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SALINITY-INDUCED CHANGES OF FREE AND BOUND POLYAMINE LEVELS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) ROOTS DIFFERING IN SALT TOLERANCE

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Abstract

The effect of salt stress (50, 100 and 150 mM NaCl) on the endogenous levels of free, bound and total polyamines was studied in root tissues of salt tolerant (Coban) and salt sensitive (Sanbro) cultivars of sunflower (*Helianthus annuus* L.) plants for the 5th, 15th and 25th days of the growth periods. The amounts of free, AS-bound and total Spm increased in root tissues of sunflower plants subjected to salt stress for different periods of time while the levels of other polyamine titers were either decreased in general or unchanged significantly. The increase in some polyamine titers in sunflower root tissues under salinization suggests their possible role in combating the adverse effect of salinity stress. The salt tolerance in sunflower plants was related to the excessive accumulation of total polyamines in root tissues of salt tolerant cultivar Coban under saline condition.

Introduction

Plant productivity is strongly influenced by stresses induced by drought, heat and osmotic as well as salt stress. Most cultivated plant species are highly sensitive and either die or display reduced productivity after they are exposed to long periods of salt stress (McKersie & Leshem, 1994). Salt stress induced physiological and biochemical changes of the whole plants and so that various compounds are synthesized by salt stress such as abscisic acid, organic acids, proline, polyols and polyamines (Yancey *et al*., 1982). Since PA level changes significantly upon exposure to environmental stress, it has been hypothesized that they are part of a plant defense mechanism against stress (Turano & Kramer, 1993). Polyamines are involved in the stress tolerance of higher plants, which encounter various abiotic stresses, including salt stress (Flores, 1991; Mattioni *et al*., 1997). The most common PAs studied in plants are the diamine Put, triamine Spd and tetraamine Spm.

In many plant systems, polyamines do not only occur as free molecular bases, but can also be linked covalently to monomers of phenolic acids, mostly hydroxycinnamic acids (acid-soluble bound polyamines) as well as to dimers and trimers of phenolic acids or other high molecular-mass molecules like proteins *via* transglutaminases (acid-insoluble bound polyamines). In most of the earlier studies, it has been shown that bound forms of PAs are associated in particular with plant developmental events such as reproductive processes or root formations (Martin-Tanguy, 1997). In recent years, attention has been focused on the possible roles of the conjugated forms of polyamines in plants exposed to unfavorable environmental conditions (Bouchereau *et al*., 1999) but even so only a few reports on the response of bound polyamines to salinity stress factors are available.

The effects of salinity stress on PA biosynthesis were studied in several plants and the pattern of PA metabolism in response to salinity stress seems to be dependent on the plant species, the plant systems used and /or duration of exposure to salinity stress (Prakash *et al*., 1988; Basu *et al*., 1988; Lin & Kao, 1995; Benavides *et al*., 1997). Since metabolism of polyamines under the saline conditions is not properly understood the role of polyamines in relation to salt tolerance still remains obscure. Moreover specific information on the effects of salinity on PA levels in sunflower roots is very scarce.

In order to understand salinity-induced changes in the titers of polyamines and to achieve a possible correlation between the levels of polyamines and the degree of salt tolerance in sunflower plants, the present study was undertaken with the objectives to evaluate the metabolic levels of free and bound (acid soluble and acid insoluble) polyamines in the roots of seedlings growing under salinity stress.

Materials and Methods

Plant material and growth of seedlings: Seeds of sunflower (*Helianthus annuus* L.) cultivars like Coban (salt tolerant) and Sanbro (salt sensitive) were used. The seeds were obtained from Seed Improvement and Certification Centre in Turkey.

Selected seeds of uniform size were surface sterilized according to Ellis *et al*., (1988) and then imbibed in saline culture solutions varying at different concentrations for 24 h. The imbibed seeds were germinated at 25±1 °C in a dark-controlled growth cabinet.

Five day-old seedlings were transferred to plastic growth boxes (26x26x11 cm) with their roots dipping in the aerated saline culture solutions. The growth experiments were also carried out in a growth cabinet where the temperature 25±1°C, relative humidity 65±5 % and 15h light/9h dark periods were set up.

During the growth period the plants were cultivated in a half-strength Hoagland solution (control) and Hoagland solution supplemented with 50, 100, 150mM NaCl. Root materials were harvested for the estimation of PA (free and bound) contents at 5, 15 and 25 days after initial salinization.

After harvesting, the fresh root material was quickly frozen with liquid nitrogen and stored at -20ºC for analyses.

Determination of polyamines: Free PAs were determined in supernatants by the method of Flores & Galston (1982). Bound polyamines were determined in acid hydrolyzed supernatants (acid soluble-bound polyamines) and acid hydrolyzed pellets (acid insoluble-bound polyamines) according to Tiburcio *et al*., (1985). Supernatant, the hydrolyzed supernatant and the hydrolyzed pellet were dansylated and separated on high performance TLC plates (Whatman LK 6D) with concentrating zone, using cyclohexane: ethylacetate (3:2, v/v) as developing solvent.

After chromatographic separation, free and bound polyamines were detected under UV light and quantified by densitometry (Pelkin Elmer) with excitation and emission at 365 and 500 nm, respectively. Polyamines were quantified by comparison of the integration areas from the samples with those of pure standards treated in the same way.

Statistical analysis: The experiments were performed in a factorial design with three replicates. Statistical variance analysis of the results was performed and compared with least-significant differences (LSD) at 5% level.

