Effects of pretreatments of some growth regulators on the stomata movements of barley seedlings grown under saline (NaCl) conditions

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ABSTRACT

In this work, the effects of double, triple and quadruple combinations of gibberellic acid, kinetin, 24-epibrassinolide and polyamines (cadaverine, putrescine, spermidine, spermine) on the stomata movements in the leaves of barley seedlings grown under saline conditions were studied. In the control seedlings, the stomata number, stomata index and stomata length increased in the upper surfaces of leaves in comparison with their lower surfaces. In addition, the epidermis cell number in the leaves of control plants were fewer in the upper surface than that in the lower surface, but the stomata were statistically in the equal width in both surfaces. As for the applyings, they generally decreased stomata number, stomata index, stomata length and epidermis cell number, while they increased the stomata width in the upper and especially in the lower surface according to the control. The growth regulators used may have served to adaptation of barley seedlings to saline conditions by causing a decrease in most of the mentioned parameters.

Keywords: barley; plant growth regulators; salt stress; stomata movements

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 (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. Soil salinity affects plant growth and development by osmotic stress, injurious effects of toxic Na^+ and Cl^- ions and nutrient imbalance caused by excess of Na^+ and Cl^- ions (Sairam and Tyagi 2004).

Salinity mostly causes alterations in leaf morphology and anatomy of plants. It is well known that high salinity decreases stomata number (Kemp and Cunningham 1981), stomata index (Bray and Reid 2002), epidermis cell number (Martins and Castro 1999), stomata length and width (Kılıç et al. 2007).

On the other hand, individual effects of gibberellic acid, kinetin, 24-epibrassinolide and polyamines (PA_S) on the stomata movements of seedlings grown under saline conditions were previously reported in our previous works (Çavuşoğlu et al. 2007a, b). However, no study has been encountered concerning various combinations of the mentioned growth regulators on stomata movements until now.

In this work, the effects of combinations composed of gibberellic acid, kinetin, 24-epibrassinolide and PA_S on the stomata movements of the seedlings from barley caryopses subjected to salinity stress were studied.

MATERIAL AND METHODS

The caryopses, NaCl concentrations and growth regulators

In this study, barley (*Hordeum vulgare* L. cv. Bülbül 89) caryopses were used. The caryopses were surface sterilized with 1% sodium hypochloride. NaCl concentration used in the experiments was 0.30M.

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Growth regulators were 900μM gibberellic acid (GA_3), 100μM kinetin (Kin), 3μM 24-epibrassinolide (EBR), 10μM a polyamine, PA (cadaverine/Cad, putrescine/Put, spermidine/Spd and spermine/Spm).

Germination of the caryopses

Germination experiments were carried out at a constant temperature (20°C), in the dark in an incubator. Barley grains in adequate amount were pretreated in the beakers containing sufficient volume of distilled water (control) or aqueous solutions of double, triple and quadruple combinations of GA_3, Kin, EBR, Cad, Put, Spd and Spm for 24 h at room temperature. At the end of this pretreatment, the solutions were filtered immediately and the grains were dried in vacuum (Braun and Khan 1976). 25 grains from every application were arranged into Petri dishes (10 cm diameter) lined by 2 sheets of Whatman No. 1 filter paper moistened with 7 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 caryopses and stomata movements

The seedlings from the grains germinated in the incubator at 20°C for 7 days were transferred into the pots with perlite including 0.30M NaCl 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). Superficial sections were taken from the second leave of 20-day-old seedlings by a microtome, in 6–7 μm thickness.

Stomata and epidermis cells in a 1-mm^2 unit area were counted to determine the stomata index. These counts were made both in the lower and upper surfaces of each leaf 10 times as 3 replicates and the averages were calculated. After the determination of the number of stomata and epidermis cells in the leaf unit area, the stomata index was estimated according to Meidner and Mansfield's method (Meidner and Mansfield 1968):

stomata index = stomata number in unit area/(stomata number in unit area + epidermis cell number in unit area) × 100

Stomata width and length 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 AND DISCUSSION

The findings related with effects of double, triple and quadruple combinations on the stomata movements of the seedlings in 0.30M salinity are presented in Table 1. The epidermis cell number in the leaves of control seedlings grown in saline medium was lower in the upper surface than that in the lower surface. The pretreatments mostly decreased the epidermis cell number, in both surfaces. While Kin + Spm and GA_3 + Kin + EBR + Put partly increased the epidermis cell number in the upper surface, GA_3 + Kin + EBR + Put caused a slight increase in the lower surface. On the other hand, many double pretreatments with GA_3 leaded to the maximum decrease in the epidermis cell number in the upper surface, whereas GA_3 + Spm and EBR + Spd in the lower surface.

