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Effects of Abscisic Acid on the Root, Stem and Leaf Anatomy of Radish Seedlings

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Abstract

In this work, the effects of various concentrations of abscisic acid on the root, stem and leaf anatomy of radish seedlings were studied. Although the concentrations of abscisic acid mostly decreased the root diameter, cortex zone thickness, endodermis cell length, vascular cylinder diameter, protoxylem and metaxylem width in comparison with roots of control seedlings grown in distilled water medium, they generally increased the root hair number and epidermis cell width. As for the stem anatomy, many of the concentrations stimulated more or less the stem diameter, epidermis cell width and cortex zone thickness while they usually reduced the epidermis cell length, vascular bundle width and trachea diameter. On the other hand, the concentrations used mostly increased the stomata number in the lower surface, stomata length in the upper surface and stomata index in both surfaces, whereas they generally decreased the epidermis cell number in the upper surface, stomata width and lenght in the lower surface, epidermis cell width in both surfaces, leaf thickness and distance between vascular bundles.

Keywords: Abscisic acid, leaf anatomy, radish, root anatomy, stem anatomy

INTRODUCTION

Abscisic acid (ABA) has been found to be a ubiquitous plant hormone in vascular plants. It has been detected in mosses, but appears to be absent in liverworts. Several genera of fungi produce ABA as a secondary metabolite [1].

ABA plays an important role in many physiological processes such as seed germination [2, 3], seedling growth [4], abscission [5], senescence [6], photosynthesis [7], transpiration [8], embryo morphogenesis [9] and synthesis of storage proteins and lipids [10]. In addition, various stress conditions such as salinity [11], drought [12], cold [13] and high temperature [14] increase endogenous amounts of ABA in plants. Thus, this hormone serves to adaptation or conservation of a plant to the mentioned environmental stresses.

ABA mostly causes also alterations in the root, stem and leaf anatomies of plants. Unfortunately, there are few studies about the effects of ABA on these subjects. Schnall and Quatrano [15] observed that exogenously applied ABA, at high concentrations, reduced the number of root hairs in *Arabidopsis thaliana*. Unyayar et al. [16] determined that exogenous ABA increased the number of xylem vessels in roots of notabilis, a hybrid species of Cupressus. Weryszko-Chmielewska and Kozak [17] demonstrated that ABA concentrations decreased the number and diameter of xylem vessel in stems of *Gloriosa rothschildiana*. Quarrie and Jones [18] found that ABA acquired xeromorphic features in *Triticum aestivum* by reducing the leaf area and size of epidermal cells and by increasing the stomatal index. Some researchers [19-21] showed that ABA increased the stomata number and thickness and area of a leaf in various species.

We were not able to encounter any work other than mentioned above on ABA and anatomy interaction.

The aim of this study is to serve the occurence of adaptive and conservative mechanisms of ABA increased in a plant under stressful conditions by examining the effects of various concentrations of exogenous ABA on the root, stem and leaf anatomy of the radish seedlings.

MATERIALS AND METHODS

The Seeds and Hormone Concentrations

In this study, radish (*Raphanus sativus* L.) seeds were used. The seeds were surface sterilized with 1 % sodium hypochloride. ABA concentrations used in the experiments were 10, 20, 30, 40 and 50 µM. These 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. 25 radish seeds were arranged into Petri dishes (10 cm diameter) lined by 2 sheets of Whatman No. 1 filter paper moistened with 6 ml of distilled water (control) and ABA solutions. 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 ABA solutions 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). Anatomical sections were taken from the root, stem and 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 [22] method:

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

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. The other parameters of root, stem and leaf anatomy 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 ABA on the root, stem and leaf anatomy of radish seedlings are presented in Table 1, 2 and 3, respectively.

