key words: amino acids, HPLC, protein patterns, salinity, *Triticum aestivum*.

Introduction

Soil salinity is one of the widespread environmental stresses that impose a serious threat to the plant growth and yield. Protein synthesis is severely affected in response to salt stress and its alteration vary within plant species. Changes in protein synthesis are attributed to changes in the gene expression induced in salt-stressed plants (*e.g.* Chen and Plant 1999). Salt-responsive genes encode proteins and other products taking part in osmoregulation (Delauney and Verma 1993), general defense (La Rosa *et al.* 1992), and cellular protection (Godoy *et al.* 1990). Accumulation of the 26 kDa protein (osmotin) is a common response to salt stress (Guerrier 1998). Moreover, alterations in protein synthesis induced by salt treatment were found to be a tissue-specific in many plant species, *e.g.*, wheat and barley (Ramagopal 1987, Gulick and Dvorak 1987). Accumulation in free amino acids is regarded as signal of disturbance induced in stressed plant cells (El-Shintinawy and El-Ansary 2000). The impact of salinity on protein synthesis includes also changes in the activation of many enzymes including antioxidant enzymes (Hernandez *et al.* 1995). Antioxidative systems including phytochromes (Velitchkova and Fedina 1998) and vitamins (El-Shintinawy and El-Shourbagy 1997, DeLong and Steffen 1998) participate in alleviating the harmful effects induced by reactive oxygen species in stressed plant cells. The information concerning the role of thiamine (vitamin B_1) as a coenzyme of the dehydrogenase enzyme complex systems which catalyze the oxidative decarboxylation of pyruvate, in repairing damage in stressed plant cells is scarce. The aim of this study was to examine a possible role played by thiamine in regulating salt-induced changes in protein synthesis in wheat seedlings.

Materials and methods

Plants: Caryopses of wheat (*Triticum aestivum* L. cv. Giza 163) were surface sterilized with 1 % sodium hypochlorite for 20 min and rinsed with distilled water, then germinated for 3 d in the dark at 25 °C in Petri dishes on filter paper soaked with water. Selected 8-d-old seedlings were placed in plastic pots with soil and sand

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and fertilized with half-strength Hoagland nutrient solution. They were divided into four groups: *1*) control plants irrigated with Hoagland solution, *2*) plants treated with 50, 100 or 150 mM NaCl, *3*) plants treated with 0.5, 1.0 or 2.0 μM thiamine and *4*) plants treated with 100 mM NaCl and 2 μM thiamine. Plants were grown under 14-h photoperiod (irradiance of 600-700 μmol m^{-2} s^{-1}) with day/night temperature of 25/15 °C and relative humidity 65 - 75 % for 3, 10 or 17 d. Every measurement was repeated 3 or 5 times.

Extraction and determination of proteins and free amino acids: Total protein content was determined according to Bradford (1976). Amino acids were analyzed by HPLC according to the method of Weibull *et al.* (1990). The free amino acids were assayed after deprotenization of the dried tissue (0.3 g prepared from ground wheat seedlings) with 5 % sulphosalicylic acid. The supernatant was filtered through *Millipore* membrane filter (0.45 μm). The filtrate was derivatized (0.025 cm^3) and injected according to the *Pico-Tag* procedure for total amino acids.

Amino acid derivatization: The filtered sample (0.025 cm^3) in 6 × 50 mm tube was placed into drying vessel and dried in *Waters Pico-Tag* workstation for 10 - 15 min. The sample was dried again in the work-station with 0.03 cm^3 of the drying solution containing 0.2 cm^3 methanol, 0.2 cm^3 0.2 M sodium acetate and 0.1 cm^3 triethy lamine. The freshly prepared derivatization reagents were added to the tube and left for 15 min. Thereafter, 0.1 cm^3 of the sample was transferred to the injection vials and for comparison the standard amino acids solutions were treated typically as the sample.

Analysis of amino acids: The apparatus used was *Spectra-Physics Analytical A0099-600* (Waters, USA) consisting of spectra focus optical scanning detector, spectra system UV 2000 detector and an ultrasphere C_{18} *Beckman* column (4.6 × 150 mm, particle size 5 μm). A gradient of *Pico-Tag* solvents at 40 °C and flow rate of 0.001 cm^3 min^{-1} was adjusted for analysis. The calibration was carried out by two injections of the standard amino acids and the retention times were determined. The separated *Pico-Tag* amino acids were detected at 254 nm.