The stomata number in control group was higher in the upper than in the lower surface. The pretreatments generally decreased the stomata number especially in the upper surface. Quadruple combinations of GA_3 caused maximum decrease of this parameter in the upper surface. Some double and triple pretreatments followed these. As for the lower surface, while some applyings reduced the stomata number, the others showed the same values as the control.

The stomata index of control was higher in the upper than in the lower surface. The pretreatments mostly decreased this index in the upper surface while they increased it the lower surface. Quadruple combinations caused maximum decrease of stomata index, except for GA_3 + Kin + EBR + Cad and GA_3 + EBR + Spm.

The stomata of control were statistically in the equal width in both the upper and lower surfaces. The applyings mostly increased the stomata width in the lower surface while they gave mostly equal results to those of the control in the upper surface. Moreover, the stomata of control were longer in the upper than in the lower surface. Most of the pretreatments reduced the stomata length in the upper surface; the biggest reduction in their length in the upper was caused by Kin + Spm and GA_3 + EBR.

Table 1. Stomata movements in the leaves of barley seedlings grown in 0.30M NaCl at 25°C for 20 days after growth regulator pretreatments

Growth regulator (μM)	Epidermis cell number		Stomata number		Stomata index		Stomata width (μm)		Stomata length (μm)	
	upper	lower	upper	lower	upper	lower	upper	lower	upper	lower
control	*17.0 ± 2.0^{e}	19.3 ± 3.3^{h}	4.7 ± 0.5$^{e, f}$	4.1 ± 1.1^{e}	21.6	17.5	7.1 ± 1.1$^{b, c}$	7.4 ± 1.1^{c}	14.9 ± 2.0^{h}	12.5 ± 1.1$^{d, e}$
GA_3 + Kin	13.0 ± 0.9^{a}	14.4 ± 1.5$^{c, d}$	3.7 ± 0.8$^{c, d}$	4.5 ± 0.9$^{e, f}$	21.1	23.8	7.2 ± 0.6$^{b, c}$	7.4 ± 0.9^{c}	12.0 ± 0.9^{e}	9.0 ± 0.9^{a}
GA_3 + EBR	13.0 ± 0.9^{a}	16.0 ± 0.6$^{e, f}$	3.4 ± 0.8^{c}	4.9 ± 0.9^{f}	19.6	23.4	9.1 ± 0.5^{f}	6.6 ± 0.8^{a}	10.3 ± 0.4^{b}	11.3 ± 1.8$^{c, d}$
GA_3 + Cad	17.0 ± 1.8^{e}	15.7 ± 1.3^{e}	3.4 ± 0.5^{c}	4.4 ± 1.1$^{e, f}$	16.6	21.8	7.6 ± 0.5$^{c, d}$	8.5 ± 1.4$^{e, f}$	12.6 ± 1.4$^{e, f}$	10.0 ± 1.2^{b}
GA_3 + Put	12.9 ± 3.4^{a}	15.3 ± 1.2$^{d, e}$	4.9 ± 1.3^{f}	4.5 ± 1.3$^{e, f}$	27.5	22.7	7.1 ± 0.5$^{b, c}$	9.0 ± 1.0^{f}	13.5 ± 2.0$^{f, g}$	12.5 ± 1.2$^{d, e}$
GA_3 + Spd	17.6 ± 1.4$^{e, f}$	15.0 ± 2.1^{d}	4.5 ± 1.0$^{d, e}$	4.2 ± 1.4^{e}	20.3	21.8	6.6 ± 0.5^{a}	6.8 ± 0.7$^{a, b}$	10.6 ± 0.6$^{b, c}$	14.8 ± 1.5^{f}
GA_3 + Spm	13.0 ± 1.1^{a}	12.9 ± 0.8^{a}	4.6 ± 0.8$^{e, f}$	4.8 ± 0.9^{f}	26.1	27.1	7.5 ± 1.2^{c}	8.3 ± 1.8^{e}	12.0 ± 1.0^{e}	12.4 ± 1.2$^{d, e}$
Kin + EBR	15.2 ± 0.6^{c}	13.3 ± 1.7^{b}	4.1 ± 1.3^{d}	4.0 ± 0.6^{e}	21.2	23.1	7.6 ± 0.9$^{c, d}$	8.5 ± 1.3$^{e, f}$	12.1 ± 1.1^{e}	12.7 ± 2.0$^{d, e}$
Kin + Cad	16.1 ± 1.4^{d}	14.1 ± 1.5^{c}	4.8 ± 0.9^{f}	3.7 ± 1.3$^{d, e}$	22.9	20.7	7.4 ± 2.0^{c}	8.3 ± 1.3^{e}	12.4 ± 1.7$^{e, f}$	11.7 ± 0.6$^{c, d}$
Kin + Put	16.1 ± 1.2^{d}	17.9 ± 2.2^{g}	4.5 ± 1.4$^{d, e}$	5.7 ± 0.8^{g}	21.8	24.1	8.3 ± 1.5$^{d, e}$	8.0 ± 1.4$^{d, e}$	12.1 ± 1.6^{e}	12.3 ± 0.8$^{d, e}$