Root Anatomy

ABA concentrations except 30 µM decreased more or less the root diameter and cortex zone thickness in comparison with the ones of control seedlings. On the other hand, 30 µM ABA increased the root diameter while it did not show a meaningful effect, statistically, on the cortex zone thickness. The levels of ABA under 50 µM increased the root hair number and epidermis cell width in the varying degrees according to control. As for the epidermis cell length and endodermis cell width, all of the concentrations used statistically showed the same values as control. All of the levels markedly reduced the endodermis cell length, vascular cylinder diameter and protoxylem width. The metaxylem width was decreased by the concentrations higher than 20 µM. Although 30 and 50 µM ABA caused a prominant increase on the trachea diameter, the others had no effect on this parameter (Table 1).

Stem Anatomy

All of the concentrations used notably increased the stem diameter according to control. Although 30 and 50 µM ABA increased the cuticle thickness, the others statistically showed the same values as control. ABA levels except 40 µM increased more or less the epidermis cell width. The epidermis cell length and trachea diameter were decreased by the concentrations higher than 30 µM. The levels of ABA except 30 µM markedly increased the cortex zone thickness. As for the vascular bundle width, the concentrations under 50 µM reduced this parameter (Table 2).

Leaf Anatomy

All of the concentrations had no effect on the stomata number and width in the upper surface and the epidermis cell number in the lower surface. In other words, these concentrations statistically showed the same values as control. The levels of ABA except 30 and 50 µM notably increased the stomata number and index in the lower surface. The concentrations under 40 µM decreased the epidermis cell number in the upper surface. 30 and 40 µM ABA slightly increased the stomata width in the lower surface while the others reduced this parameter. All of the concentrations decreased more or less the stomata length in the lower surface, whereas 30 µM ABA in the upper surface. All of the levels increased the stomata index in the upper surface. As for the epidermis cell width, the concentrations except 10 µM reduced this parameter in both surfaces. The leaf thickness and distance between vascular bundles were notably decreased by the concentrations higher than 20 µM (Table 3).

DISCUSSION

As known, exogenous ABA is a potent inhibitor of growth and development in many species [23, 24]. ABA can perform this preventive effect in many ways. ABA may interfere with growth and development events by changing the water status of plants [25], by limiting the availability of energy and nutrients [26], by inhibiting cell extensibility [27] and division [28] or by reducing synthesis of protein [29] and nucleic acid [30].

In this study, the concentrations of ABA mostly decreased the root diameter, cortex zone thickness, endodermis cell length, vascular cylinder diameter, protoxylem and metaxylem width in comparison with roots of control seedlings. In addition, they generally increased the root hair number and epidermis cell width while they have not shown a meaningful effect, statistically, on the epidermis cell length, endodermis cell width and trachea diameter (Table 1). These results indicate that radish roots acquire both xeromorphic (for example, the decrease in cortex zone thickness) and halosucculence (for example, the increase in epidermis cell width) features [18, 31] in ABA medium. Therefore the radish seedlings can provide adaptation to ABA by decreasing the protoxylem and metaxylem width or by increasing the root hair number and thus water uptake and transportation take place more easily.

Although many of the concentrations stimulated the stem diameter, epidermis cell width and cortex zone thickness, they usually reduced the epidermis cell length, vascular bundle width and trachea diameter (Table 2). These observations introduce that radish stems, as the case of roots, acquire halosucculence (for example, the increase in epidermis cell width and cortex zone thickness) properties [18, 31] in ABA medium. Moreover, the radish seedlings can provide adaptation to ABA by increasing the stem diameter or by reducing the vascular bundle width and trachea diameter.

