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

Key words: Jasmonic acid, leaf anatomy, radish, root anatomy, stem anatomy

INTRODUCTION

Jasmonic acid (JA) and its derivatives including methyl jasmonate (JA-Me) have been regarded as endogenous plant growth regulators because of their ubiquitous occurence in plant kingdom and their pleiotropic effects on plant growth and development [1, 2]. These compounds were isolated from fungi [3, 4], algae [5, 6] and many higher plants [7].

JA plays an important role in many physiological processes such as seed germination [8], pollen germination [9], seedling growth [10], fruit ripening [11], senescence [12], abscission [13], embryogenesis [14], carotenoid biosynthesis [15], chlorophyll formation [16], photosynthesis [17], respiration [18] and synthesis of proteins and nucleic acids [19].

JA mostly causes also alterations in the root, stem and leaf anatomies of plants. Unfortunately, there are few studies about the effects of JA on these subjects. Zhu et al. [20] and Tung et al. [21] observed that exogenously applied JA increased the number of root hair in roots of tomata and Arabidopsis. Weryszko-Chmielewska and Kozak [22] reported that exogenous JA decreased the number and diameter of xylem vessel in stems of *Gloriosa rothschildiana*. Some researchers [17, 23] determined that JA stimulated the stomata closure in various species.

The aim of this study is to examine the influences of various concentrations of JA on the root, stem and leaf anatomy of the radish seedlings.

MATERIALS AND METHODS

The Seeds and Growth Regulator Concentrations

In this study, radish (*Raphanus sativus* L.) seeds were used. The seeds were surface sterilized with 1 % sodium hypochloride. JA concentrations used in the experiments were 100, 500, 1000, 1500 and 2000 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 JA 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 JA 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 [24] method:

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

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

JA 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	*89±5.4^{b}	24±4.1^{c}	1.7±0.4^{ab}	1.5±0.5^{a}	30±3.5^{cd}	1.4±0.4^{a}	1.9±0.2^{b}	27±2.7^{c}	2.2±0.2^{bc}	3.7±0.6^{ab}	1.2±0.2^{bc}
100	76±6.5^{a}	16.6±2.3^{b}	1.9±0.2^{b}	1.7±0.4^{ab}	19±2.2^{a}	1.3±0.2^{a}	1.7±0.4^{ab}	19.6±3.2^{ab}	2.5±0.5^{c}	5.3±0.5^{c}	1.4±0.4^{c}
500	89±7.4^{b}	30±7.1^{d}	3.8±0.8^{d}	2.8±0.8^{c}	26±4.1^{bc}	3.1±0.5^{b}	3.6±0.5^{d}	22.6±3.7^{b}	1.8±0.2^{ab}	4.3±0.2^{b}	1.2±0.2^{bc}
1000	92±5.7^{c}	28±4.1^{cd}	2.2±0.2^{c}	2.3±0.4^{bc}	22±2.1^{ab}	2.7±0.4^{b}	2.5±0.5^{c}	28±2.7^{c}	1.5±0.5^{a}	3.5±0.5^{a}	0.8±0.3^{ab}
1500	116±8.5^{d}	27.6±2.5^{cd}	1.3±0.4^{a}	1.1±0.2^{a}	36±6.5^{e}	1.2±0.2^{a}	1.4±0.4^{ab}	19±2.6^{ab}	1.3±0.4^{a}	3.2±0.2^{a}	0.8±0.2^{ab}
2000	92±1.5^{c}	7.2±2.1^{a}	1.2±0.2^{a}	1.2±0.1^{a}	35±3.5^{de}	0.9±0.2^{a}	1.2±0.2^{a}	16.2±1.3^{a}	2.6±0.5^{c}	3.3±0.4^{a}	0.7±0.4^{a}