Kin + Spd	16.4 ± 2.2^{d}	13.4 ± 1.2^{b}	3.8 ± 1.1$^{c, d}$	4.4 ± 0.8$^{e, f}$	18.8	24.7	7.2 ± 0.7$^{b, c}$	8.3 ± 0.9^{e}	13.4 ± 1.0$^{f, g}$	13.9 ± 1.4$^{e, f}$
Kin + Spm	18.4 ± 2.1^{f}	14.6 ± 0.9$^{c, d}$	4.3 ± 0.8$^{d, e}$	3.8 ± 0.6$^{d, e}$	18.9	20.6	7.6 ± 0.9^{c}	10.2 ± 0.6^{h}	9.8 ± 0.7^{a}	10.9 ± 1.6^{c}
EBR + Cad	14.6 ± 1.7^{b}	14.5 ± 0.9$^{c, d}$	2.5 ± 0.9^{b}	4.5 ± 0.9$^{e, f}$	14.6	23.6	9.2 ± 1.2^{f}	9.5 ± 0.5^{g}	14.2 ± 1.6$^{g, h}$	15.0 ± 0.8^{f}
EBR + Put	14.7 ± 1.6^{b}	14.1 ± 0.9^{c}	3.8 ± 1.1$^{c, d}$	1.9 ± 0.5$^{a, b}$	20.5	11.8	7.8 ± 1.0$^{c, d}$	10.0 ± 0.4^{h}	13.2 ± 1.8^{f}	16.0 ± 2.2^{g}
EBR + Spd	15.4 ± 1.8^{c}	13.1 ± 0.8$^{a, b}$	3.6 ± 0.9$^{c, d}$	3.5 ± 0.5^{d}	18.9	21.0	8.5 ± 1.3$^{d, e}$	9.0 ± 1.8^{f}	11.7 ± 1.2$^{d, e}$	15.8 ± 1.6^{g}
EBR + Spm	16.3 ± 0.4^{d}	14.2 ± 1.1^{c}	4.0 ± 1.2^{d}	4.1 ± 1.3^{e}	19.7	22.4	8.0 ± 1.8^{d}	11.5 ± 1.3^{i}	14.8 ± 1.7^{h}	12.1 ± 1.2^{d}
GA_3 + Kin + EBR	17.2 ± 1.0^{e}	13.6 ± 0.8$^{b, c}$	3.2 ± 0.7$^{b, c}$	2.3 ± 0.6^{b}	15.6	14.4	7.8 ± 1.0$^{c, d}$	8.6 ± 0.9$^{e, f}$	12.4 ± 2.1$^{e, f}$	12.0 ± 1.0^{d}
GA_3 + Kin + Cad	15.5 ± 1.9^{c}	16.4 ± 1.1^{f}	3.0 ± 1.0$^{b, c}$	2.7 ± 0.6$^{b, c}$	16.2	14.1	8.7 ± 1.0^{e}	7.5 ± 0.7^{c}	11.8 ± 1.3$^{d, e}$	13.7 ± 1.5$^{e, f}$
GA_3 + Kin + Put	15.8 ± 1.4$^{c, d}$	15.7 ± 1.1^{e}	3.1 ± 0.8$^{b, c}$	3.1 ± 1.2^{c}	16.4	16.4	6.9 ± 1.4$^{a, b}$	8.6 ± 1.1$^{e, f}$	11.3 ± 1.4$^{c, d}$	14.2 ± 1.2$^{e, f}$
GA_3 + Kin + Spd	15.3 ± 1.4^{c}	14.3 ± 1.0^{c}	3.0 ± 0.6$^{b, c}$	3.6 ± 0.9^{d}	16.3	20.1	6.5 ± 0.7^{a}	8.5 ± 0.8$^{e, f}$	13.7 ± 1.0^{g}	10.8 ± 0.7^{c}
GA_3 + Kin + Spm	15.9 ± 1.3$^{c, d}$	15.3 ± 1.3$^{d, e}$	3.4 ± 0.9^{c}	3.3 ± 0.6$^{c, d}$	17.6	17.7	8.1 ± 0.9^{d}	7.9 ± 0.5^{d}	12.5 ± 1.0$^{e, f}$	11.0 ± 0.9$^{c, d}$
GA_3 + EBR + Cad	13.1 ± 1.4^{a}	14.7 ± 0.9$^{c, d}$	4.8 ± 0.7^{f}	4.6 ± 0.6$^{e, f}$	26.8	23.8	8.0 ± 1.0^{d}	8.4 ± 1.2^{e}	10.9 ± 0.9^{c}	13.4 ± 1.0^{e}
GA_3 + EBR + Put	14.4 ± 1.3^{b}	15.9 ± 2.3$^{e, f}$	3.5 ± 1.0^{c}	4.8 ± 1.3^{f}	19.5	23.1	7.4 ± 0.9^{c}	8.4 ± 1.0^{e}	10.6 ± 0.8$^{b, c}$	13.5 ± 1.4^{e}
GA_3 + EBR + Spd	16.9 ± 0.8$^{d, e}$	15.4 ± 0.9$^{d, e}$	4.2 ± 0.9^{d}	2.4 ± 0.5^{b}	19.9	13.4	8.0 ± 1.4^{d}	7.0 ± 0.6^{b}	14.1 ± 1.2$^{g, h}$	12.0 ± 1.1^{d}
GA_3 + EBR + Spm	15.9 ± 1.1$^{c, d}$	14.5 ± 1.4$^{c, d}$	2.7 ± 1.4^{b}	4.1 ± 1.1^{e}	14.5	22.0	6.6 ± 0.6^{a}	7.0 ± 0.9^{b}	14.0 ± 1.2$^{g, h}$	11.3 ± 1.0$^{c, d}$
GA_3 + Kin + EBR + Cad	16.8 ± 1.1$^{d, e}$	14.3 ± 0.8^{c}	4.4 ± 1.0$^{d, e}$	3.6 ± 0.8^{d}	20.7	20.1	7.8 ± 1.0$^{c, d}$	7.8 ± 0.6^{d}	12.4 ± 0.8$^{e, f}$	13.6 ± 1.8^{e}
GA_3 + Kin + EBR + Put	19.0 ± 1.4^{g}	21.0 ± 1.4^{i}	2.0 ± 0.6^{a}	1.7 ± 0.8^{a}	10.5	10.4	8.0 ± 2.0^{d}	7.1 ± 0.7^{b}	11.0 ± 1.2^{c}	10.0 ± 1.2^{b}
GA_3 + Kin + EBR + Spd	15.1 ± 1.5$^{b, c}$	15.5 ± 0.8$^{d, e}$	2.7 ± 1.0^{b}	3.5 ± 0.8^{d}	15.1	18.4	7.0 ± 1.0$^{a, b}$	7.9 ± 1.5^{d}	10.9 ± 2.1^{c}	12.4 ± 1.1$^{d, e}$
GA_3 + Kin + EBR + Spm	16.2 ± 1.1^{d}	16.4 ± 1.6^{f}	2.7 ± 0.6^{b}	1.6 ± 0.8^{a}	14.2	18.0	6.9 ± 1.0$^{a, b}$	7.8 ± 1.6^{d}	11.5 ± 1.0^{d}	9.4 ± 1.4$^{a, b}$

