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Effect of salt stress on free amino acid and polyamine content in cereals

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ABSTRACT The effect of salt stress on the level of free amino acids and polyamines in cereal samples was investigated. Different cereal seedlings were exposed to 0.1 M NaCl. Quantitative determination of amino acids and polyamines were accomplished by chromatographic methods. The results showed genotype dependent changes in free amino acid and polyamine contents. The salt stress caused increases in proline, glutamic acid, aspartic acid, arginine, ornithine and γ-amino-butyric acid levels. With respect to polyamine content, salt stress increased the levels of putescine and decreased the concentration of spermidine.

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KEY WORDS

amino acids
polyamines
salt stress
wheat

High concentrations of salts cause ionic, osmotic and associated secondary stresses to plants. Plant responses to these primary and secondary stresses are complex but can be grouped into three general categories: homeostasis, detoxification and growth control (Zhu 2000). Homeostatic responses include activities that help restore both ionic and osmotic balances in plant cells. Examples of detoxification responses are induction of metabolites and stress proteins that alleviate oxidative damage, and up-regulation of proteins that help to renature or remove denatured proteins that increase under stress. Growth control refers to the coordination of stress adaptation and the rate of cell division and expansion. Polyamines are involved in the growth control of the cells subjected to salt stress. In salt-tolerant cultivars of rice high level of spermidine and spermine was maintained, while in the salt-sensitive genotype the putrescine was accumulated during salt stress (Krishnamurthy and Bhagwat 1989). This observation indicates that the individual polyamines may have different roles during the response of plants to salt stress. Several investigations have shown that, besides other solutes, the level of free amino acids, especially proline, increases during adaptation to various environmental stresses. Significant positive correlations between proline level and frost tolerance have been found in a broad spectrum of genotypes e.g. winter barley (Dobslaw and Bielka 1988) and winter wheat (Dörffling et al. 1990).

Materials and Methods

Plant material and growth conditions. The salt-tolerant *Triticum aestivum* L. cv. Sakha, the salt-sensitive *T. aestivum* cv. Regina, the moderately salt-tolerant *T. aestivum* cv. Chinese Spring, the Chinese Spring 5AL-8, -10, -20 deletion lines, the frost-tolerant *Triticum monococcum* L. accessions G3116 and G2528, the frost-sensitive *Triticum monococcum* L. accession DV92, the frost-tolerant *Hordeum vulgare* L. cv. Nure and the frost-sensitive *Hordeum vulgare* L. cv. Tremois were used in the experiments. The seeds were obtained from the Martonvásár Cereal Gene Bank (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary).

	Control	0.1M NaCl
Nure	0,98	1,89
Tremois	0,99	2,42
DV92	1,37	10,59
G3116	1,16	4,21
G2528	1,05	4,31
Sakha	1,46	3,66
Regina	1,22	3,51
Chinese Spring	1,19	5,03
5AL-10	3,29	4,95
5AL-8	1,97	5,36
5AL-20	1,89	4,29

Table 1. Total free amino acid content of cereal samples (mg/g)

	Control	0.1M NaCl
Nure	3,01	3,53
Tremois	6,07	4,96
DV92	4,43	2,36
G3116	17,23	10,74
G2528	13,06	-
Sakha	10,60	5,86
Regina	14,30	4,64
Chinese Spring	7,64	5,77
5AL-10	5,78	1,09
5AL-8	5,60	2,28
5AL-20	10,10	4,08

Table 2. Total free polyamine content of cereal samples (μg/g)

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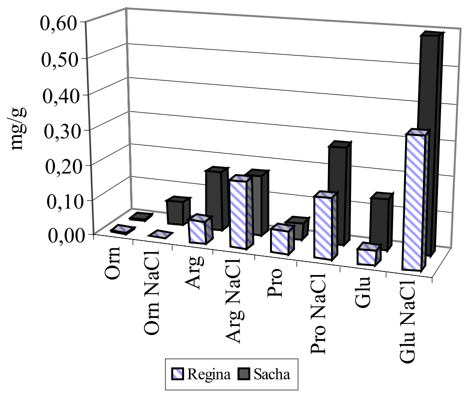

Figure 1. Changes in some amino acid contents in wheat varieties during salt stress.

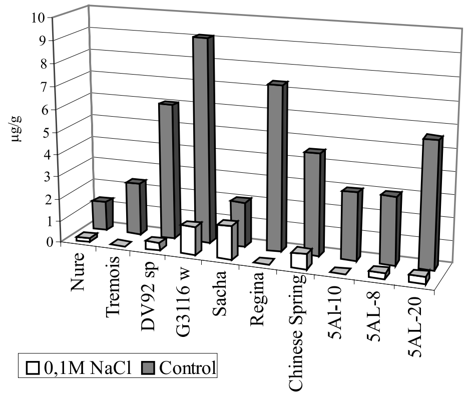

Figure 3. Changes of spermidine content in cereals during salt stress.

