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# Effects of Some Plant Growth Regulators on Leaf Anatomy of Radish Seedlings Grown Under Saline Conditions

Kürşat ÇAVUŞOĞLU[1*] Semra KILIÇ[1] Kudret KABAR[1]

[1]Süleyman Demirel University, Faculty of Arts and Science, Biology Department, 32260-Isparta/Turkey

*Corresponding Author
e-mail: kursat@fef.sdu.edu.tr

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**Abstract**

In this work, the effects of gibberellic acid, ethylene, 24-epibrassinolide, triacontanol and polyamine (cadaverine, putrescine, spermidine, spermine) pretreatments on the leaf anatomy of radish seedlings grown under saline conditions were studied. Salt stress decreased the stomata number, epidermis cell number and width, leaf thickness and distance between vascular bundles in the varying degrees in the control seedlings non-pretreated with the growth regulators, in comparison with leaves of the ones in distilled water medium. On the other hand, it was observed that the growth regulator pretreatments affected in different degrees on the leaf anatomy of radish seedlings, and this difference was significant.

**Key words:** Leaf anatomy, plant growth regulators, radish, salt stress

## INTRODUCTION

Salinity is one of the most important problems in the agriculture areas of the world. Nearly 20 % of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity [21] 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 [2, 10] 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 [18] In addition, it is evident that there are big changes in leaf morphology and anatomy of the plants growing in saline soils. It is well known that high salinity mostly causes alterations in stomata width and length [14], stomata index [4], leaf thickness [12], distance between vascular bundles [6], epidermis cell number and stomata number [9]

On the other hand, the effects of gibberellic acid, ethylene, 24-epibrassinolide, triacontanol and polyamines on the leaf anatomy of monocot seedlings grown under saline conditions had already been reported in our previous works [6-8, 14] However, it has not been encountered any study concerning effects of the mentioned growth regulators on the leaf anatomy of dicot seedlings grown in both distilled water and saline medium until now.

In this work, the influences of gibberellic acid, ethylene, 24-epibrassinolide, triacontanol and polyamines on the leaf 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 ($GA_3$), 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 $GA_3$, 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 [3] 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 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). 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 [16] method:

$$\text{Stomata index} = \frac{\text{Stomata number in unit area}}{\text{Stomata number in unit area + epidermis cell number in unit area}} \times 100$$

Stomata width and length, epidermis cell width, leaf thickness and distance between vascular bundles 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 some parameters of the leaf anatomy of radish seedlings grown in distilled water and saline medium are presented in Table 1.

In distilled water medium, most of the growth regulator pretreatments decreased the stomata number in both surfaces in comparison with control seedlings. EBR and Cad caused important increases in the upper surface and also $GA_3$, E and Spm in the lower one. The applyings causing the most increase in the epidermis cell number were E, TRIA, EBR, Put and Spm in the upper surface and also only E in the lower one. The growth regulators slightly reduced the stomata width and length in the upper surface while in the lower one particularly E, TRIA, Spd and Spm leaded to important reductions in the stomata width, and also E, Spd, Spm in the stomata length. The pretreatments mostly decreased the stomata index in the upper surface while they generally increased this index in the lower one. As for the epidermis cell width, the applyings mostly increased the epidermis cell width in the upper surface while they decreased this parameter in the lower one. All of the pretreatments except TRIA and Spd increased the leaf thickness in a great extent according to the control. Cad was statistically the most effective applying on this parameter. Many growth regulators, specially $GA_3$, EBR and Cad applyings notably increased the distance between vascular bundles. The most effective regulator on this parameter was again Cad (Table 1).

A salinity of 0.25 M decreased the stomata number, epidermis cell number and width, leaf thickness and distance between vascular bundles in the varying degrees in the control seedlings non-pretreated with the growth regulators, in comparison with leaves of the ones in distilled water medium. This salt level leaded to the slight reductions in the stomata width and length in both surfaces. In addition, it markedly decreased the stomata index in the upper surface, but increased a small amount in the lower one (Table 1).

