Effects of some plant growth regulators on stem anatomy of radish seedlings grown under saline (NaCl) conditions

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ABSTRACT

In this work, effects of gibberellic acid, 2-chloroethylphosphonic acid (ethephon), triacontanol, 24-epibrassinolide and polyamine (cadaverine, putrescine, spermidine, spermine) pretreatments on the stem anatomy of radish seedlings grown under saline conditions were studied. Salt stress decreased the stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, cambium thickness, xylem width, trachea diameter and phloem width in the seedlings non-pretreated with the growth regulators, in comparison with the control seedlings grown in distilled water medium. In addition, it slightly increased the cuticle thickness. On the other hand, many of the growth regulator pretreatments more or less stimulated the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control seedlings grown on saline medium. Moreover, they generally reduced the cuticle thickness, epidermis cell length and cambium thickness.

Keywords: plant growth regulators; radish; salt stress; stem anatomy

Excess amount of salt in the soil adversely affects plant growth and development. Nearly 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu 2001). Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of production (Sairam and Tyagi 2004). The salt-affected soils contain excess salts which affect plants by decreasing the osmotic potential of the soil solution (osmotic stress), interfering with normal nutrient uptake, inducing ionic toxicity, and associating nutrient imbalances (Dudley 1992).

It is evident that there are big changes in morphology and anatomy of plants growing in saline soils. The effect of salinity on root (Valenti et al. 1991, Reinhardt and Rost 1995, An et al. 2003) and leaf anatomy (Hu and Schmidhalter 2001, Çavuşoğlu et al. 2007, Kılıç et al. 2007) of plants had already been reported in previous works. Unfortunately, there are fewer studies on the effect of salinity on stems than on leaves and roots. Casenave et al. (1999) observed that cotton seedlings subjected to the higher salinity levels had a significantly smaller cortex. In addition, the same researchers reported that with an increase in salinity there was a decrease in the development of the xylem. Pimmongkol et al. (2002) stated that the width of vascular bundles and diameters of rice stems decreased in NaCl medium. Junghans et al. (2006) showed that high salt concentrations reduced the cambial activity in *Populus euphratica*.

However, no study has been encountered concerning effects of the plant growth regulators used in this work on the stem anatomy of seedlings grown under saline conditions until now.

In this work, the influences of gibberellic acid, ethephon, triacontanol, 24-epibrassinolide and

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polyamines on stem anatomy of the seedlings from radish seeds subjected to salinity stress were studied.

MATERIAL AND METHODS

The seeds, NaCl concentrations and growth regulators

In this study, radish (*Raphanus sativus* L. cv. Spring radish) seeds were used. The seeds were surface sterilized with 1% sodium hypochloride. NaCl concentration used in the experiments was 0.25M.

Growth regulators were 900 µmol gibberellic acid (GA_3, Fluka), 400 µmol ethephon (E, Fluka), 10 µmol triacontanol (TRIA, Fluka), 3 µmol 24- epibrassinolide (EBR, Sigma), 10 µmol polyamine, PA (cadaverine/Cad and putrescine/Put, Sigma; spermidine/Spd and spermine/Spm, Fluka).

Salt and growth regulator concentrations were determined in a preliminary study. 0.25M salt, in the case of control, was the level that prevented final germination percentage and gave way to study of the selected parameters of stem anatomy. Also the concentrations of the used growth regulators were at levels which were relatively successful in alleviation of the salt-induced inhibition on the germination and seedling growth (Çavuşoğlu and Kabar 2007b).

Germination of the seeds

Germination experiments were carried out at a constant temperature (20°C), in the dark in an incubator. Radish seeds were pretreated in the beakers containing 100 ml of distilled water (control, C) or aqueous solutions of GA_3, E, EBR, TRIA, Cad, Put, Spd and Spm for 24 h at room temperature. At the end of this pretreatment, the solutions were filtered immediately and the seeds were dried in vacuum (Braun and Khan 1976). 25 seeds from every application were arranged into Petri dishes (10 cm diameter) lined by 2 sheets of Whatman No. 1 filter paper moistened with 6 ml of salt solution. After sowing, Petri dishes were placed into an incubator for germination for 7 days.