Growth conditions. After germination in Petri dishes (25 °C, 3 d) the seedlings were cultivated in pots containing half-strength Hoagland solution (Hoagland and Arnon 1950) for 11 d at 18/15°C day/night temperature with 16 h illumination at 260 µmol m^{-2} s^{-1} in an autumn-winter type growth chamber (Conviron PGV-36, Controlled Env. Ltd., Winnipeg, Canada). During the first week of the salt treatment the plants were cultivated on a half-strength Hoagland solution supplemented with 0.05 m NaCl. On the second week 0.1 M NaCl was added to the nutrient solution and maintained for the third week. The control plants were cultivated without NaCl during the whole experiments. The plant material for the biochemical analysis was collected at the end of the experiments.

Sample preparation for amino acid determination. Samples were homogenized in cold 7% (v/v) perchloric acid at a ratio of 300 mg/cm^3. The supernatant was collected after centrifugation at 12000 x g for 10 min. Aliquots of the supernatant were injected to an amino acid analyser (Biotronik LC 3000, Germany).

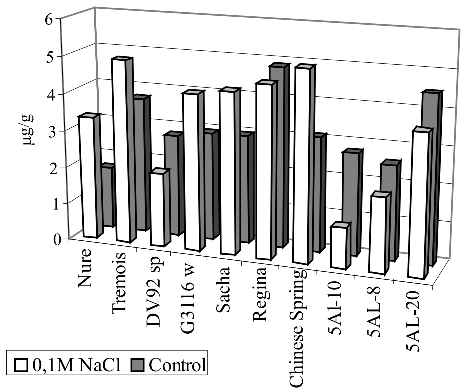

Figure 2. Changes of putrescine content in cereals during salt stress.

Sample preparation for polyamine determination. Aliquots of the same supernatant as for amino acid analysis were derivatised with dansyl chloride (Simon-Sarkadi and Galiba 1988). Dansyl polyamines were separated by over-pressured layer chromatogtahy (OPLC BS 50 Chromato-graph, OPLC-NIT Co. Ltd. Hungary). Quantitative evaluati-on was accomplished at 313 nm by means of a densitometer (Camag TLC Scanner 3, Switzerland) (Simon-Sarkadi et al. 2001).

Results and Discussion

The results of amino acid determination are shown in Table 1. The total free amino acid content increased in all varieties during salt stress. The highest free amino acid accumulation was found in the case of the frost-sensitive *Triticum monococcum* L. accession DV92 and the moderately salt-tolerant *T. aestivum* cv. Chinese Spring. The main amino acids detected were Asp, Glu, Pro, Arg, Orn and γ-aminobutyric acid. The levels of free Pro (7–27 times), Orn (30–130 times), Glu (4–20 times) and Asp (5-6 times) increased predominantly in *Triticum monococcum* L. varieties. Significant differences were found between the salt-tolerant *Triticum aestivum* L. cv. Sakha and the salt-sensitive *T. aestivum* cv. Regina. In the salt tolerant variety a higher accumulation rate of Orn (19 times) and Pro (6 times) was observed whereas in the sensitive variety the level of Glu (9 times) and of Arg (3 times) showed higher accumulation rate during salt stress (Fig. 1).

The total free polyamine content showed decrease in the 0.1 M NaCl samples compared to the control ones (Table 2). The most pronounced decrease was observed by the salt-sensitive *T. aestivum* cv. Regina (0.3 times). Put and Spd were the major free polyamines in all varieties. With regard to the changes induced by salt stress on the free polyamine levels, the putrescine levels (Fig. 2) increased (1.5–2 times), whereas the spermidine levels (Figure 3) decreased (0.1 – 0.05 times). There was significant difference between the salt-tolerant *Triticum aestivum* L. cv. Sakha and the salt-sensitive *T. aestivum* cv. Regina concerning Spd concentration. The decrease of Spd was less pronounced in Sakha, than in Regina variety. It seemed that the accumulation of Spd may be good marker of salt sensitivity. In the case of the frost-sensitive *Triticum monococcum* L. accession DV92 a decline in the putrescine level ran parallel to a high accumulation of Orn (135 times), Pro (17 times), Arg (12 times) under salt stress. It can be suggested that putrescine catabolism can contribute to compatible osmolyte accumulation. The salt-induced changes in the free amino acid and polyamine content play an important role in the response to salt stress of plants, since these alterations were several times in a relationship with the stress tolerance of cereals investigated.

Acknowledgments

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