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Effect of Inhibitors of Ethylene Production on Growth and Gravitropism Inhibited by Oryzalin in Arabidopsis Roots

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Oryzalin is a herbicide that disrupts the arrangement of microtubules by binding to tubulin, thereby blocking the anisotropic growth of plant cells. Microtubules and microfilaments are cytoskeleton components that have been implicated in plant growth through their influence on the formation of cell walls. Microtubules also play roles in the sedimentation of amyloplasts in the root tip columella cells; this sedimentation is related to gravity sensing and results in downward root growth in the soil for absorption of water and minerals. However, the orientation of microtubules changes depending on the level of ethylene in plant cells. A recent study reported that oryzalin stimulated ethylene production via 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase and caused a concentration-dependent inhibition of root growth and gravitropic responses. The aim of the present study was to investigate the possibility that oryzalin-induced inhibition might be recovered by the application of inhibitors of ethylene production, such as 10^{-4} M cobalt ions and 10^{-8} M aminoethoxyvinylglycine (AVG). The inhibition of root growth and gravitropic response was overcome by 10-20% by an 8 hr treatment with cobalt ions or AVG. These results suggest that ethylene levels could regulate root growth and gravitropic responses in Arabidopsis.

Key words : Arabidopsis root, AVG, cobalt ions, gravitropic response, oryzalin

Introduction

Oryzalin is a dinitroaniline herbicide that acts on the disruption of microtubules [12, 15]. According to Kim et al. [6], oryzalin inhibited root growth and gravitropic response in maize, and the primary root tip was swelled in the agar plate containing 10^{-4} M oryzalin. They suggested that the arrangement of microtubule might be one of the important factors to determine the cell shape. From these results, they concluded that the effect of oryzalin could be related to the oryzalin-induced stimulation of ethylene production in maize roots. In addition, we found that the growth and gravitropism have been inhibited by oryzalin, and its inhibition is related to the increase of ethylene production in Arabidopsis roots (data submitted).

Polko et al. [13] suggested that the orientation of microtubule is changed depending on the level of ethylene in plant cells, and ethylene-induced hyponastic leaf movement is related to the unequal growth rates between adaxial and abaxial of petiole. Their research concluded that ethylene stimulates cell expansion in a tissue-specific manner with the arrangement of cortical microtubules along the petiole. Le et al. [9] reported that microtubule reorientation is related to the early response of the root epidermis to ethylene in Arabidopsis root. There are some studies reported that the microtubule arrangement regulated the alignment of cellulose microfibrils across the root, which made the uniform mechanical structure [3, 5]. Recent study by Wang et al. [16] reported that microtubule reorientation was induced by ethylene, and it inhibited root growth in Arabidopsis.

Ethylene, one of the stress hormone, participates in various plant developments and differentiation reactions including seed germination, fruit ripening and senescence [1, 18]. Ethylene synthesis begins from methionine via two major intermediates, S-adenosylmethionine (AdoMet) and 1-aminocycopropane-1-carboxylic acid (ACC), in sequence. The enzyme of ACC synthase (ACS) and ACC oxidase (ACO) regulate the steps from AdoMet to ACC and from ACC to ethylene, respectively. Several factors regulate these two enzymes, especially auxin which stimulates the ethylene production through increasing the expression level of the ACS gene [4]. The formation of ACC from AdoMet is regulated by ACC synthase (ACS), and ACS is strongly inhibited by

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aminoethoxyvinylglycine (AVG), which is a known inhibitor of pyridoxal phosphate-mediated enzyme reactions [2]. The last step in the ethylene biosynthesis pathway is the conversion of ACC to ethylene by ACC oxidase (ACO), and cobalt ions suppress the ACO activity [1].

Based on these facts, we tried to figure out that the inhibition of root growth and gravitropic response by oryzalin could be recovered by cobalt ions and AVG, inhibitors of ethylene production.

Material and Methods

Plant material

The sterilized seeds of *Landsberg erecta* (Ler), *Arabidopsis thaliana* were planted on the agar medium with half-strength of MS salts, 1% sucrose and 1 mM MES (pH 5.8). The seeds were incubated in vertical position at 4℃ for 1 day and then were incubated for another 6 days at 22℃.