Growth conditions of the seedlings from the seeds and anatomical observations

The seedlings from the seeds germinated in the incubator at 20°C for 7 days were transferred into the pots with perlite including 50 ml (Hoagland + 0.25M NaCl) of solution prepared with Hoagland recipe and were grown in a growth chamber for 20 days. Growth conditions were: photoperiod 12 h, temperature 25 ± 2°C, relative humidity 60 ± 5%, light intensity 160 mol/m^2/s PAR (white fluorescent lamps). The materials for anatomical study were fixed in FAA for 24 h and then preserved in 70% alcohol in the field. Anatomical observations were performed on transverse sections of stem and leaves cut by microtome (8–10 µm). The sections were stained with safranin/fast green for 24 h and mounted in glycerine-gelatine to make permanent slides. All measurements and observations were made three and four times.

Stem diameter, cuticle thickness, epidermis cell size, cortex zone thickness, vascular bundle width, cambium thickness, xylem width, trachea diameter and phloem width were determined in µm by using ocular micrometer. Statistical evaluation concerning all parameters was realized by using the SPSS program according to Duncan's multiple range test.

RESULTS AND DISCUSSION

The findings related with effects of growth regulator pretreatments on the stem anatomy of radish seedlings grown in distilled water and saline medium are presented in Figure 1.

In distilled water medium, GA_3, E, TRIA and Spm applications increased the stem diameter in statistically the same degree in comparison with the C seedlings, but EBR reduced this parameter. The others showed statistically the same values as the C. The most effective regulator of this parameter was Spm although all of the pretreatments except EBR and Spm (insignificantly) decreased the cuticle thickness. GA_3, E and EBR increased the epidermis cell width while the others had a reductive effect on this parameter. As for the epidermis cell length, all of the growth regulators had reductive effect on this parameter in the varying levels. All treatments increased the cortex zone thickness. The most effective regulator of this parameter was Spm again, as in the case of stem diameter. TRIA, GA_3 and E increased the vascular bundle width, but EBR and Spd reduced it. TRIA was statistically the most effective for this parameter. All of the pretreatments decreased the cambium thickness. TRIA, GA_3 and E increased the xylem width, the others had statistically the same values as the C. Put, Cad, GA_3, E and TRIA reduced the trachea