Protein analysis: Wheat plant organs were ground to a fine powder using sterilized pestle and mortar. Total soluble proteins were extracted by mixing 0.5 g sample powder and 1 cm^3 extraction Tris-HCl buffer [0.2 M Tris, 10 % sucrose and 2 % sodium dodecyl sulphate (SDS), pH 8]. After 2 h at 4 °C, centrifugation was achieved at 15 000 *g* for 20 min. The supernatant was taken as total protein extract. The SDS polyacrylamide gel electrophoresis (SDS-PAGE) was carried out for protein separation according to the method described by Laemmli (1970) using *Bio-Rad* (England) electrophoresis large unit. The gels were stained with the staining solution for 1 - 2 h followed by immersion in a destaining solution (10 cm^3 acetic acid and 90 cm^3 of water:methanol) over night. Destained gels were photographed while wet and the protein patterns were analyzed quantitatively using a laser densitometer. An output indicating the different data was obtained using a computerized *Gel Document* system.

Results and discussion

Changes in growth and development were observed in wheat plants treated with different concentrations of NaCl or thiamine. NaCl reduced fresh mass of the plant roots and shoots (Fig. 1), higher reduction was detected at higher NaCl concentrations or in plants treated for a longer time. Addition of 2 μM thiamine to the nutrient solution containing 100 mM NaCl alleviate the reduction of growth more in roots than in shoots. These data suggest that shoots are more affected by NaCl-stress than roots while roots are more responsive to the thiamine treatments. Salinity-induced growth inhibition more pronounced in shoots than roots was also found in maize (Izzo *et al.* 1991) and wheat (Mansour and Salama 1996).

The most abundant amino acids constituting about 55 % of the total free amino acids in control wheat were cysteine (27.3 %), arginine (14.1 %) and methionine (13.2 %) (Table 1). In response to NaCl-stress, a pronounced reduction in the above mentioned amino acids from 55 to 33 % was detected. Other amino acids (valine, aspartic acid, isoleucine and proline) were accumulated under NaCl treatment. In addition, treatment with 100 mM NaCl increased the total free amino acid contents compared to the control. However, addition of 2 μM thiamine reversed the alterations in free amino acid composition induced by salinity. Thiamine addition raised cysteine, arginine, and methionine contents (Table 1). Leucine and valine are metabolically cross-linked via their precursor pyruvate. It is known that the alternative oxidase is activated under stress, with the increase in the content of pyruvate. However, the first step of the biosynthetic pathway of valine and leucine involves acetohydroxy acid synthase, a thiamine pyrophosphate requiring enzyme. This enzyme has been inhibited in soybean stressed with herbicide (Scarponi *et al.* 1995) leading to alterations in the contents of valine, leucine and isoleucine. Therefore, we speculate that acetohydroxy acid synthase could be inactivated under NaCl-stress due to the competition with alternative

oxidase, resulting in the accumulation of valine in the expense of leucine, and addition of thiamine could restore the changes in the contents of both amino acids. The impact of salinity on the composition of free amino acids in wheat plants was observed earlier in many plant species (*e.g.* El-Shourbagy *et al.* 1980, Guerrier 1998). In response to NaCl treatment, the reduction in cysteine was found to be associated with a pronounced inhibition in both glutamic acid and glycine contents. Such three amino acids are known as precursors of glutathione, one

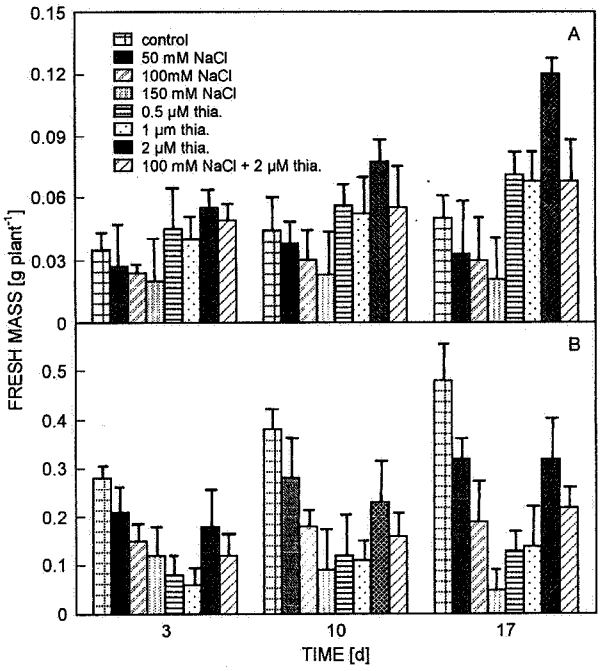

Fig. 1. Changes in fresh mass of roots (*A*) and shoots (*B*) of wheat plants at different age exposed to NaCl and thiamine (thia.) at various concentrations. Means ± SE, $n = 5$.