Measurement of ethylene production

Ethylene production was measured in 100 root segments (10 mm). The root segments were placed in vials containing 1 ml of MES buffer (100 mM, pH 6.8, 50 μg/ml chloramphenicol) with the test compounds. The vials were shaken in the dark at 27℃ in an incubator. To measure the ethylene production, 1-ml of gas sample was withdrawn from the vial using a syringe and injected to the gas chromatograph (HP5890 Series II; Hewlett-Packard, USA) equipped with an alumina column (80/100 Porapak-Q; 1.8-m × 2.1-mm).

Measurement of root growth and gravitropic curvature

The seedlings were placed in vertical or horizontal position in petri dishes, depending on the experiments. Growth and gravitropic curvature were measured using a camera (Rexsa, DS-400 PC-camera) with the time-interval software (SupervisionCam ver. 3.2.2.4; http://supervisioncam.com). Images were recorded every 15 min and analyzed using UTHSCSA Image Tool Program (ver. 3.0; http://comdent.uthscsa.edu/dig/itdes.html).

Observation of root external structure

Roots were placed in agar medium containing 10^{-6} M oryzalin in vertically, and observed roots with the microscope (Olympus SZ51) at 45 folds after 24 hr.

Statistical analysis

All experiments were conducted at least three times, with no fewer than 30 primary roots each. All data were computed to establish statistical significance at p of < 0.05 using two-way ANOVA and Tukey test.

Results and Discussion

Effect of cobalt ions and AVG on the oryzalin-induced ethylene production

It has been known that external treatment of oryzalin increased ethylene production in maize roots [6]. The stimulation of ethylene production by oryzalin was due to increase of ACO and ACS activities, which act on the ethylene production pathway.

We examined the effect of oryzalin on the external structure in Arabidopsis root. The application of oryzalin for 24 hr caused root tip swelling (Fig. 1). This result suggested that oryzalin disrupt the microtubule formation especially in the meristem regions. However, oryzalin-induced swelling in the meristem region was reduced by the treatment of ethylene biosynthesis inhibitors such as Co^{2+} and AVG. The swelling of tissue in plants is one of the key roles of ethylene action [1].

Ethylene synthesis starts from methionine via two intermediates such as S-adenosyl-methionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). The formation of ACC from AdoMet is regulated by ACC synthase (ACS), and ACS is strongly inhibited by AVG, which is a known inhibitor of pyridoxal phosphate-mediated enzyme reactions [2]. The last step in the ethylene biosynthesis pathway is the conversion of ACC to ethylene by ACC oxidase (ACO), and cobalt ions suppress the ACO activity [1].

In this study, we measured how oryzalin-induced ethylene production was regulated by the treatment of cobalt ions and AVG (Fig. 2). Along with the previous results, treatment of oryzalin resulted in 35% increase in ethylene production at 4 hr [6]. However, the stimulated ethylene production by oryzalin decreased by AVG and cobalt ions. When 10^{-6} M cobalt ions and 10^{-4} M AVG were treated, the ethylene production was inhibited 52% and 47% by cobalt ions and AVG at 4 hr, respectively.

From these results, we examined the effect of cobalt ions and AVG on the oryzalin induced inhibition of growth and gravitropic response in Arabidopsis roots.