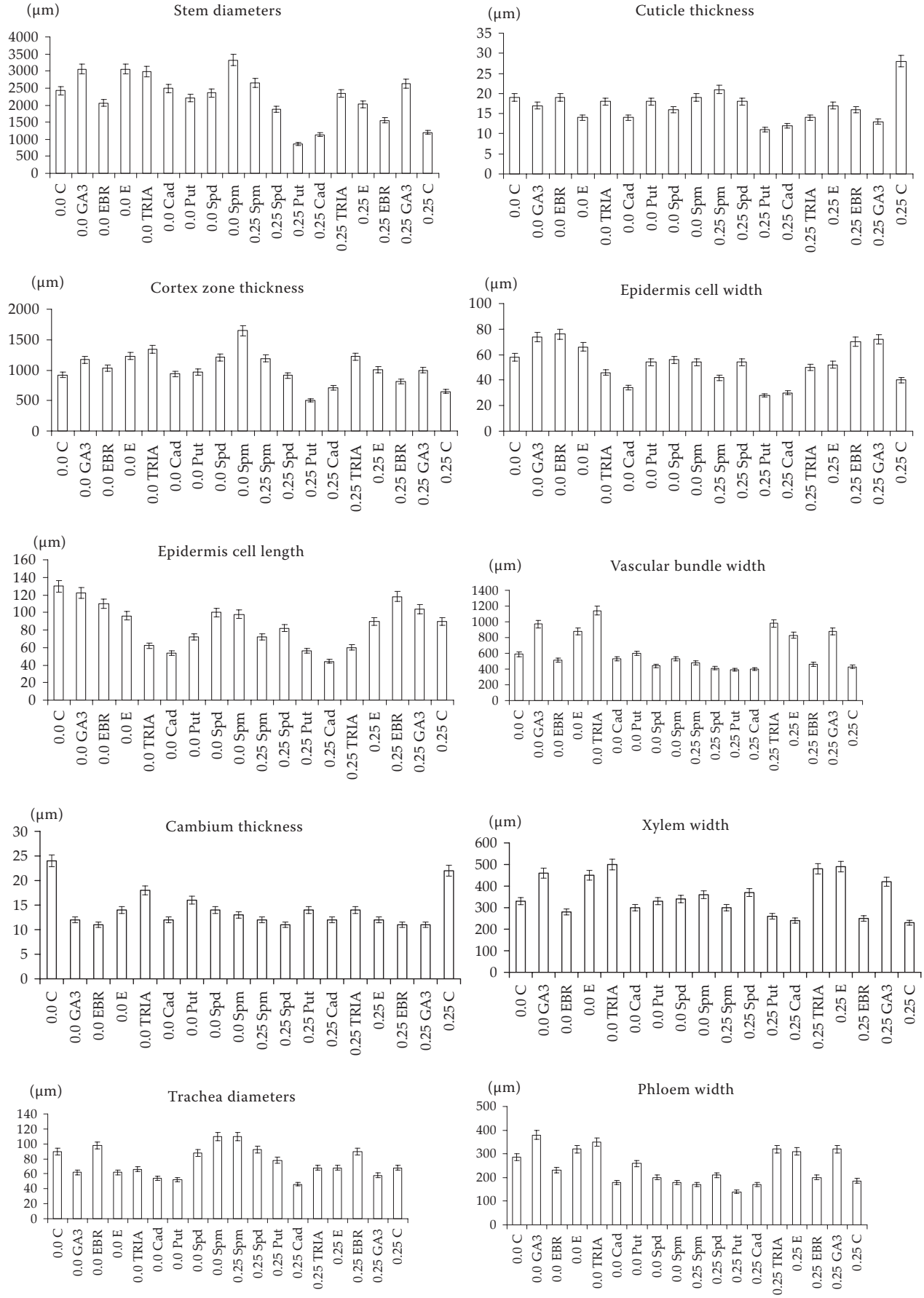

Figure 1. Some parameters of stem anatomy of radish seedlings grown in 0.0 and 0.25M NaCl at 25°C for 20 days after growth regulator pretreatments

diameter to a great extent in comparison with the C seedlings while Spm enhanced it, and the other two pretreatments, Spd and EBR, had the similar values to the C. GA_3 and TRIA increased the phloem width, the others decreased it.

In the C group, salinity of 0.25M, in comparison with distilled water medium, notably decreased the stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in the seedlings. In addition, this salt level rather increased the cuticle thickness and slightly reduced the cambium thickness.

On the other hand, all of the growth regulators except Cad and Put increased the stem diameter and epidermis cell width in comparison with the control seedlings grown in 0.25M salinity. Spm, GA_3 and TRIA were the most effective treatments for the stem diameter, whereas GA_3 and EBR for epidermis cell width. Almost all treatments decreased the cuticle and cambium thickness to a great extent. Although GA_3 and EBR rather increased the epidermis cell length, the others reduced it in general. All of the growth regulators except Put evidently increased cortex zone thickness. The most effective regulators in this case were TRIA and Spm. TRIA, GA_3 and E markedly enhanced the vascular bundle while the others gave statistically similar values as C. The treatments except EBR, Cad and Put clearly increased the xylem width. Particularly E, TRIA and GA_3 showed a prominent increase compared to the C. EBR, Spd and Spm pretreatments importantly increased the trachea diameter while Cad reduced it, and the effects of others were statistically in the same degree as C. Although Put decreased the phloem width, GA_3, E and TRIA increased it, and the other treatments gave the similar results as C.

It has been reported previously that saline conditions negatively affect growth and development events in general, even in halophytes. However, the effect of salinity mechanisms has not been completely clarified so far (Gill and Singh 1985, Schmidhalter and Oertli 1991).

