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Effects of Growth Regulators on Anatomy of Radish Roots Under Saline Conditions

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Abstract

In this work, effects of gibberellic acid, benzyladenine, kinetin, ethylene, 24-epibrassinolide, triacontanol and polyamine (cadaverine, putrescine, spermidine, spermine) pretreatments on the root anatomy of radish seedlings grown under saline conditions were studied. Salt stress had a reductive effect on many of the examinated anatomy parameters. On the other hand, a lot of pretreatments showed an inductive effect on the anatomy parameters.

Key words: Plant growth regulator, radish, root anatomy, salt stress

INTRODUCTION

Soil salinity 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 [1]. 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 [2]. 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 [3].

It is evident that there are big changes in root morphology and anatomy of the plants growing in saline soils. Since soil salinity is detected first by roots, Lacan and Durand [4] pointed out that the primary and important processes of plant salt tolerance may reside in roots. Bernstein and Hayward [5] reported that salt accumulation in leaves was controlled by roots. Casenave et al. [6] observed that cotton seedlings subjected to salt stress had a significantly smaller cortex. In addition, same researchers determined that salinity caused a decrease in the development of the xylem. Valenti et al. [7] showed that salt stress reduced the root diameter. In constrast, Neuman [8] found that saline conditions was increased the root diameter. Other workers [9, 10] reported that salinity induced the endodermis cell size and vascular cylinder diameter in roots of various plant species.

On the other hand, it has not been encountered any study concerning effects of the growth regulators used in this work on the root anatomy of seedlings grown under saline conditions until now.

In this work, the influences of gibberellic acid, benzyladenine, kinetin, ethylene, 24-epibrassinolide, triacontanol and polyamines on root anatomy of the seedlings from radish seeds subjected to salinity stress were studied.

MATERIALS and METHODS

The Seeds, Salt Concentrations and Growth Regulators

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

Growth regulators were 900µM gibberellic acid (GA_3), 100µM benzyladenine (BA), 100µM kinetin (Kin), 400µM ethylene (E), 3µM 24-epibrassinolide (EBR), 10µM triacontanol (TRIA), 10µM a polyamine, PA (cadaverine/Cad, putrescine/Put, spermidine/Spd and spermine/Spm). Salt and growth regulator concentrations were determined in a preliminary study.

Germination of the Seeds

Germination experiments were carried out at a constant temperature (20°C), in the dark in an incubator. Radish seeds in adequate amount were pretreated in the beakers containing sufficient volume of distilled water (control) or aqueous solutions of GA_3, BA, Kin, 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 [11]. 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 the 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 0.25 M NaCI 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 µmol/m^2/s PAR (white fluorescent lamps). Anatomical sections were taken

Table 1. Some of parameters of root anatomy of radish seedlings grown in 0.0 and 0.25 M NaCl at 25 ºC for 20 d after growth regulator pretreatments