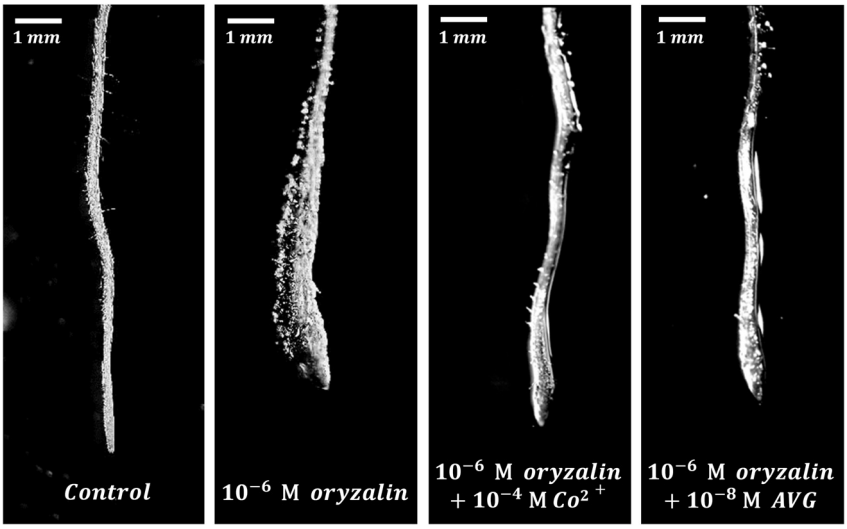

Fig. 1. Morphology of vertically-grown roots treated with 10^{-6} M oryzalin, 10^{-6} M oryzalin + 10^{-4} M Co^{2+}, 10^{-6} M oryzalin + 10^{-8} M AVG for 24 hr. Swelling in oryzalin-treated roots occurred in the root tip which is the meristem.

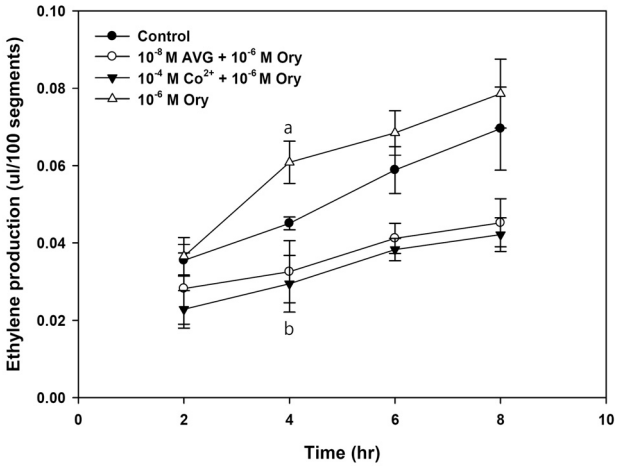

Fig. 2. Effect of inhibitors of ethylene production on oryzalin-induced ethylene production in the root segments of Arabidopsis for 8 hr. Root segments (10 mm) were incubated in solution containing 10^{-6} M oryzalin, 10^{-6} M oryzalin + 10^{-4} M cobalt ions, 10^{-6} M oryzalin + 10^{-8} M AVG. At every 2 hr, 1 ml of gas sample was withdrawn from the vials for measuring the production of ethylene. Symbols are mean values ± SE from 3 independent experiments. Different letters indicate significant difference based on two-way ANOVA and Tukey test at $p<0.05$.

Effect of IAA and AVG in root growth and gravitropic response

It is well known that IAA could promote ethylene production through an activation of ACS, and resulted in inhibition of root growth [1]. Kim and Mulkey [7] reported that ethylene antagonists such as AVG and silver ions recovered the IAA-induced inhibition of root elongation in maize roots. And Ma et al [11] suggested that ethylene regulates root growth and gravitropic responses via the alignment of microtubule. Thus, we examined the effect of IAA and AVG on root growth and gravitropic response. According to the preliminary data, the inhibition of root growth and gravitropic response were depended on IAA concentrations (data not shown). Root growth was further inhibited as the IAA concentration increased, and the onset of the gravitropic response was delayed compared to the control (data now shown).

Based on these results, we examined the effect of IAA and AVG in Arabidopsis roots on the growth and gravitropic response. When treated with 10^{-7} M IAA, root growth was suppressed 80% compared to the control, but the treatment of the 10^{-8} M AVG recovered the inhibition induced by the IAA (Fig. 3A). Gravitropic curvature was also reduced compared to the control when 10^{-9} M IAA was treated. The curvature reduced by IAA was recovered to the degree of control roots by the treatment of 10^{-8} M AVG (Fig. 3B). These results suggested that root growth and gravitropic response could be regulated by ethylene in part.

Therefore, we conducted an experiment to see whether the inhibition of root growth and gravitropic response by oryzalin were related to the ethylene production.

