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Endogenous Auxin is Required but Supraoptimal for Rapid Growth of Rice (*Oryza sativa* L.) Seminal Roots, and Auxin Inhibition of Rice Seminal Root Growth is Not Caused by Ethylene

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Abstract The dual effects of auxin and ethylene on rice seminal root growth were investigated in this study. Low concentrations of exogenous indole-3-acetic acid (IAA) had no effect on rice seminal root growth, whereas higher concentrations (≥0.003 μM) were inhibitory. In contrast, low concentrations of the auxin action inhibitor *p*-chlorophenoxyisobutyric acid (PCIB), ranging from 0.5 to 50 μM, promoted rice seminal root growth, whereas high concentrations of PCIB (≥500 μM) and the polar auxin transport inhibitor 2,3,5-triiodobenzoic acid (TIBA) inhibited rice seminal root growth. These results suggest that endogenous auxin is required but supraoptimal for rapid growth of rice seminal roots. In addition, although rice seminal root growth was inhibited by the exogenous ethylene-releasing compound ethephon or the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) as well as exogenous IAA, the 50% inhibition of growth (I_{50}) caused by ethephon or ACC was weakened by certain concentrations of the ethylene action inhibitor Ag^+ (0.016-0.4 μM). However, the I_{50} caused by exogenous IAA was strengthened by Ag^+ or the ethylene biosynthetic inhibitor aminoethoxyvinylglycine (AVG) and weakened by certain concentrations of PCIB (0.5-50 μM). Together, the inhibitory mechanisms of auxin and ethylene on rice seminal root growth should be different, and auxin inhibition of rice seminal root growth should not be caused by ethylene. Furthermore, our results indicated that a certain threshold level of ethylene was required to maintain rice seminal root growth, and that ethylene within the threshold may antagonize auxin inhibition of rice seminal root growth.

Keywords Auxin · Ethylene · Inhibition · Rice · Seminal root growth · Threshold level

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Introduction

The plant hormone auxin is involved in diverse plant growth and developmental processes, including cell division and elongation, root initiation and growth, gravitropic and phototropic responses, vascular tissue differentiation, and apical dominance. Exogenous auxin promoted growth of isolated stem segments (Kutschera and Niklas 2007) or intact stems of *lkb* mutants in which auxin levels were lower than wild-type control plants (McKay and others 1994). However, evidence of the promotion of root growth in intact plants by exogenous auxin is lacking, although exogenous auxin has been reported to promote root growth in decapitated *Arabidopsis* plants in which auxin levels were suboptimal (Fu and Harberd 2003). In addition, many results have indicated that exogenous auxin did not promote but inhibited root growth of wild-type control plants (Mulkey and others 1982a; Rahman and others 2007). Based on these results, we hypothesized that in intact roots of wild-type control plants, a threshold level of auxin was required for root growth, and that endogenous levels of auxin are probably optimal or supraoptimal in roots; thus, the application of exogenous auxin does not promote but actually inhibits root growth. Rice, a model monocotyledonous plant, was used to test the hypothesis, and the effects of exogenous indole-3-acetic acid (IAA, an auxin),

2,3,5-triiodobenzoic acid (TIBA, a polar auxin transport inhibitor), and *p*-chlorophenoxyisobutyric acid (PCIB, an auxin action inhibitor) on seminal root growth were investigated.

It has been reported that auxin application induced the biosynthesis of ethylene, and the inhibition of root growth by auxin has been attributed to ethylene (Chadwick and Burg 1967; Steen and Chadwick 1973). Zimmerman and Wilcoxon (1935) first reported that tomato plants treated with IAA could produce a gas that caused an epinastic response in ethylene-sensitive plants. Later, it was demonstrated that the gas was ethylene (Morgan and Hall 1962), and the auxin-induced ethylene production was mediated through the production of specific enzymes required for the enhanced synthesis of ethylene (Abeles 1966). However, the specific enzymes induced by auxin were unclear before the determination of the ethylene biosynthetic pathway. Interestingly, ethylene was subsequently shown to be synthesized from the amino acid methionine by the consecutive action of three enzymatic activities: S-adenosyl-L-methionine (SAM) synthase, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, and ACC oxidase (Yang and Hoffman 1984). ACC synthase catalyzes the main regulatory step in this biosynthetic pathway, the conversion of SAM to ACC (Wang and others 2002). In the model dicot *Arabidopsis*, auxin has been shown to stimulate ethylene production by activating the particular biosynthetic step of SAM to ACC (Abel and others 1995). Moreover, transcription of eight of the nine *ACS* genes was upregulated by auxin (Tsuchisaka and Theologis 2004). In the model monocotyledon rice, upregulation of the *OsACS1* and *OsACS3* genes contributed to auxin-stimulated ethylene production (Zarembinski and Theologis 1993).