Results and Discussion

In the root tissues of Sanbro and Coban cultivars grown in saline cultures for 5, 15 and 25 days, the levels of free Spm were found to increase as compared to their controls ($p<0.05$) (Table 1). The findings concerning the increase in free Spm levels of sunflower roots as a result of salt application are very similar to those of Yamamoto *et al*., (2003).

The change of free Spm in both cultivars revealed that the level increased more in root tissues with 15 and 25 days of growth period in comparison to those with 5 days. This increase was more evident in the roots of 15 day old plants. Furthermore, the level of free Spm was much higher in 5 day-old Coban roots than Sanbro, whereas the level of free Spm appeared to be higher in 15 and 25 day-old Sanbro roots than Coban ($p<0.05$).

Application of salt stress also led to increase of free Spd level only in 5 day-old salt sensitive Sanbro roots as compared to their controls ($p<0.05$). But it led to decrease in the level of free Spd in Sanbro and Coban roots with 15 and 25 days of growth periods. In the control samples with 5, 15 and 25 days period, the level of free Spd increased with the age of plants, whereas this fact was not observed clearly in the salt treated plants ($p<0.05$). On the other hand increased salt stress led to decrease in free Put levels of 5, 15 and 25 days old Sanbro and Coban roots ($p<0.05$). It was also observed that free Spm, Spd and Put levels in 5 day-old Coban roots grown in Hoagland solution were higher than that of Sanbro roots (Table 1) ($p<0.05$). This finding is similar to those of Léfevre *et al*., (2001).

In general, the findings obtained about the levels of free polyamine in the root tissue of sunflower that were subjected to salt stress condition had some similarities to the findings of several other studies. Tattini *et al*., (1993) concluded that salt stress led to the accumulation of Spd and Spm whereas the level of Put decreased in olive roots. Reggiani *et al*., (1994) found that salt stress decreased the level of Put especially in root with increasing NaCl concentrations in three wheat cultivars. Foster & Walters (1991) also reported that the level of Put decreased whereas the level of Spm increased in wheat roots grown under 100 and 150mM NaCl. All these findings support our findings presented above. However the findings reported recently (Chattopadhyay, 1997; Ali, 2000) on salinity stress induced changes of PA levels are not similar to ours.

On the other hand, as our findings indicated any clear trend was not observed in the change of both bound and total polyamine levels in the roots of plants grown under the salt stress. The age of the plants and the type of cultivars did not have a clear effect on neither bound nor total polyamine levels. However in all experiments, the levels of AS-bound, AINS-bound and total Spm in 25 day-old Sanbro and Coban roots were found to be lower than that of 15 days old plant roots. These results are consistent with those of Shen & Galston (1985) who studied with corn, tomato, and pea roots.

Our findings related to AS-bound and AINS-bound Put levels were found to be higher than those of Felix & Harr (1987). Furthermore, in some cases it was found that bound polyamines could be higher in the plant tissue. For instance in Sanbro roots total bound PA (AS-bound+AINS-bound) level formed 42.5% of total free PA level and similar ratio (42.3%) was found for Coban roots grown in Hoagland solution. This finding is consistent with those reported by Königshofer & Lechner (2002).

Changes in total polyamine (free+AS-bound+AINS-bound) level in Sanbro and Coban cultivar showed that only total Spm level in both cultivars increased as the salt stress increase. This is regarded as a adaptive reaction of the plant to the stress. Tattini *et al*., (1993), Foster & Walters (1991) also support this view.

In our experiment, the levels of total Put in Sanbro and Coban roots with three different growth periods decreased in relation to increase in salt stress. It is known that salt stress affects Put catabolism and the synthesis of Spd and Spm and it may influence the amount of polyamine synthesis in the roots. On the other hand generally, in root tissues of sunflower plants the amount of free polyamine increased but the level of bound polyamine decreased. This finding with those of Shiozaki *et al*., (2000) support the assumption that some bound polyamines may convert into free form.

Erdei *et al*., (1996) suggested that the initiation of PA accumulation needs osmotic signal. Under salt stress, the ions may contribute to osmotic adjustment and thus the onset of PA biosynthesis is delayed or does not take place. Similarly Reggiani *et al*., (1993) concluded that when cation levels in plant tissues (K^+, Na^+, Ca^{++}) increase, the level of PA decreases. As Erdei *et al*., (1996) stated, the presence of Na and Cl ions in sunflower tissues might provide osmotic regulation in our study. Thus PA biosynthetic process becomes closer to oxidative destruction.

According to the present findings it is possible to suggest that treatment of NaCl may limit Put synthesis and other amines and therefore it can inhibit the plant growth and development. The findings of Strogonov *et al*., (1972) showed that salinization limited Put accumulation in sunflower and barley also support our study.

The findings also suggest that the current information cannot provide a full explanation on the effects of polyamines upon seedling development under saline conditions. These results may imply that salinity affects PA metabolism through in different ways, such as the plant species, the developmental stage, strength of the stress, duration of the treatment, light and temperature.

The stress-induced modifications in PA concentrations reported in the present study concern only long-term exposure however the short-term effects of stress may be quite different (Das *et al*., 1995). Accumulation of free, AS-bound and total Spm under saline conditions suggest that they may have a potential role in combating the adverse effect of salt stress. However, the detailed studies relating to the underlying mechanism of PA accumulation and their function in higher plants are essential in order to define the exact role of polyamines in environmental stress.

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