Effect of cobalt ions and AVG on oryzalin-induced inhibition of root growth

For this experiment, roots pretreated with 10^{-4} M oryzalin for 2 hr was transferred to the agar plate containing 10^{-4} M cobalt ions or 10^{-8} M AVG. As we expected, 10^{-4} M oryzalin inhibited root growth 62% by compared to the control at 2 hr (Fig. 4A). The effect of oryzalin on root growth inhibition was further increase for 8 hr. Cobalt ions, an ethyl-

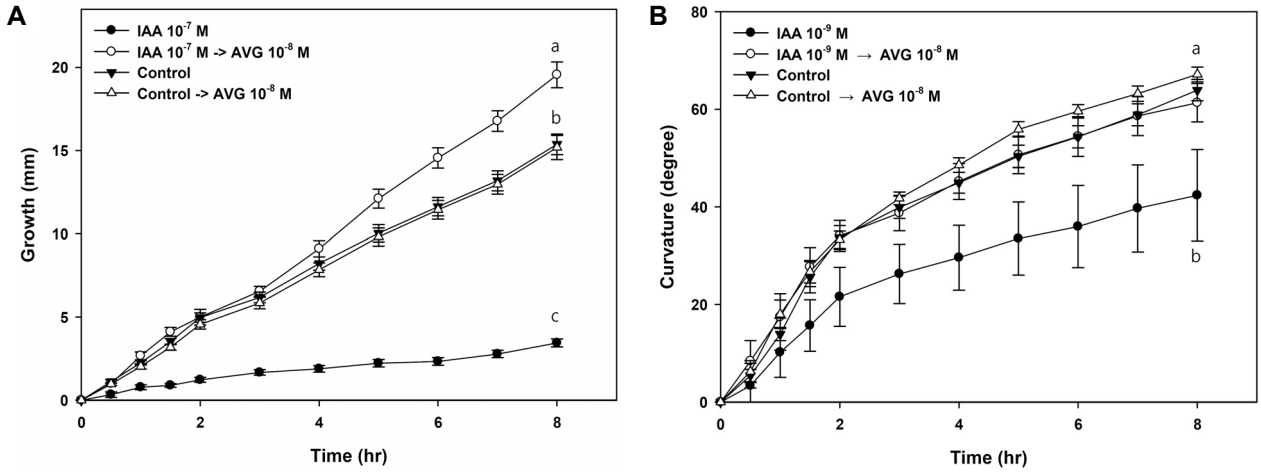

Fig. 3. Effect of AVG on the pretreated with IAA in root growth (A) and gravitropic response (B) for 8 hr in the Arabidopsis root. Roots were pretreated with 10^{-7} M IAA for 2 hr vertically, and then transferred to the agar plate containing 10^{-8} M AVG. The growth was measured for 8 hr using a camera as described in Material and Methods. Symbols are mean values ± SE from 10 independent experiments. Different letters indicate significant difference based on two-way ANOVA and Tukey test at p <0.05.

ene-producing inhibitor, was treated to see whether the root growth inhibition by oryzalin would recover or not. Cobalt ion recovered the root growth inhibited by oryzalin by 40% over 8 hr (Fig. 4A). Even in the control root, cobalt ions recovered 38% of root growth. These data suggested that ethylene might play a role of the inhibition in the root growth.

Some studies suggested that ethylene promoted IAA biosynthesis and increased the capacity of IAA transport by regulating the transcription of AUX1 and PIN2, which is an auxin transport component [14]. The increase in auxin biosynthesis could alter the distribution of auxin from root cap to the elongation zone. Therefore, ethylene could inhibit the root growth elongation in plants.

We applied with AVG, which is another inhibitor of ethylene production to confirm the role of ethylene in root growth. As cobalt ions did, treatment of AVG restored root growth inhibited by oryzalin (Fig. 4B). Roots pretreated with or without 10^{-4} M oryzalin was transferred to the agar medium including 10^{-8} M AVG, and then root growth was measured for 8 hr. Both of these were expected to be in-