NaCl (M)	Pretreatment (µM)	Root diameter (µm)	Root hair number	Epidermis cell size (µm)		Cortex zone thickness (µm)	Endodermis cell size (µm)		Vascular cylinder diameter (µm)	Protoxylem width (µm)	Metaxylem width (µm)	Trachea diameter (µm)
				Width	Length		Width	Length				
0.0	**Control**	*144± 9.3 ^{hkl}	56±8.4 ^{n}	2.0±0.1 ^{bc}	2.6±0.5 ^{d}	52.5±5.4 ^{hk}	1.7±0.4 ^{e}	1.7±0.2 ^{bc}	25.5±6.8 ^{a}	3.6±0.5 ^{hk}	7.8±0.5 ^{ghk}	1.7±0.4 ^{de}
	GA_3	132±8.2 ^{e}	61±8.4 ^{n}	4.4±0.5 ^{k}	4.3±0.6 ^{fg}	53.5±4.7 ^{hk}	2.5±0.5 ^{f}	2.3±0.4 ^{d}	33±4.8 ^{bcde}	3.8±0.4 ^{kl}	7.5±0.4 ^{gh}	1.6±0.4 ^{cde}
	BA	151±9.0 ^{kl}	19±3.9 ^{bcd}	3.5±0.5 ^{fg}	4.2±0.4 ^{fg}	68.2±4.2 ^{no}	1.4±0.4 ^{abcde}	1.7±0.4 ^{bc}	35±5.2 ^{cdefg}	3.6±0.5 ^{hk}	6.2±0.7 ^{de}	1.5±0.5 ^{bcde}
	Kin	91.5± 8.1 ^{b}	26.5±5.7 ^{def}	2.9±0.7 ^{e}	2.0±0.6 ^{bc}	34±5.6 ^{b}	1.4±0.2 ^{abcde}	1.0±0.1 ^{a}	36±3.9 ^{cdefgh}	4.2±0.7 ^{lm}	8.0±0.6 ^{hk}	1.1±0.2 ^{ab}
	E	133.5±13.3 ^{efg}	57±8.2 ^{n}	2.9±0.1 ^{e}	5.2±0.7 ^{hk}	56±1.7 ^{hkl}	1.4±0.2 ^{abcde}	1.8±0.6 ^{bc}	43±9.7 ^{hk}	4.4±0.5 ^{m}	7.0±0.4 ^{fg}	2.3±0.4 ^{fg}
	EBR	120±9.1 ^{cd}	39.5±12.1 ^{hkl}	1.8±0.5 ^{ab}	2.4±0.5 ^{cd}	45±6.2 ^{efg}	1.1±0.3 ^{ab}	1.6±0.4 ^{bc}	43.5±1.5 ^{k}	1.4±0.4 ^{a}	5.7±0.3 ^{bcd}	1.4±0.5 ^{abcd}
	TRIA	127±6.7 ^{de}	40.5±14.9 ^{kl}	1.6±0.4 ^{ab}	2.4±0.5 ^{cd}	55±4.1 ^{hk}	1.1±0.2 ^{ab}	1.7±0.4 ^{bc}	37.5±4.8 ^{efghk}	2.2±0.4 ^{cde}	7.8±1.4 ^{ghk}	1.4±0.3 ^{abcd}
	Cad	170±6.8 ^{m}	47±5.3 ^{lm}	1.7±0.1 ^{ab}	1.8±0.4 ^{ab}	71.5±4.1 ^{o}	1.0±0.1 ^{a}	1.4±0.4 ^{ab}	29±6.1 ^{abc}	1.8±0.3 ^{abc}	3.9±0.8 ^{a}	1.1±0.2 ^{ab}
	Put	90.2±7.9 ^{b}	28±7.5 ^{efg}	3.1±0.5 ^{ef}	2.7±0.6 ^{de}	39±6.5 ^{bcde}	1.3±0.4 ^{abcd}	1.4±0.5 ^{ab}	25.5±3.6 ^{a}	3.0±0.4 ^{efg}	6.6±0.6 ^{ef}	1.7±0.5 ^{de}
	Spd	130±10.5 ^{e}	35.5±6.4 ^{ghk}	1.3±0.4 ^{a}	1.4±0.5 ^{a}	35±6.2 ^{bc}	0.9±0.1 ^{a}	1.0±0.1 ^{a}	38±1.1 ^{efghk}	2.0±0.3 ^{bcd}	6.0±0.8 ^{cde}	2.9±0.4 ^{hk}
	Spm	111±9.9 ^{c}	29.5±4.3 ^{efg}	4.1±0.5 ^{hk}	4.2±0.4 ^{fg}	35.5±1.3 ^{bc}	1.6±0.4 ^{cde}	1.7±0.4 ^{bc}	36.5±4.1 ^{defgh}	3.8±0.6 ^{kl}	6.7±0.6 ^{ef}	1.9±0.5 ^{ef}
0.25	**Control**	59±13.2 ^{a}	14.7±4.1 ^{abc}	1.6±0.5 ^{ab}	1.9±0.7 ^{abc}	38±7.1 ^{bcd}	1.5±0.2 ^{bcde}	1.3±0.3 ^{ab}	24±4.5 ^{a}	2.3±0.6 ^{cde}	7.5±0.5 ^{gh}	1.4±0.5 ^{abcd}
	GA_3	116±8.0 ^{c}	54±9.2 ^{mn}	4.2±0.6 ^{k}	4.8±0.6 ^{gh}	40.5±5.5 ^{cdef}	2.7±0.4 ^{f}	3.0±0.6 ^{e}	33±5.2 ^{bcde}	2.3±0.4 ^{cde}	7.1±0.9 ^{fg}	1.4±0.2 ^{abcd}
	BA	153.5±10.1 ^{l}	13.5±4.7 ^{ab}	3.8±0.4 ^{gh}	4.1±0.4 ^{f}	57±3.4 ^{kl}	1.2±0.4 ^{abc}	1.6±0.5 ^{bc}	43±3.4 ^{hk}	3.3±0.6 ^{ghk}	5.1±0.5 ^{b}	1.5±0.5 ^{bcde}
	Kin	131±6.1 ^{e}	13.3±4.3 ^{ab}	2.6±0.5 ^{de}	1.8±0.4 ^{ab}	40.5±2.8 ^{cdef}	1.4±0.5 ^{abcde}	2.0±0.4 ^{cd}	28±2.5 ^{ab}	3.2±1.1 ^{gh}	7.0±1.0 ^{fg}	1.3±0.4 ^{abcd}
	E	143.5±19.8 ^{ghkl}	42.5±15.2 ^{kl}	3.0±0.4 ^{e}	5.6±0.5 ^{k}	61.5±6.6 ^{lm}	1.5±0.3 ^{bcde}	2.4±0.5 ^{d}	51±6.5 ^{l}	4.7±0.6 ^{m}	7.6±0.5 ^{gh}	2.7±0.5 ^{h}
	EBR	135±11.7 ^{efgh}	38±18.7 ^{hk}	2.0±0.4 ^{bc}	2.6±0.5 ^{d}	68±5.8 ^{no}	1.4±0.5 ^{abcde}	1.4±0.4 ^{ab}	40.5±2.8 ^{fghk}	1.6±0.5 ^{ab}	6.7±0.4 ^{ef}	1.4±0.4 ^{abcd}
	TRIA	136±11.4 ^{efgh}	9±1.0 ^{a}	2.6±0.5 ^{de}	3.2±0.6 ^{e}	50±5.2 ^{gh}	1.3±0.4 ^{abcd}	1.4±0.3 ^{ab}	40±5.2 ^{fghk}	2.6±0.5 ^{ef}	8.5±1.1 ^{k}	1.0±0.1 ^{ab}
	Cad	142±10.5 ^{fghk}	32±3.4 ^{fgh}	1.5±0.5 ^{ab}	1.4±0.4 ^{a}	63±4.2 ^{mn}	1.0±0.1 ^{a}	1.0±0.1 ^{a}	42±1.1 ^{ghk}	1.6±0.4 ^{ab}	3.8±0.7 ^{a}	0.9±0.0 ^{a}
	Put	63±13.1 ^{a}	22±4.3 ^{cde}	2.4±0.6 ^{cd}	5.0±0.8 ^{h}	20±5.2 ^{a}	1.7±0.4 ^{e}	2.0±0.6 ^{cd}	36±7.3 ^{cdefgh}	3.5±0.2 ^{ghk}	7.0±0.6 ^{fg}	2.4±0.6 ^{gh}
	Spd	147.5±8.2 ^{kl}	42±7.1 ^{kl}	1.5±0.4 ^{ab}	1.8±0.4 ^{ab}	42.5±2.6 ^{def}	1.2±0.3 ^{abc}	1.3±0.4 ^{ab}	50±4.01 ^{l}	2.5±0.5 ^{def}	9.4±0.9 ^{l}	1.9±0.3 ^{ef}
	Spm	96±8.4 ^{b}	23.5±4.1 ^{de}	4.3±0.4 ^{k}	4.8±0.4 ^{gh}	45.5±5.9 ^{efg}	2.3±0.4 ^{f}	2.4±0.5 ^{d}	29.5±6.4 ^{abcd}	3.4±0.5 ^{ghk}	5.3±0.4 ^{bc}	1.8±0.5 ^{ef}