Table 1. Some of parameters of root anatomy of radish seedlings grown in various concentrations of ABA at 25 ºC for 20 d

Hormone concentration (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**				
Control	*90±3.5 ^{cd}	24±4.5 ^{ab}	1.5±0.5 ^{ab}	1.5±0.2 ^{a}	29±2.8 ^{c}	1.3±0.4 ^{a}	1.9±0.2 ^{b}	27±2.5 ^{c}	2.0±0.3 ^{c}	3.6±0.5 ^{bc}	1.1±0.2 ^{a}
10	71±2.1 ^{b}	31±2.5 ^{b}	1.7±0.4 ^{b}	1.8±0.4 ^{a}	23±2.7 ^{b}	1.4±0.5 ^{a}	1.5±0.4 ^{a}	20±1.3 ^{b}	1.2±0.2 ^{a}	4.6±0.5 ^{d}	0.8±0.2 ^{a}
20	87±4.4 ^{c}	45±6.3 ^{c}	1.8±0.2 ^{b}	1.7±0.3 ^{a}	27±3.7 ^{bc}	1.2±0.2 ^{a}	1.1±0.2 ^{a}	17±2.7 ^{a}	1.4±0.5 ^{ab}	3.8±0.4 ^{c}	1.1±0.3 ^{a}
30	97±8.3 ^{d}	34±6.5 ^{b}	1.9±0.2 ^{b}	1.4±0.5 ^{a}	30±3.5 ^{c}	0.9±0.2 ^{a}	1.1±0.4 ^{a}	22±2.6 ^{b}	1.8±0.4 ^{bc}	2.8±0.4 ^{a}	1.7±0.4 ^{b}
40	57±8.2 ^{a}	53±4.4 ^{c}	1.7±0.4 ^{b}	1.4±0.2 ^{a}	17±2.7 ^{a}	1.1±0.2 ^{a}	1.2±0.7 ^{a}	16±2.2 ^{a}	1.6±0.5 ^{abc}	3.4±0.5 ^{abc}	0.9±0.2 ^{a}
50	72±7.5 ^{b}	18±2.7 ^{a}	1.1±0.2 ^{a}	1.3±0.4 ^{a}	28±5.7 ^{bc}	0.9±0.2 ^{a}	1.2±0.2 ^{a}	15±4.1 ^{a}	1.1±0.2 ^{a}	3.0±0.7 ^{ab}	2.0±0.3 ^{b}

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

Table 2. Some of parameters of stem anatomy of radish seedlings grown in various concentrations of ABA at 25 ºC for 20 d

Hormone concentration (µM)	Stem diameter (µm)	Cuticle thickness (µm)	Epidermis cell size (µm)		**Cortex zone thickness (µm)**	**Vascular bundle width (µm)**	**Trachea diameter (µm)**
			Width	**Length**			
Control	*194±2.4 ^{a}	1.1±0.2 ^{a}	8.6±0.5 ^{ab}	9.0±1.0 ^{abc}	107±8.3 ^{ab}	90±3.5 ^{b}	9.4±0.5 ^{d}
10	326±3.6 ^{d}	1.2±0.2 ^{a}	11.4±0.8 ^{d}	9.6±0.8 ^{c}	158±13 ^{cd}	55±7.1 ^{a}	13.8±0.8 ^{e}
20	307±2.3 ^{cd}	1.1±0.2 ^{a}	10.6±1.8 ^{cd}	9.2±0.8 ^{bc}	141±4.4 ^{cd}	58±5.7 ^{a}	13.2±0.5 ^{e}
30	229±1.3 ^{b}	1.5±0.3 ^{ab}	9.6±1.1 ^{bc}	7.6±1.5 ^{a}	104±8.2 ^{a}	51±5.4 ^{a}	7.2±0.8 ^{c}
40	285±20 ^{c}	1.3±0.4 ^{a}	7.2±0.8 ^{a}	7.8±0.8 ^{ab}	133±1.3 ^{bc}	84±9.6 ^{b}	5.4±0.6 ^{b}
50	391±15 ^{e}	1.8±0.2 ^{b}	9.6±1.1 ^{bc}	7.8±1.1 ^{ab}	163±8.3 ^{d}	119±1.6 ^{c}	4.2±1.3 ^{a}