Table 1. Free amino acids [µmol g^{-1}(d.m.)] in 25-d-old wheat seedlings treated for 17 d with 100 mM NaCl or 100 mM NaCl plus 2 µM thiamine. Mean values from three experiments ± SE.

Amino acids	Control	NaCl	NaCl+ thiamine
Aspartic acid	0.12 ± 0.03	1.89 ± 0.30	1.32 ± 0.40
Glutamic acid	2.05 ± 0.20	0.42 ± 0.20	1.99 ± 0.02
Serine	0.90 ± 0.40	0.17 ± 0.10	0.80 ± 0.04
Glycine	0.29 ± 0.20	0.08 ± 0.30	0.63 ± 0.02
Histidine	1.95 ± 0.01	2.72 ± 0.30	1.85 ± 0.03
Arginine	4.21 ± 0.20	2.96 ± 0.40	4.90 ± 0.06
Therionine	1.49 ± 0.30	1.28 ± 0.60	0.66 ± 0.08
Alanine	0.36 ± 0.10	0.54 ± 0.50	0.44 ± 0.20
Proline	0.55 ± 0.05	1.98 ± 0.60	1.20 ± 0.30
Tyrosine	1.67 ± 0.08	0.37 ± 0.10	1.08 ± 0.06
Valine	0.25 ± 0.06	8.12 ± 0.04	1.90 ± 0.04
Methionine	3.94 ± 0.01	2.84 ± 0.02	4.13 ± 0.04
Cysteine	8.17 ± 0.20	9.60 ± 0.06	8.99 ± 0.05
Isoluecine	1.82 ± 0.30	8.06 ± 0.05	5.04 ± 0.20
Luecine	1.04 ± 0.20	0.95 ± 0.20	0.83 ± 0.03
Phenylalanine	0.53 ± 0.30	3.62 ± 0.06	0.80 ± 0.03
Lysine	0.61 ± 0.20	1.51 ± 0.05	0.75 ± 0.02
Total	29.95	47.11	37.31

of the antioxidant enzymes detected at a higher rate in stressed plant cells (Rennenberg 1982). Thus, the decline in the contents of these amino acids may affect the rate of glutathione biosynthesis that has a role in protecting the plant cell from oxidative damage induced under salinity stress (Hernandez *et al.* 1993). The reduction in arginine and glutamic acid contents that regarded as proline precursors of many plant species (Guerrier 1998) was accompanied with two-fold increase in the percent content of proline (Table 1). Such enhancement of proline content by salt stress is a common metabolic response of higher plants (Kuznetsov and Shevyakova 1997). Arginine and methionine are suggested to be the persecutors of polyamine biosynthesis, therefore, reduction in their contents under NaCl stress is expected to affect polyamine content, which in turn inhibits plant growth and development (Bouchereau *et al.* 1999). Also, the accumulation of aspartic acid (10 fold of control level) under NaCl stress reflecting the inhibition of transferases may give an insight into the alterations in the protein metabolism in stressed plants (El-Shintinawy and El-Ansary 2000). It would be expected that thiamine addition can alleviate the above mentioned alterations in amino acid composition minimizing total free amino acid content almost to that of control.