In contrast, investigation in maize roots indicated that the increase in ethylene production due to auxin was quite small, and that the level of auxin-induced ethylene was too low to affect root growth. Thus, it was difficult to conclude that auxin inhibition of root growth was mediated by ethylene (Bucher and Pilet 1983). Moreover, studies of pea roots suggested that ethylene was not responsible for the inhibition of root growth by auxin (Eliasson and others 1989).

To provide further insight into the debate over whether the inhibition of root growth by auxin is caused by ethylene, in this study we investigated and compared the inhibitory effects of auxin and ethylene on rice seminal root growth. Because ACC is the direct precursor of ethylene and can be converted to ethylene by ACC-forming enzyme in plants. (Boller and others 1979), and ethephon is an ethylene-releasing compound that can release ethylene in the presence of water and at a pH above 3.5 (Lorbiecke and Sauter 1999), we used ACC and ethephon as precursors for ethylene production to investigate the inhibitory effects of ethylene on rice seminal root growth. Furthermore, to determine whether auxin inhibition on rice seminal root growth is caused by ethylene, Ag^+ and aminoethoxyvinylglycine (AVG) were applied in the presence of exogenous IAA (the main auxin in higher plants). Ag^+ is a very effective inhibitor of ethylene action and is thought to occupy the binding site of the ethylene receptor (Elmo and Beyer 1976; Rodríguez and others 1999), whereas AVG is an inhibitor of ACC synthase and can markedly decrease the level of endogenous ethylene in rice (Adams and Yang 1979; Métraux and Kende 1983). If auxin inhibition of rice seminal root growth is caused by auxin-induced ethylene, auxin inhibition of rice seminal root growth should be weakened by Ag^+ or AVG. However, our results revealed that auxin inhibition of rice seminal root growth was strengthened by Ag^+ and AVG, suggesting that auxin inhibition of rice seminal root growth is not caused by ethylene.

Materials and Methods

Plant Material and Growth Conditions

The seeds of indica rice 9311 (*Oryza sativa* L.) were used in this study. Rice 9311 is an elite inbred variety that is widely used in China. Rice 9311 seeds were surface sterilized in a solution of 5% (v/v) NaOCl for 20 min, rinsed six times with distilled water, and then soaked in distilled water for 1 day at 26°C. Subsequently, rice seeds were germinated for another day at 26°C, and then the germinated seeds were incubated on plastic screens floated on different culture solutions. Experiments were carried out in an artificial climate incubator (HP 1000 GS) under the following conditions: in light for 12 h at 29°C and then in darkness for 12 h at 26°C every day.

Chemicals and Treatments

TIBA, PCIB, IAA, ACC, and AVG were purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd. Ethephon was purchased from Nanjing Adobe Fournier Biological Technology Co., Ltd. $AgNO_3$ was purchased from Shanghai Chemical Reagent Factory. TIBA and PCIB were used as a polar auxin transport inhibitor (Yin and others 2007), and an auxin action inhibitor (Biswas and others 2007), respectively. ACC, ethephon, AVG, and Ag^+ were used as an ethylene precursor (Swarup and others 2007), an ethylene-releasing compound (Lorbiecke and Sauter 1999), an ethylene biosynthetic inhibitor (Swarup and others 2007), and an ethylene action inhibitor (Guzmán and Ecker 1990), respectively.

Ethephon and ACC were directly dissolved in distilled water; TIBA, IAA, and PCIB were dissolved in 100% ethanol; and AVG was dissolved in 1 M HCl. All the stock

solutions were stored at 4°C. When used, the stock solutions were diluted to the required concentrations with distilled water. Ag^+ was provided by $AgNO_3$ solution which was prepared immediately before use.

The germinated seeds were incubated with solutions of plant growth substances or distilled water as a control. The final concentration of ethanol was 0.4 ml/l or less, and the pH was regulated to 6.5 for each culture solution. All the culture solutions were refreshed every 2 days, and the seminal root lengths were measured with a ruler after 4 days of incubation.