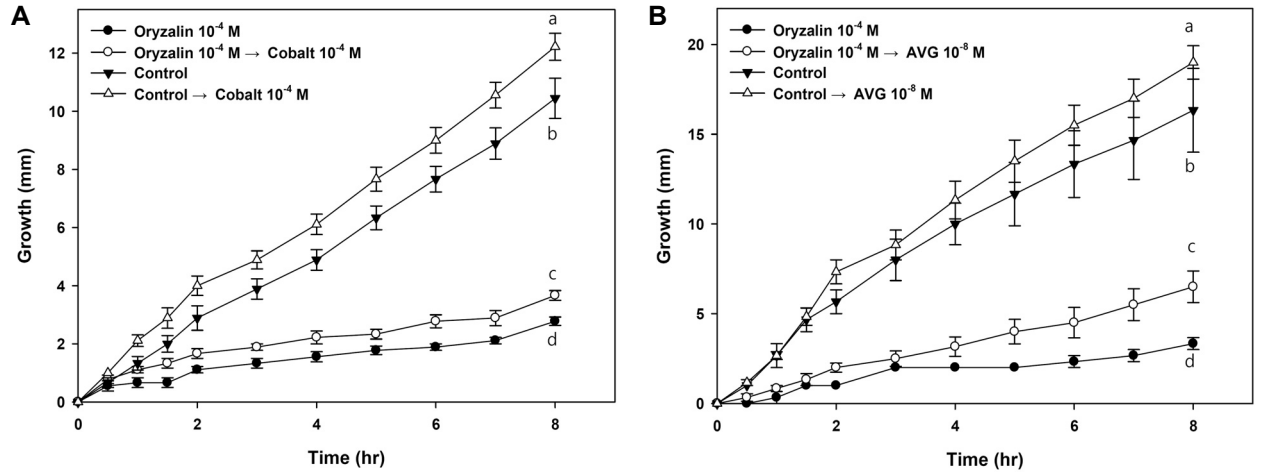

Fig. 4. Effect of cobalt ions (A) and AVG (B) on the pretreated with oryzalin in root growth for 8 hr in the Arabidopsis root. Roots were pretreated with 10^{-4} M oryzalin for 2 hr vertically, and then transferred to the agar plate containing 10^{-4} M cobalt ions or 10^{-8} M AVG. The growth was measured for 8 hr using a camera as described in Material and Methods. Symbols are mean values ± SE from 15 independent experiments. Different letters indicate significant difference based on two-way ANOVA and Tukey test at p<0.05.

hibited from ethylene production, and root growth was increased compared to roots that was not pretreated with AVG as we predicted. When roots pretreated with oryzalin was transferred to AVG agar medium, root growth was recovered from the oryzalin-induced inhibition of root growth 58% at 4 hr and 100% at 8 hr.

Effect of cobalt ions and AVG on oryzalin-induced inhibition of root gravitropic response

We measured the effect of cobalt ions and AVG on oryzalin-induced inhibition of gravitropic response in Arabidopsis roots. For this experiment, roots were pretreated with 10^{-6} M oryzalin for 2 hr in vertical position, and then were transferred to the agar plate containing 10^{-4} M cobalt ions or 10^{-8} M AVG horizontally. Gravitropic response did not occurred when 10^{-4} M oryzalin were treated (data not shown). Therefore, we used 10^{-6} M oryzalin to measure the gravitropic response instead of 10^{-4} M oryzalin which was used for the root growth.

Application of 10^{-4} M cobalt ions restored the inhibition of gravitropic response by oryzalin as well as in root growth (Fig. 5A). The curvature was recovered by 6~8% in the control root, and 10~20% in roots pretreated with 10^{-6} M oryzalin over 8 hr.

Further, we examined the effect of 10^{-8} M AVG on the oryzalin-induced inhibition of gravitropic response (Fig. 5B). The curvature was recovered by 22% in roots pretreated with 10^{-6} M oryzalin at 4 hr. The curvature of roots pretreated with 10^{-6} M oryzalin was recovered to the same degree as the control at 8 hr.

Several researches suggested that ethylene regulates growth and gravitropism in plants. Lee et al. [10] suggested that ethylene affects the gravity induced-lateral auxin transport, resulted in regulation of gravitropic curvature. And an optimal concentration of ethylene might be required for the regulation of gravitropism in maize roots [8]. Further, there are several reports that ethylene changed the arrangement of microtubule in plants [17].

In conclusion, oryzalin inhibited both gravitropic response and growth of Arabidopsis roots, and increased the ethylene production via activation of ACO and ACS. The increased ethylene might regulated the orientation of microtubule, resulting in inhibition of root growth and gravitropic sensing. Oryzalin, a known inhibitor of microtubule arrangement, promoted the ethylene production resulted in the inhibition of root growth and gravitropic response in Arabidopsis root. And this inhibition by the treatment of oryzalin could be recovered by cobalt ions and AVG which were inhibitors of ethylene production. These results suggest that the oryzalin-induced inhibition both of growth and gravitropic response were recovered by the treatment of oryzalin. This study suggested that the effect of oryzalin connected with the ethylene production and root growth and gravitropic response could be regulated according to the level of ethylene.