To assess the inhibitory impact of salinity stress and its alleviation by thiamine on protein synthesis, an electrophoretic profile of proteins was detected for wheat roots and shoots (Fig. 2) treated for 17 d with different treatments. Scanning of the gel indicated the occurrence of at least 14 protein bands having molecular masses ranging from 130 to 13 kDa in control wheat. Compared to the control, the 100 mM NaCl treatment increased the intensities of two protein bands at 26 and 44 kDa in roots, whereas a slight accumulation of only one band at 26 kDa in shoots. Compared to the 100 mM NaCl treatment, the protein band at 13 kDa representing a relatively high concentration of control shoot protein (50 %) was reduced to 38 % while in roots it decreased from 21 to 15 %. Another decrease in the intensity of the 20 kDa protein band was observed in salinized shoots and roots and the band at 52 kDa was also reduced in salinized roots. These results suggest that the impact of salinity is organ-specific: the accumulation of the 26 kDa band was more pronounced in roots while the intensities of the 13 and 20 kDa bands were more reduced in shoots. The 26 kDa protein has been speculated to represent osmotin (Delauney and Verma 1993). This protein which accumulated in response to salt stress is involved in the rapid accumulation of proline and glycinebetaine during stress (Weigel *et al.* 1986). The induction of the 26 kDa protein in NaCl-treated wheat has been shown earlier by Dell'Aquila and Spada (1992) who concluded that the expression of salt-induced proteins being related to the adaptation of seeds to the genetic constitution of a selected salt-tolerant genotype to salinity. The 24 kDa protein disappeared under NaCl stress was initiated again by the addition of 2 μM thiamine to the 100 mM NaCl-treated roots. Moreover, addition of different thiamine concentrations (0.5 and 2 μM) to the non-salinized roots has developed the 24 kDa band. A marked accumulation of the 20 kDa protein was also observed in roots and shoots affected by NaCl and thiamine. A protein band at 26 kDa that showed a marked increase in salinized roots has disappeared completely with all thiamine treatments. All the above alterations in the protein patterns were found to be dependent on the type of organ as well as the concentration of salt or thiamine in the culture media. The marked variations in the protein patterns shown in our work may reflect the changes in the expression of some salt responsive genes as reported by Gulick and Dvorak (1987) in wheat and by Hurkman *et al.* (1989) in barley. In addition, the salt-induced changes in protein patterns that are more pronounced in roots compared to shoots observed in the present work was shown earlier by Ramagopal (1987). This finding reflects the direct exposure of roots to salt and suggests that they are the primary organs affected in salt-exposed plants (Chen and Plant 1999). Thus, the lesser number of induced and over-accumulated proteins detected in stressed shoots compared to roots may be responsible for the more dramatic injury induced in these shoots. Moreover, roots are more responsive to thiamine than shoots in counteracting the damage induced by salinity may be due to their high ability to accumulate the 20 kDa protein (it constituted 46 % of protein in roots). Thus, thiamine may be mediated in reversing salt-induced changes in protein patterns via the induction of certain low molecular mass proteins. These protein chaperons are known to have a protective role in cellular repair of stressed plants (Lopez *et al.* 1994).

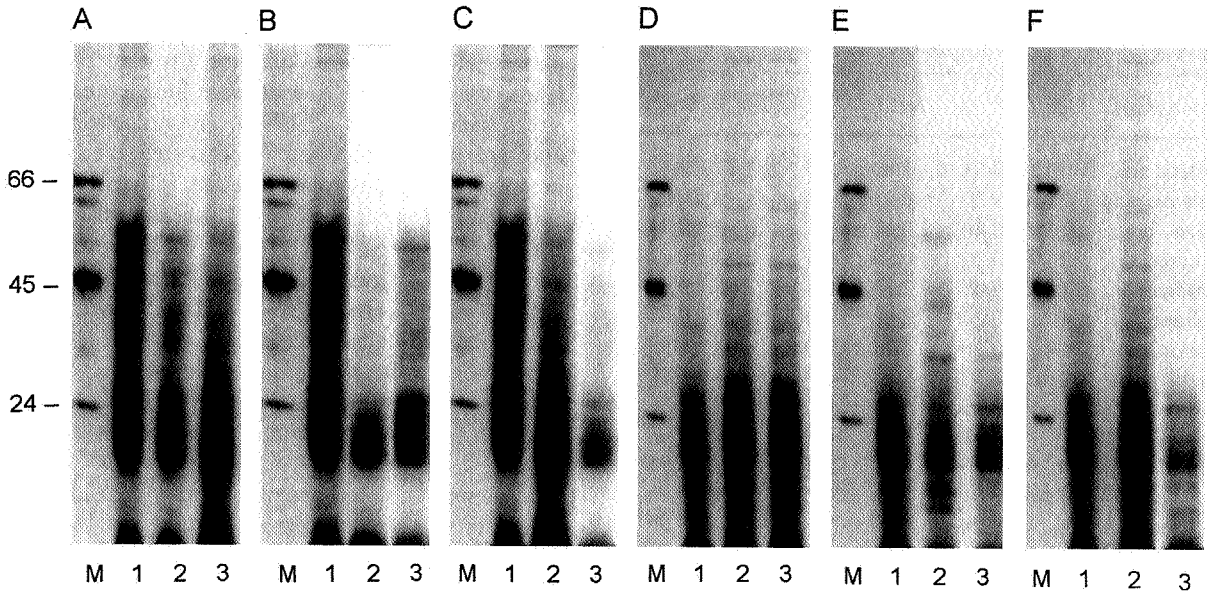

Fig. 2. Protein banding patterns of 25-d-old wheat roots (*A*, *B* and *C*) and shoots (*D*, *E* and *F*) treated for 17 d with different treatments. (*A*, *D*) represent: (1) control, (2) 50 mM NaCl, (3) 100 mM NaCl. (*B*, *E*) represent: (1) control, (2) 0.5 μM thiamine, (3) 2 μM thiamine. (*C*, *F*) represent: (1) control, (2) 100 mM NaCl, (3) 100 mM NaCl+2 μM thiamine. Data were analyzed using the *Gel Document* system.

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