On the other hand, E, EBR and Put dramatically increased the stomata number in the upper surface of the leaves of the seedlings grown in 0.25 M salinity. As for the lower surface, E and TRIA reduced the stomata number, but Spm increased this parameter. In the upper surface E, EBR, Put and Spm, and also in the lower surface EBR, Put, Spd and Spm, clearly increased the epidermis cell number compared to the control, but others were ineffective on this parameter. Stomata width was reduced by many growth regulators particularly in the lower surface at this salt level. Although E and Put decreased the stomata length in the upper surface, the others statistically showed the same values as the control in both surfaces. E and Spd reduced the stomata index in the upper surface while EBR and Cad increased this index. As for the lower surface, all of the pretreatments except Spm reduced the stomata index, but Spm increased this parameter. In the upper surface E, TRIA and EBR, and in the lower surface E and TRIA, increased the epidermis cell width. The others statistically exhibited the same values as the control. All of the pretreatments except Spm increased the leaf thickness according to the control. On the other hand, all pretreatments stimulated the distance between vascular bundles compared to the 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 [1, 11]

Salinity of the medium caused changes in the anatomic properties of the seedlings' leaves. Stomata number, epidermis cell number and width, leaf thickness and distance between vascular bundles of the control seedlings in 0.25 M salinity decreased in comparison with those of distilled water medium (Table 1). The results we got comply with research findings arguing that salinity reduces stomata number [13], epidermis cell number [15] and leaf thickness [12] On the other hand, reducing effects of salt stress on stomata width, stomata length and stomata index were reported previously [6]

These observations indicate that radish leaves acquire both succulent (for example, in the lower surface the decrease in stomata number) and xeromorphic (for example, in the lower surface the reduce in epidermis cell width) properties [19]

On the other hand, stomata can close as a response to salt stress due to an increase of $Na^+$ and $CI^-$ ions and also a decrease in $K^+$ amount in leaves. Plants then survive because transpiration and water loss decrease [17, 20] Moreover, an increase in ABA content of the leaves under salt stress is known to cause stomata closing [5]

In this study, the growth regulators used generally reduced the stomata width and index in saline medium especially in the lower surface, compared to the control, but increased the epidermis cell number, leaf thickness and distance between vascular bundles (Table 1). These pretreatments can provide adaptation to salt stress by decreasing the stomata width and index, and thus by reducing the transpiration.

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. 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 leaf anatomy. Our data may serve to provide new conceptual tools for designing hypotheses of salt tolerance in plants.