* The difference between values with the same letter in each column is not significant at the level 0.05 (± Standard deviation)

from the roots of 20-day-old seedlings by a microtome, in 6-7 µm thickness.

Root hair numbers in a 1-mm^2 unit area were counted by using ocular micrometer. These counts were made in each root 10 times as 3 replicates and the averages were calculated. Root diameter, epidermis cell size, cortex zone thickness, endodermis cell size, vascular cylinder diameter, protoxylem width, metaxylem width and trachea diameter were also determined in µm by using ocular micrometer.

Statistical evaluation concerning all parameters was realized by using SPSS program according to Duncan's multiple range test.

RESULTS

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

In distilled water medium, all of the pretreatments except Cad notably reduced the root diameter in comparison with control seedlings. The applyings except GA_3 and E caused a prominant decrease on the root hair number. EBR, TRIA, Cad and Spd slightly reduced the epidermis cell width while the others increased this parameter. GA_3 was statistically the most effective applying on the epidermis cell width. As for the epidermis cell length, GA_3, BA, EBR, Put and Spm increased this parameter while the others decreased it. Although BA, E and Cad increased the cortex zone thickness, Kin, EBR, Put, Spd and Spm reduced this parameter. GA_3 and TRIA statistically showed the same values as control. The most effective regulator was again Cad on the cortex zone thickness as the case of root diameter. The pretreatments except GA_3 decreased the endodermis cell width and length. All of growth regulators except Put increased the vascular cylinder diameter. EBR was statistically the most effective applying on this parameter. GA_3, Kin, E and Spm partly increased the protoxylem width while the others reduced this parameter. The applyings except Kin and TRIA decreased the metaxylem width. E, Spd and Spm increased the trachea diameter while the others reduced this parameter. The most effective regulator was E on the trachea diameter (Table 1).