*the difference between values with the same letter in each column is not significant at the level 0.05

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

In this study, combinations composed of the growth regulators generally increased the stomata width in the saline medium in the upper and especially in the lower surface, compared to the control, but decreased the epidermis cell number in both surfaces. Moreover, they reduced the stomata number, stomata index and stomata length, particularly in the upper surface (Table 1).

It is evident that salinity causes significant changes in stomata movements (Shennan et al. 1987, Martins and Castro 1999). Reducing effects of salt stress on epidermis cell number (Curtis and Lauchli 1987), stomata number (Flowers et al. 1986, Hwang and Chen 1995), stomata index (Bray and Reid 2002) and stomata width (Çavuşoğlu et al. 2007b) were reported previously.

Our findings on the epidermis cell number, stomata number and stomata index in the leaves of seedlings from barley caryopses pretreated with various growth regulators are consistent with those of the above-mentioned authors focusing on salinity; the results indicate that barley leaves acquire succulent properties (Strogonov 1964) under saline conditions by pretreatments with growth regulators. These pretreatments can provide adaptation to salt stress by decreasing the stomata number and length in the upper surface, and thus by reducing the transpiration. In addition, the pretreatments can serve to the same aim by causing a reduction of leaf area as a result of decreasing the epidermis cell number of both surfaces.

On the other hand, there are also reports indicating that salinity increases epidermis cell number (Bray and Reid 2002), stomata number (Curtis and Lauchli 1987) and stomata length (Kılıç et al. 2007). Stomata can close as a response to salt stress due to an increase of Na^+ and Cl^- ions and also a decrease in K^+ amount in leaves. Plants then survive because transpiration and water loss decrease (Robinson et al. 1983). Moreover, an increase in ABA content of the leaves under salt stress is known to cause stomata closing (Cramer and Quarrie 2002).

It is surprising that many pretreatments with plant growth regulators used in this work are successful in the adaptation of barley 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 stomata movements. Our data may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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