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

| NaCl (M) | Pretreatment- | Stomata number | | Epidermis cell number | | Stomata width (µm) | | Stomata length (µm) | | Stomata index | | Epidermis cell width (µm) | | Leaf thickness (µm) | Distance between vascular bundles (µm) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower | | |
| 0.0 | Control | *13.5±5.1$^{ef}$ | 15.9±5.3$^{gh}$ | 26.8±2.5$^{de}$ | 33.3±4.0$^{g}$ | 5.4±1.5$^{c}$ | 6.1±0.9$^{fg}$ | 7.4±1.5$^{cde}$ | 8.3±1.2$^{ef}$ | 33.4 | 32.3 | 8.6±1.9$^{cdef}$ | 7.4±1.8$^{ef}$ | 85.3±8.6$^{ab}$ | 81.0±7.4$^{b}$ |
| | $GA_3$ | 10.5±2.1$^{bcde}$ | 19.7±3.5$^{i}$ | 27.3±3.7$^{de}$ | 32.4±1.5$^{ef}$ | 4.9±0.5$^{bc}$ | 6.4±1.1$^{fg}$ | 7.2±1.3$^{cde}$ | 8.6±2.0$^{ef}$ | 27.7 | 37.8 | 9.6±1.6$^{gh}$ | 6.8±1.4$^{cde}$ | 127.1±8.3$^{cde}$ | 98.1±4.4$^{d}$ |
| | E | 14.8±2.3$^{f}$ | 21.1±6.2$^{i}$ | 37.6±3.2$^{g}$ | 38.0±5.7$^{h}$ | 4.5±0.8$^{abc}$ | 5.1±0.5$^{cde}$ | 6.5±0.9$^{abc}$ | 6.8±0.4$^{abcd}$ | 28.2 | 35.7 | 10.6±0.8$^{ghi}$ | 9.0±1.0$^{g}$ | 134.2±16.3$^{def}$ | 91.3±7.4$^{bcd}$ |
| | TRIA | 11.3±2.9$^{cde}$ | 14.1±4.3$^{efg}$ | 34.8±7.0$^{g}$ | 28.6±7.0$^{d}$ | 4.4±1.0$^{ab}$ | 5.0±1.1$^{cde}$ | 6.8±1.2$^{bcde}$ | 7.7±1.4$^{de}$ | 24.5 | 33.0 | 9.4±1.3$^{gh}$ | 6.8±1.0$^{cde}$ | 81.6±12.9$^{a}$ | 92.3±7.5$^{bcd}$ |
| | EBR | 19.8±4.6$^{gh}$ | 12.4±2.1$^{defg}$ | 34.1±9.1$^{g}$ | 29.3±2.3$^{de}$ | 4.9±0.5$^{bc}$ | 5.6±1.0$^{f}$ | 7.0±0.9$^{bcde}$ | 7.2±1.2$^{abcde}$ | 36.7 | 29.7 | 6.4±0.5$^{a}$ | 4.4±0.5$^{a}$ | 111.4±13.4$^{bcd}$ | 95.6±8.6$^{cd}$ |
| | Cad | 20.4±2.5$^{h}$ | 18.0±2.1$^{hi}$ | 29.0±2.8$^{ef}$ | 33.2±2.8$^{g}$ | 5.4±0.9$^{c}$ | 5.8±0.9$^{fg}$ | 7.3±1.2$^{cde}$ | 9.2±1.5$^{f}$ | 41.2 | 35.1 | 13.8±1.0$^{k}$ | 10.4±0.5$^{h}$ | 179.1±7.4$^{gh}$ | 144.3±4.1$^{f}$ |
| | Put | 11.3±2.7$^{cde}$ | 13.6±1.8$^{efg}$ | 33.1±3.7$^{fg}$ | 27.4±2.2$^{cd}$ | 5.0±0.6$^{bc}$ | 6.8±1.2$^{g}$ | 7.8±1.1$^{e}$ | 9.4±1.2$^{f}$ | 25.4 | 33.1 | 7.4±1.1$^{abcde}$ | 5.0±0.7$^{ab}$ | 123.2±6.7$^{cde}$ | 85.6±10.0$^{bc}$ |
| | Spd | 10.0±2.8$^{bcd}$ | 13.2±4.1$^{defg}$ | 22.8±2.3$^{cd}$ | 22.2±3.5$^{b}$ | 4.9±0.5$^{bc}$ | 3.2±1.1$^{a}$ | 7.4±1.1$^{cde}$ | 5.9±1.2$^{a}$ | 30.4 | 39.3 | 6.6±0.8$^{ab}$ | 5.5±0.7$^{ab}$ | 82.5±4.1$^{a}$ | 81.1±14.3$^{b}$ |