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

Table 3. Some of parameters of leaf anatomy of radish seedlings grown in various concentrations of ABA at 25 ºC for 20 d

Hormone concentration (µM)	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		
Control	*11.8±1.4 ^{a}	13.8±2.5 ^{b}	30.4±3.0 ^{bcd}	32±6.0 ^{a}	4.4±0.5 ^{a}	4.8±0.8 ^{bc}	5.6±0.8 ^{ab}	7.4±1.6 ^{c}	27.96	30.13	8.6±0.8 ^{c}	7.5±0.5 ^{c}	163±12 ^{c}	86±4.1 ^{b}
10	11.6±2.3 ^{a}	22.2±4.3 ^{cd}	22.2±2.2 ^{a}	32.8±2.7 ^{a}	4.8±0.8 ^{a}	2.8±0.4 ^{a}	5.4±0.5 ^{ab}	3.6±0.5 ^{a}	34.31	34.15	8.2±1.2 ^{c}	6.9±0.7 ^{c}	169±7.4 ^{c}	87±11 ^{b}
20	11.4±4.0 ^{a}	20.2±4.1 ^{c}	27±3.3 ^{abc}	35.6±3.7 ^{a}	4.4±0.5 ^{a}	4.2±0.4 ^{b}	6.1±0.7 ^{b}	5.8±1.0 ^{b}	29.68	36.20	5.6±0.8 ^{ab}	4.9±0.6 ^{ab}	189±15 ^{d}	121±7.4 ^{c}
30	12.2±2.5 ^{a}	8.8±2.2 ^{a}	25.2±3.1 ^{ab}	30.4±2.8 ^{a}	4.3±0.2 ^{a}	5.2±0.8 ^{cd}	4.8±0.8 ^{a}	6.4±0.5 ^{bc}	32.25	22.44	4.7±0.6 ^{a}	5.8±1.3 ^{b}	144±6.5 ^{b}	71±15 ^{a}
40	13.6±2.6 ^{a}	25.8±2.7 ^{d}	33±5.4 ^{d}	30±7.9 ^{a}	5.1±1.0 ^{a}	5.8±0.4 ^{d}	6.2±1.4 ^{b}	6.8±0.4 ^{bc}	41.69	46.23	6.1±0.7 ^{b}	4.5±0.5 ^{a}	76±6.5 ^{a}	67±7.9 ^{a}
50	14.4±4.0 ^{a}	10.8±2.7 ^{ab}	32.4±6.2 ^{cd}	35.6±3.7 ^{a}	5.2±0.8 ^{a}	4.3±0.7 ^{b}	6.2±0.8 ^{b}	5.8±0.8 ^{b}	30.76	23.27	6.2±0.5 ^{b}	4.7±0.4 ^{a}	86±6.3 ^{a}	63±7.5 ^{a}

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

As for the leaf anatomy, the concentrations used mostly increased the stomata number in the lower surface, stomata length in the upper surface and stomata index in both surfaces while they generally decreased the epidermis cell number in the upper surface, stomata width and lenght in the lower surface, epidermis cell width in both surfaces, leaf thickness and distance between vascular bundles. On the other hand, these concentrations usually had no effect on stomata number and width in the upper surface and the epidermis cell number in the lower surface (Table 3). These findings emphasize that radish leaves, as the case of roots, acquire xeromorphic (for example, the decrease in epidermis cell width and the increase in stomata index) features [18, 31] in ABA medium. In addition, the radish seedlings can provide adaptation to ABA by reducing the stomata width and length especially in the lower surface and so decrease transpiration and water loss. They can serve to the same aim by causing a reduction of leaf area as a result of decreasing the epidermis cell number of the upper surface. Moreover, the seedlings make water and food transportation easy by decreasing the distance between vascular bundles in ABA medium.

There are few literature yet on the effects of ABA on the root, stem and leaf anatomies of seedlings. There is a need for more comprehensive and detailed researches for this subject to be made clear.

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