Statistical Analysis

Statistical analysis was carried out using SPSS 13.0 statistical software (SPSS, Inc., Chicago, IL, USA). The mean values were compared using Duncan's test and the differences were considered significant at $p < 0.05$. All data presented are means ± SE.

Results

Endogenous Auxin is Required but Supraoptimal for Rapid Growth of Rice Seminal Roots

TIBA, a polar auxin transport inhibitor that can inhibit auxin transport from its source in the shoot (or tip) regions to basal sink tissues such as the root, and PCIB, a commonly used inhibitor of auxin that is assumed to inhibit auxin action by competing with auxin for the auxin receptor binding site (Oono and others 2003), were used in this experiment to determine whether endogenous auxin was required, but supraoptimal, for rapid growth of rice seminal roots.

As shown in Fig. 1a, high concentrations of PCIB significantly inhibited seminal root growth ($p < 0.05$), and the lengths of seminal roots were inhibited by 38, 62, 77, and 93% by the application of 500, 1000, 1500, and 2000 μM PCIB, respectively. Similarly, seminal root growth was significantly inhibited ($p < 0.05$) by application of TIBA at concentrations ranging from 0.1 to 2.5 μM, and the lengths of seminal roots were inhibited by 38, 51, and 70% by the application of 0.1, 0.5, and 2.5 μM TIBA, respectively (Fig. 1b). These results suggest that a certain threshold level of endogenous auxin is required, and auxin within the threshold may act as an active regulator during rapid growth of rice seminal roots.

In contrast, low concentrations of PCIB, ranging from 0.5 to 50 μM, significantly promoted seminal root growth ($p < 0.05$), and the lengths of the seminal roots were increased by 10, 26, and 66% by the application of 0.5, 5, and 50 μM PCIB, respectively (Fig. 1a). Moreover, low concentrations of IAA (≤0.0003 μM) did not inhibit seminal root growth, whereas high concentrations of IAA, ranging from 0.003 to 30 μM, inhibited seminal root growth. The