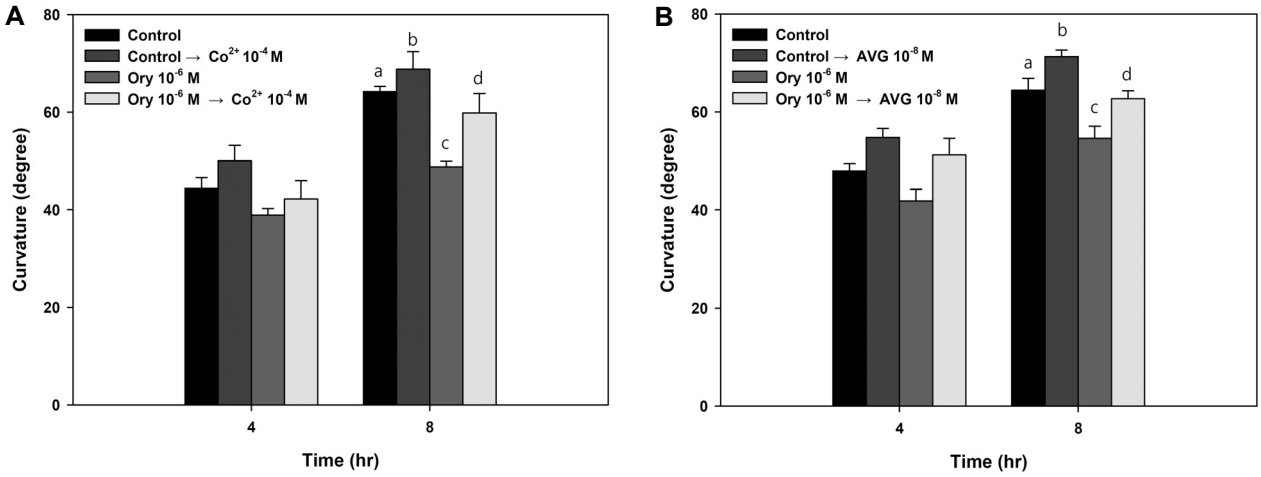

Fig. 5. Effect of cobalt ions (A) and AVG (B) on the pretreated with oryzalin in gravitropic response for 8 hr in the Arabidopsis root. Roots were pretreated with 10^{-6} M oryzalin for 2 hr vertically, and then transferred to the agar plate containing 10^{-4} M cobalt ions or AVG. The growth was measured for 8 hr using a camera as described in Material and Methods. Symbols are mean values ± SE from 15 independent experiments. Different letters indicate significant difference based on two-way ANOVA and Tukey test at $p<0.05$.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 애기장대 뿌리에서 ethylene 생성 억제제가 oryzalin에 의해 억제된 뿌리 생장과 굴중성 반응에 미치는 영향

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Oryzalin은 미세소관의 형성을 억제하는 dinitroaniline계 제초제이다. Oryzalin은 튜불린에 결합해 식물의 미세소관 배열을 무질서하게 하여 식물 세포의 비등방성 성장을 억제한다. 미세소관과 미세섬유는 세포벽을 구성하고 columella 세포에서 녹말체 침강에 관여하는 세포골격이다. 녹말체는 뿌리 끝에 있는 columella 세포에서 중력을 인지하여 물과 무기염류를 흡수하기 위하여 토양 속으로 자라도록 한다. 식물세포에서 미세소관의 배열은 에틸렌 수준에 따라 조절된다. Oryzalin이 ACC synthase와 ACC oxidase를 활성화시켜 에틸렌 생성을 촉진한다고 알려졌다. 또한 oryzalin은 농도에 의존적으로 뿌리 생장과 굴중성 반응을 억제한다고 보고 되었다. 이 결과에 따라, 본 연구는 Arabidopsis 뿌리에서 이 억제 효과가 에틸렌 생성 억제제인 10^{-4} M cobalt ions과 10^{-8} M aminoethoxyvinylglycine (AVG)를 처리하여 회복될 가능성에 초점을 두었다. 뿌리 생장과 굴중성 억제는 cobalt ions과 AVG에 의해 10-20% 회복되었다. 이 결과는 뿌리 생장과 굴중성 반응은 에틸렌의 수준에 따라 조절될 가능성을 제시하였다.