0.25 M salinity decreased the root diameter, root hair number, epidermis cell size, cortex zone thickness, endodermis cell size, protoxylem and metaxylem width and trachea diameter in varying degrees in the control seedlings non-pretreated with the growth regulators, in comparison with roots of the ones in distilled water medium, but not show a meaningful effect, statistically, on the vascular cylinder diameter (Table 1).

On the other hand, all of the applyings except Put markedly increased the root diameter and cortex zone thickness in comparison with the control seedlings grown in 0.25 M salinity. BA and EBR statistically showed the most positive effects on these parameters, respectively. BA, Kin and TRIA reduced the root hair number while the others increased this parameter. The most effective regulator was GA_3 on the root hair number. The pretreatments except Cad and Spd increased the epidermis cell width. GA_3 and Spm were the most effective applyings on this parameter. Kin, Cad and Spd partly decreased the epidermis cell length while the others notably increased this parameter. The most effective pretreatment was E on the epidermis cell length. The growth regulators mostly reduced the endodermis cell width while they mostly increased the endodermis cell length. GA_3 and Spm caused a maximum increase on the endodermis cell width, whereas GA_3 on the endodermis cell length. All of the applyings markedly increased the vascular cylinder diameter. E and Spd were the most effective regulators on this parameter. Although the pretreatments mostly increased the protoxylem width, they mostly decreased the metaxylem width. EBR and Cad caused a prominant reduce on the protoxylem width, whereas Cad on the metaxylem width. E, Put, Spd and Spm increased the trachea

diameter while TRIA and Cad decreased this parameter. The others statistically showed same values as control (Table 1).

DISCUSSION

It was reported previously that saline conditions negatively affect growth and development events in general, even in halophytes. However, the effect mechanism of salinity has not been completely clarified so far [12, 13].

After a 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 transfered into pots containing Hoagland solution, and those that were in saline medium into pots containing NaCI in the same concentration prepared with Hoagland solution. After that they were grown for 20 days.

Salinity of the medium caused changes in the anatomic properties of the seedlings' roots. Root diameter, root hair number, epidermis cell size, cortex zone thickness, endodermis cell size, protoxylem and metaxylem width and trachea diameter in 0.25 M salinity decreased in comparison with those of distilled water medium. In addition, this salt level had no effect on the vascular cylinder diameter (Table 1). These results indicate that radish roots acquire xeromorphic (for example, the decrease in cortex zone thickness and epidermis cell size) features [14] in salinity medium.

Among the most cited studies related to anatomical modifications induced by salinity stress is Strogonov's classic research [14], which could not detect differences in the root diameter of seedlings growth under saline conditions, but this author reported that salinity was associated with a greater number of small diameter xylem vessels. In contrast, Neumann [8] found an increase in the root diameter in saline medium and suggested that a reduction in cell size, an increase in root diameter and a smaller plant size could be adaptive advantages for prolonged survival in saline or dry soils. Kalaji and Pietkiewicz [9] and Shannon et al. [10] determined that salt stress increased the endodermis cell size, diameter of root and vascular cylinder. Casenave et al. [6] observed that salinity caused a decrease in the development of the cortex and xylem. In addition, same researchers pointed out that the lower development of the xylem was probably caused by a repression in the development of metaxylem vessels. Similar results were reported by Reinhardt and Rost [15] who examined different concentrations of salt on the growth of cotton.

In this study, the growth regulators used generally increased the root diameter, root hair number, epidermis cell size, cortex zone thickness, endodermis cell length, vascular cylinder diameter and protoxylem width in saline medium in comparison with control while they usually decreased the endodermis cell width, metaxylem width and trachea diameter (Table 1). These observations indicate that radish roots acquire halosucculence (for example, the increase in cortex zone thickness and epidermis cell size) properties [14] in saline medium by pretreatments of growth regulators. Therefore these pretreatments can provide adaptation to salt stress by decreasing the metaxylem width and trachea diameter or by increasing the root diameter and root hair number and so ease water uptake and transportation.

It is surprising that many pretreatments of plant growth regulators used in this work are successful in the adaptation of radish seedlings to salt stress. This indicates that salt tolerance in plants caused by absolute presence or absence of a growth regulator may not be 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 may be needed to alleviate salt stress on root anatomy. Our data may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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