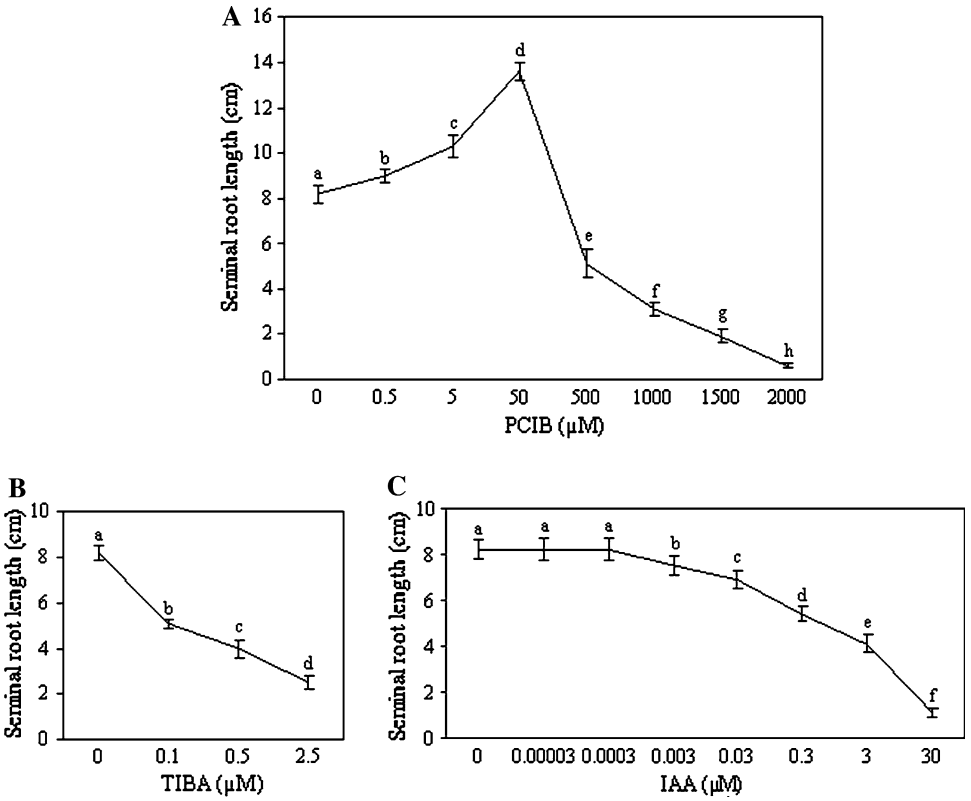

Fig. 1 Effects of exogenous auxin (IAA), a polar auxin transport inhibitor (TIBA), and an auxin action inhibitor (PCIB) on rice seminal root growth. Germinated seeds were incubated with different solutions or distilled water as a control. The lengths of the seminal roots were determined after 4 days of incubation; each result represents the mean ± SE ($n = 10$). Different letters above the bars indicate a significant difference at $p < 0.05$. **a** Low concentrations of PCIB promote rice seminal root growth, whereas high concentrations are inhibitory. **b** TIBA inhibits rice seminal root growth. **c** Exogenous IAA application does not promote but rather inhibits rice seminal root growth. PCIB, *p*-chlorophenoxyisobutyric acid; TIBA, 2,3,5-triiodobenzoic acid; IAA, indole-3-acetic acid

lengths of the seminal roots were inhibited by 9, 16, 34, 50, and 87% by the application of 0.003, 0.03, 0.3, 3, and 30 μM IAA, respectively (Fig. 1c). These results led to the suggestion that endogenous auxin was not optimal but supraoptimal for rapid growth of rice seminal roots, and supraoptimal auxin may actually act as an inhibitory regulator in rice seminal root growth. Thus, the application of exogenous auxin might make the auxin level more supraoptimal, which in turn inhibits seminal root growth.

Auxin Inhibition of Rice Seminal Root Growth is Not Caused by Ethylene

In this experiment, ACC and ethephon were used as precursors for ethylene production, and Ag^+ was used to alleviate the inhibitory effects of ACC and ethephon on rice seminal root growth. Furthermore, PCIB, Ag^+, and AVG were applied in the presence of exogenous IAA to determine whether IAA inhibition of rice seminal root growth was caused by ethylene.

As shown in Table 1, seminal root growth was significantly inhibited ($p < 0.05$) by the application of ACC (≥0.12 μM) or ethephon (≥0.01 μM), and seminal root length was inhibited by 50% by the application of 3 μM ACC or 1 μM ethephon. However, the I_{50} of seminal root growth caused by ACC or ethephon was weakened by the application of Ag^+, in the range of 0.016 to 0.4 μM, and the I_{50} caused by ACC or ethephon was completely eliminated by 0.08 μM Ag^+. Although seminal root length was also inhibited by 50% by the application of 3 μM IAA, the I_{50} of seminal root growth caused by IAA was not weakened but strengthened by the application of Ag^+; in contrast, the I_{50} caused by IAA was weakened significantly ($p < 0.05$) by the application of PCIB, ranging from 0.5 to 50 μM, and the I_{50} caused by IAA was completely eliminated by 50 μM PCIB (Table 2). In addition, although low concentrations of AVG, ranging from 0.0025 to 0.05 μM, did not affect the I_{50} of seminal root growth caused by IAA, high concentrations of AVG (≥1 μM) strengthened it. Taken together, the inhibitory mechanisms of auxin and ethylene on seminal root growth should differ, and a certain threshold level of ethylene is required to maintain seminal root growth in the presence of high concentrations of auxin (3 μM IAA); ethylene levels within the threshold may antagonize the inhibition of seminal root growth by auxin. Thus, ethylene is apparently not responsible for the inhibition of seminal root growth by auxin.

Table 1 An ethylene precursor (ACC) and an ethylene-releasing compound (ethephon) inhibit seminal root growth, but the inhibition was weakened by certain concentrations of an ethylene action inhibitor (Ag^+)

Treatment	Length (cm)
Control	8.2 ± 0.4^{a}
0.12 μM ACC	7.7 ± 0.3^{b}
0.6 μM ACC	7.0 ± 0.2^{c}
3 μM ACC	4.1 ± 0.4^{d}
3 μM ACC + 0.016 μM Ag^+	$5.2 \pm 0.2^{e,g}$
3 μM ACC + 0.08 μM Ag^+	8.5 ± 0.5^{a}
3 μM ACC + 0.4 μM Ag^+	$5.0 \pm 0.4^{e,f,g}$
3 μM ACC + 2 μM Ag^+	1.9 ± 0.2^{h}
0.01 μM ethephon	7.6 ± 0.3^{b}
0.1 μM ethephon	$4.7 \pm 0.2^{f,i}$
1 μM ethephon	4.1 ± 0.2^{d}
1 μM ethephon + 0.016 μM Ag^+	$4.8 \pm 0.3^{f,g,i}$
1 μM ethephon + 0.08 μM Ag^+	9.0 ± 0.4^{j}
1 μM ethephon + 0.4 μM Ag^+	6.2 ± 0.3^{k}
1 μM ethephon + 2 μM Ag^+	1.8 ± 0.1^{h}