After 7-day germination period of seedlings coming out of seeds treated or not treated with the growth regulators, those that were in distilled water were transferred into pots containing Hoagland solution, and also those that were in saline medium into pots containing NaCl in the same concentration prepared with Hoagland solution. After that, they were grown for 20 days, salinity of the medium added to dramatic changes in the anatomic properties of the seedlings' stems. Stem diameter, epidermis cell size, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phyloem width decreased in comparison with those of distilled water medium (Figure 1). Reducing effects of salt stress on stem diameter (Pimmongkol et al. 2002), epidermis cell width and length (Curtis and Lauchli 1987), cortex zone thickness (Casenave et al. 1999), vascular bundle width (Pimmongkol et al. 2002), xylem width (Baum et al. 2000) and trachea diameter (Bass et al. 1983, Reinhardt and Rost 1995) were reported previously. Moreover, some researchers (Liphschitz and Waisel 1970, Shannon et al. 1994) determined that salinity increased cuticle thickness and decreased cambium thickness. The results obtained in this work are consistent with the above mentioned research findings. The changes caused by the salinity of medium on the mentioned parameters of the stem anatomy during the growth of radish seedlings followed the germination of the C seeds untreated with growth regulators. They essentially appeared as a results of osmotic effect and the difficulty of water uptake from the saline medium and are probably ecologically functional, and serve to give better protection to a plant against the salt stress. The presence of coordination or cooperation (among structure, function and environment) in all the living things including plants is very well known. It is therefore of interest to reveal the physiological basis of these anatomical changes; an increase in the capillarity of the xylem elements occurs, water potential of the cells lowers and thus, water uptake and conductance relatively easily take place in spite of the existence of salt. However, despite these salt-induced anatomical alterations, the values of the parameters studied were below those of distilled water medium.

It has been known for long that salinity stress during seed germination and seedling growth may be alleviated by pretreatments with various growth regulators (Braun and Khan 1976, Kabar 1990, Kaur et al. 1998, Gulzar and Khan 2002). Unfortunately, we did not find a study related to the interaction between growth regulators used in this work and salt stress influencing the stem anatomy of seedlings grown under saline conditions. The growth regulator pretreatments mostly increased the stem diameter, epidermis cell width, cortex zone thickness, vascular bundle width, xylem width, trachea diameter and phloem width in comparison with the control seedlings grown in saline medium. In addition, they generally decreased the cuticle thickness, epidermis cell length

and cambium thickness (Figure 1). These anatomical changes indicate that salt stress on the stems of radish may be reduced by growth regulators. Actually, Çavuşoğlu and Kabar (2007b), in their work with radish, observed that the growth regulators alleviated the salt-induced inhibition on seed germination, percentage of hypocotyl and water uptake. In addition, Çavuşoğlu and Kabar (2007a) found that the applications of all mentioned growth regulators except TRIA dramatically overcame ABA (abscisic acid)-inhibition on germination of radish seeds.

The mentioned large cells of the stems of salinized seedlings from the seeds pretreated with growth regulators may results from increased cell wall extensibility together with higher turgor pressures (Strogonov 1962, Jennings 1976, Munns and Termaat 1986). Cell expansion is an expression of water uptake and cell wall extension. The working view is that a biochemical loosening of the cell wall under turgor pressure initiates cell expansion followed by water and solute uptake (Cosgrove 1997, Boyer 2001). Growth regulators such as GA_3 and EBR may not require an increase in cuticle thickness or a reduction in stem diameter, epidermis cell size and other anatomical parameters studied by alleviating the growth inhibitive-effect of ABA increase induced by salinity (Zhao et al. 1992, Hu et al. 2005), compared to the other regulators such as Cad or Put.

Salinity, as known, affects synthesis of carbohydrates as well as transport of photosynthetic products and their utilization in the formation of new tissues. By the pretreatments with growth regulators, both important physiological processes may be stimulated or ameliorated in the presence of salinity which inhibits these processes and salt-alternated anatomical changes may be recovered as if there were no stress conditions but a slight stress.

Strogonov (1962) suggested that halosucculence could be defined as the hydration of plant tissues by a salt effect. Our observations that cortex zone thickness and epidermis cell width increased under saline conditions by pretreatments with growth regulators could fit with this Strogonov's (1962) concept. Similary, Poljakoff-Mayber (1975) defined halosucculence as an increase in cell size. In addition, these pretreatments can have provided adaptation to salt stress by increasing the stem diameter.

It is surprising that many pretreatments with plant growth regulators used in this work are successful in the adaptation of radish seedlings to salt stress. The similarity of the effects of growth regulators used on the anatomical parameters of stem seems like insurance for adaptation to salt stress. It indicates that salt tolerance in plants by absolute presence or absence of a growth regulator is not probable. It may be more accurate to think of a common pool of growth regulators against salt stress; one or several of these growth regulators might alleviate salt stress on stem anatomy. The mechanisms by which salinity inhibits growth are complex and controversial. Moreover, they may vary according to species and cultivar. A universal mechanism has not been established yet. Although the causes of salinity have been characterized, our understanding of the mechanisms by which salinity prevents plant growth is still rather poor. This work may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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