* 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 JA at 25 ºC for 20 d

JA 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	*182±2.8 ^{ab}	1.1±0.2 ^{a}	8.6±0.5 ^{c}	9.1±0.7 ^{c}	110±6.1 ^{ab}	91±4.1 ^{bc}	9.4±0.5 ^{c}
100	251±2.4 ^{c}	1.1±0.1 ^{a}	9.3±0.4 ^{cd}	8.8±0.6 ^{c}	88±4.3 ^{a}	95±3.5 ^{c}	9.3±0.4 ^{c}
500	261±11.4 ^{c}	1.1±0.5 ^{a}	9.5±0.5 ^{d}	8.9±0.7 ^{c}	109±8.9 ^{ab}	93±4.4 ^{c}	9.6±0.5 ^{c}
1000	201±13.2 ^{b}	1.2±0.2 ^{ab}	9.6±0.5 ^{d}	8.8±0.8 ^{c}	93±4.4 ^{a}	85±5.1 ^{b}	5.6±0.8 ^{b}
1500	172±10.3 ^{a}	1.4±0.4 ^{ab}	7.2±0.8 ^{b}	7.2±7.1 ^{b}	88±2.7 ^{a}	73±4.4 ^{a}	4.1±0.7 ^{a}
2000	301±14.3 ^{d}	1.6±0.3 ^{b}	6.1±0.7 ^{a}	5.8±0.8 ^{a}	133±8.3 ^{c}	74±8.2 ^{a}	4.8±0.8 ^{ab}

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

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

Root Anatomy

JA concentrations except 100 and 500 µM markedly increased the root diameter in comparison with the ones of control seedlings. On the other hand, 100 µM JA decreased this parameter while 500 µM JA statistically showed the same value as control. Although 100 and 2000 µM JA notably reduced the root hair number, the others partly increased this parameter. The levels of JA under 1500 µM increased the epidermis cell width and length in the varying degrees according to control while the mentioned levels decreased the cortex zone thickness. 500 and 1000 µM JA caused a prominant increase on the endodermis cell width, but the others did not show a meaningful effect, statistically, on this parameter. As for the endodermis cell length, 500 and 1000 µM JA markedly increased this parameter while the others reduced it. The concentrations except 1000 µM decreased the vascular cylinder diameter. Although 100 and 2000 µM JA slightly increased the protoxylem width, the others notably reduced this parameter. The metaxylem width and trachea diameter were decreased by the levels of JA higher than 500 µM (Table 1).

Stem Anatomy

The concentrations of JA except 1500 µM increased the stem diameter according to control. The levels of JA higher than 500 µM slightly increased the cuticle thickness while the mentioned levels markedly decreased the trachea diameter. Although 1500 and 2000 µM JA reduced the epidermis cell width, the others increased this parameter. As for the epidermis cell length, 1500 and 2000 µM JA caused a maximum decrease on this parameter, but the others statistically showed the same values as control. The concentrations except 500 and 2000 µM notably decreased the cortex zone thickness. The vascular bundle width was reduced by the levels higher than 500 µM (Table 2).

Leaf Anatomy

The concentrations except 500 and 1000 µM decreased the stomata number and index in the varying degrees in the upper surface. As for the lower surface, the levels of JA except 500 and 1500 µM increased more or less these parameters. 500 and 1000 µM JA increased the epidermis cell number in the upper surface, whereas 100 µM JA in the lower surface. The concentrations except 100 and 2000 µM slightly reduced the stomata width in the upper surface while all of the concentrations had no effect on this parameter in the lower surface. The levels of JA except 500 and 2000 µM did not show a meaningful effect, statistically, on the stomata length in the upper surface. On the other hand, 500 µM JA decreased this parameter, but 2000 µM JA increased it. As for the lower surface, the concentrations higher than 500 µM partly reduced the stomata length. JA levels higher than 100 µM decreased the epidermis cell width in the lower surface, whereas 1500 and 2000 µM JA in the upper surface. The concentrations except 100 and 2000 µM caused a prominant increase on the leaf thickness. The distance between