ACC = 1-aminocyclopropane-1-carboxylic acid

Germinated seeds were incubated with different solutions or distilled water as a control. The lengths of the seminal roots were determined after 4 days of incubation; the data show means ± SE ($n = 10$)

Means denoted by different letters are significantly different at $p < 0.05$

Table 2 Inhibition of seminal root growth by auxin (IAA) was weakened by certain concentrations of an auxin action inhibitor (PCIB) but strengthened by an ethylene action inhibitor (Ag^+) and an ethylene biosynthetic inhibitor (AVG)

Treatment	Length (cm)
Control	8.2 ± 0.4^{a}
3 μM IAA	4.1 ± 0.3^{b}
3 μM IAA + 0.5 μM PCIB	5.8 ± 0.3^{c}
3 μM IAA + 5 μM PCIB	7.7 ± 0.4^{d}
3 μM IAA + 50 uM PCIB	8.5 ± 0.4^{e}
3 μM IAA + 0.016 μM Ag^+	3.7 ± 0.1^{f}
3 μM IAA + 0.08 μM Ag^+	3.2 ± 0.2^{g}
3 μM IAA + 0.4 μM Ag^+	0.6 ± 0.1^{h}
3 μM IAA + 2 μM Ag^+	0.5 ± 0.1^{h}
3 μM IAA + 0.0025 μM AVG	3.9 ± 0.3^{b}
3 μM IAA + 0.05 μM AVG	4.0 ± 0.2^{b}
3 μM IAA + 1 μM AVG	3.1 ± 0.2^{g}
3 μM IAA + 20 μM AVG	1.1 ± 0.2^{h}

IAA = indole-3-acetic acid; PCIB = *p*-chlorophenoxyisobutyric acid; AVG = aminoethoxyvinylglycine

Germinated seeds were incubated with different solutions or distilled water as a control. The lengths of the seminal roots were determined after 4 days of incubation; the data show means ± SE ($n = 10$)

Means denoted by different letters are significantly different at $p < 0.05$

A Certain Threshold Level of Ethylene is Required for Rapid Growth of Rice Seminal Roots

It remains unclear whether a certain threshold level of ethylene is required for rapid growth of rice seminal roots grown under normal conditions, not in the presence of a high concentration of auxin. To test this, different concentrations of the ethylene action inhibitor, Ag^+, and the ethylene biosynthetic inhibitor, AVG, were used. As shown in Fig. 2a, b, low concentrations of Ag^+ (0.016-0.8 μM) and AVG (0.0025-0.05 μM) significantly promoted seminal root growth, whereas higher concentrations of Ag^+ (≥0.4 μM) and AVG (≥1 μM) significantly inhibited seminal root growth. These results indicate that even for control rice plants grown in auxin-free solution, a certain threshold level of ethylene was required for the rapid growth of rice seminal roots.

Discussion

Effects of Auxin on Root Growth

To determine whether endogenous auxin is required, but supraoptimal, for rapid growth of rice seminal roots, the effects of IAA, TIBA, and PCIB were investigated. Treatment with TIBA and high concentrations of PCIB (≥500 μM) significantly inhibited rice seminal root growth (Fig. 1a, b), suggesting that TIBA and high concentrations of PCIB might result in suboptimal auxin levels and auxin action in roots for seminal root growth, respectively. Thus, we conclude that a certain threshold level of endogenous auxin is required for rapid growth of rice seminal roots. However, seminal root growth was inhibited by exogenous IAA and promoted by low concentrations of PCIB, suggesting that the level of endogenous auxin was already supraoptimal for the rapid growth of rice seminal roots. Thus, exogenous IAA treatment inhibited seminal root growth (Fig. 1c), whereas low concentrations of PCIB might promote seminal root growth by decreasing the auxin action, down to an optimal level (Fig. 1a). Based on these results, we concluded that endogenous auxin was required, but was supraoptimal, for the rapid growth of rice seminal roots. Our conclusion is consistent with previous findings that in rice, the seminal roots of auxin-resistant mutants (*arm1* and *arm1 arm2*) are longer and the seminal roots of auxin-overproducing plants are shorter, compared with wild-type plants (Chhun and others 2003; Yamamoto and others 2007). On the other hand, lateral root formation in auxin-resistant mutants (*arm1* and *arm1 arm2*) is inhibited and adventitious root formation in auxin-overproducing plants is promoted in rice (Chhun and others 2003; Yamamoto and others 2007). In addition, Zhou and others (2003) reported that in rice, TIBA treatment resulted in fewer and shorter adventitious roots, and exogenously supplemented 1-naphthalene acetic acid (NAA) partially restored the formation of adventitious roots. These findings indicate that in rice a certain level of auxin is also required for adventitious and lateral root formation, but the optimal level of auxin for these processes may be higher than that for seminal root growth.