| | Spm | 10.6±2.5$^{bcde}$ | 25.4±3.4$^{k}$ | 36.1±4.7$^{g}$ | 34.3±3.8$^{g}$ | 4.6±1.0$^{abc}$ | 5.0±0.8$^{cde}$ | 6.6±0.9$^{bcd}$ | 6.6±0.8$^{abcd}$ | 22.6 | 42.4 | 11.0±1.0$^{hi}$ | 8.6±0.8$^{fg}$ | 120.6±7.0$^{cde}$ | 93.4±12.0$^{bcd}$ |
| 0.25 | Control | 7.8±2.4$^{ab}$ | 11.8±3.5$^{cdef}$ | 17.4±2.0$^{ab}$ | 22.2±5.6$^{b}$ | 5.1±1.1$^{bc}$ | 5.6±0.6$^{f}$ | 7.3±1.0$^{cde}$ | 7.4±1.8$^{bcde}$ | 30.9 | 32.9 | 7.2±1.6$^{abcd}$ | 6.2±1.0$^{bcde}$ | 74.0±9.6$^{a}$ | 65.3±5.0$^{a}$ |
| | $GA_3$ | 9.1±2.3$^{abc}$ | 9.9±2.1$^{bcd}$ | 19.7±3.6$^{abc}$ | 22.3±1.7$^{b}$ | 5.3±1.4$^{bc}$ | 5.7±1.4$^{f}$ | 7.7±1.0$^{de}$ | 7.5±2.2$^{cde}$ | 31.5 | 30.7 | 8.8±1.3$^{def}$ | 6.2±1.3$^{bcde}$ | 115.3±10.0$^{cd}$ | 95.1±7.0$^{cd}$ |
| | E | 13.5±5.6$^{ef}$ | 7.1±2.8$^{ab}$ | 34.9±1.3$^{g}$ | 20.5±4.7$^{b}$ | 4.5±0.5$^{abc}$ | 5.5±1.4$^{f}$ | 6.0±0.8$^{ab}$ | 7.7±1.7$^{de}$ | 27.8 | 25.7 | 11.8±1.3$^{i}$ | 9.6±0.5$^{gh}$ | 145.4±22.6$^{ef}$ | 94.3±8.2$^{cd}$ |
| | TRIA | 8.5±1.7$^{abc}$ | 6.0±2.2$^{a}$ | 19.6±1.2$^{abc}$ | 16.7±3.2$^{a}$ | 5.0±0.6$^{bc}$ | 5.6±1.4$^{f}$ | 7.3±1.1$^{cde}$ | 8.3±1.9$^{ef}$ | 30.2 | 26.4 | 13.4±0.8$^{k}$ | 10.8±1.3$^{h}$ | 114.1±8.2$^{cd}$ | 101.2±5.4$^{d}$ |
| | EBR | 14.7±1.6$^{f}$ | 11.9±1.8$^{cdef}$ | 23.3±2.5$^{cd}$ | 38.9±2.0$^{h}$ | 5.2±0.6$^{bc}$ | 4.5±0.5$^{bcd}$ | 7.8±0.7$^{e}$ | 6.0±0.9$^{ab}$ | 38.4 | 23.4 | 9.0±0.7$^{fg}$ | 5.8±0.8$^{abcd}$ | 116.3±11.4$^{cde}$ | 98.1±2.7$^{d}$ |
| | Cad | 8.3±2.4$^{abc}$ | 11.3±3.9$^{cde}$ | 15.7±2.4$^{a}$ | 23.9±3.0$^{bc}$ | 4.6±0.6$^{abc}$ | 5.4±1.4$^{cdef}$ | 7.5±1.1$^{cde}$ | 7.7±1.1$^{de}$ | 34.5 | 32.1 | 8.2±1.3$^{bcdef}$ | 6.8±1.0$^{cde}$ | 193.2±4.4$^{h}$ | 123.6±15.6$^{e}$ |
| | Put | 12.8±3.4$^{def}$ | 8.6±1.8$^{abc}$ | 27.3±1.8$^{de}$ | 30.1±2.9$^{def}$ | 3.7±0.8$^{a}$ | 3.9±0.5$^{ab}$ | 5.5±0.8$^{a}$ | 6.2±1.4$^{abc}$ | 31.9 | 22.2 | 8.6±1.3$^{cdef}$ | 7.0±0.7$^{de}$ | 156.6±11.4$^{fg}$ | 132.1±7.5$^{e}$ |
| | Spd | 6.9±1.6$^{a}$ | 8.5±2.7$^{abc}$ | 21.7±2.4$^{bc}$ | 29.8±2.9$^{def}$ | 4.9±0.9$^{bc}$ | 4.3±1.0$^{bc}$ | 6.9±0.8$^{bcde}$ | 6.3±1.0$^{abcd}$ | 24.1 | 22.1 | 7.0±1.1$^{abc}$ | 5.4±1.1$^{abc}$ | 99.1±18.1$^{abc}$ | 98.7±9.0$^{d}$ |
| | Spm | 10.7±1.7$^{bcde}$ | 15.3±5.0$^{fgh}$ | 23.9±2.0$^{cd}$ | 26.6±2.8$^{cd}$ | 4.5±0.5$^{abc}$ | 4.1±0.9$^{abc}$ | 6.5±0.9$^{abc}$ | 6.3±1.1$^{abcd}$ | 30.9 | 36.5 | 7.6±0.5$^{abcde}$ | 5.4±1.1$^{abc}$ | 78.5±5.7$^{a}$ | 81.3±2.2$^{b}$ |

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