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

JA 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	*11.6±1.8^{c}	13.2±2.8^{ab}	30.6±2.6^{cd}	31±5.6^{ab}	4.6±0.5^{ab}	4.6±0.8^{a}	5.6±0.8^{ab}	6.8±1.9^{abc}	27.48	29.86	5.8±0.5^{bc}	6.5±0.5^{c}	161±7.4^{ab}	148±7.5^{c}
100	4.4±2.1^{a}	16.6±3.5^{bc}	13.6±3.2^{a}	35.6±2.3^{b}	5.0±1.2^{ab}	5.6±0.5^{a}	6.4±1.8^{ab}	8.3±1.2^{c}	24.44	31.80	8.9±0.7^{d}	6.6±0.8^{c}	159±13^{ab}	92±13^{ab}
500	12.4±2.5^{c}	10.4±2.7^{a}	32±4.4^{d}	28.8±2.7^{a}	4.4±0.5^{a}	5.6±1.1^{a}	5.2±0.8^{a}	7.4±1.5^{bc}	27.92	26.53	6.6±0.7^{c}	4.6±0.5^{ab}	177±14^{b}	94±4.1^{ab}
1000	18±3.5^{d}	15.2±3.1^{b}	38.2±3.7^{e}	28.6±5.1^{a}	4.3±0.5^{a}	4.6±0.5^{a}	5.4±0.5^{ab}	5.8±0.8^{ab}	32.02	34.70	6.5±0.5^{c}	4.5±0.5^{ab}	176±18^{b}	97±9.6^{b}
1500	7.2±1.9^{ab}	14.4±2.9^{ab}	26±4.8^{bc}	28.2±5.4^{a}	4.4±0.3^{a}	4.8±0.4^{a}	6.2±0.8^{ab}	6.1±0.7^{ab}	21.68	30.25	4.2±0.4^{a}	5.2±0.3^{b}	176±10^{b}	90±3.5^{ab}
2000	8.4±1.5^{b}	20.2±3.1^{c}	23.6±2.7^{b}	33.6±4.1^{ab}	5.4±0.4^{b}	4.6±0.5^{a}	6.8±0.8^{b}	5.6±0.8^{a}	25.41	37.31	5.4±0.4^{b}	4.0±0.7^{a}	149±7.4^{a}	80±15^{a}

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

vascular bundles was notably reduced by all of the levels used (Table 3).

DISCUSSION

JA is a growth regulator preventing growth and development in plants [25]. JA can perform this preventive effect in many ways. JA may interfere with growth and development events by changing the water status of plants [26], by limiting the availability of energy and nutrients [27], by inhibiting cell division [28] or by reducing synthesis of protein and nucleic acid [19].

In this work, JA concentrations mostly increased the root diameter, root hair number and epidermis cell size in comparison with roots of control seedlings while they generally decreased the cortex zone thickness, endodermis cell length, vascular cylinder diameter, protoxylem and metaxylem 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 size) features [29, 30] in JA medium. Therefore radish seedlings can provide adaptation to JA by decreasing the protoxylem and metaxylem width and trachea diameter or by increasing the root diameter and root hair number and thus water uptake and transportation take place more easily.

Although many of the concentrations stimulated more or less the stem diameter, cuticle thickness and epidermis cell width, they usually reduced the cortex zone thickness, vascular bundle width and trachea diameter (Table 2). These observations introduce that radish stems, as the case of roots, acquire both xeromorphic (for example, the decrease in cortex zone thickness) and halosucculence (for example, the increase in epidermis cell width) properties [29, 30] in JA medium. Moreover, radish seedlings can provide adaptation to JA by increasing the stem diameter and cuticle thickness or by decreasing the vascular bundle width and trachea diameter and so decrease water loss and ease water transportation.

As for the leaf anatomy, the concentrations used mostly increased the stomata number and index in the lower surface, epidermis cell width in the upper surface and leaf thickness while they generally decreased the stomata number, width and index in the upper surface, stomata length and epidermis cell width in the lower surface, epidermis cell number in both surfaces and distance between vascular bundles (Table 3). These findings emphasize that radish leaves, as the case of roots and stems, acquire both xeromorphic (for example, on the lower surface the decrease in epidermis cell width) and halosucculence (for example, on the upper surface the increase in epidermis cell width) features [30] in JA medium. In addition, radish seedlings can provide adaptation to JA by reducing the stomata number, width and index especially in the upper surface and stomata length especially in the lower surface or by increasing the leaf thickness and thus 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 both surfaces. Therefore the seedlings make water and food transportation easy by reducing the distance between vascular bundles in JA medium.

There are few literature yet on the effects of JA 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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