Root elongation growth is attributed to cell division and cell elongation. Below the threshold level, auxin can promote cell division and cell elongation. In *Arabidopsis*, auxin

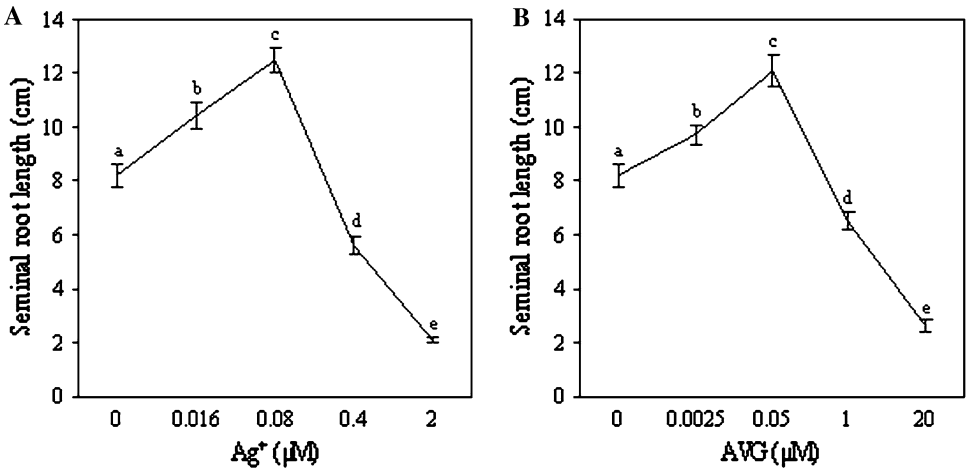

Fig. 2 Effects of an ethylene action inhibitor (Ag^+) and an ethylene biosynthetic inhibitor (AVG) on rice seminal root growth. Germinated seeds were incubated with different solutions or distilled water as a control. The lengths of the seminal roots were determined after 4 days of incubation; each result represents the mean ± SE ($n = 10$). Different letters above the bars indicate a significant difference at $p < 0.05$. **a** Low concentrations of Ag^+ promoted rice seminal root growth, whereas high concentrations were inhibitory. **b** Low concentrations of AVG promoted rice seminal root growth, whereas high concentrations were inhibitory. AVG, aminoethoxyvinylglycine

promotes cell division by inducing the expression of various mitotic cyclins: *CYCA2;1*, *CYCA2;2*, *CYCB1;1*, *CYCB2;1*, *CYCB2;2* (Ferreira and others 1994a, b; Richard and others 2002), and *CDKA;1* (Richard and others 2002). Similarly, D-type cyclin levels are also highly responsive to auxin (Soni and others 1995). In particular, auxin can induce telomerase activity during S-phase, which, in turn, supplies plant cells with sufficient telomeric DNA, thereby guaranteeing prolonged cell proliferation capacity (Vanneste and others 2005). Furthermore, it has been suggested that E2FB is one of the key targets for auxin to determine whether cells proliferate or whether they exit the cell cycle, enlarge, and endoreduplicate their DNA. Auxin can increase E2FB abundance through stabilization of the protein; correspondingly, the elevated E2FB advances cells into both S-phase and mitosis, thereby shortening cell cycle duration and promoting the production of new cells (Magyar and others 2005). In rice, the effects of auxin on the expression of cell cycle genes have been investigated in detail by Guo and others (2007); their results revealed that 25 cell cycle genes were upregulated by auxin treatment. The upregulated genes were *Orysa;CycA2;1*, *Orysa;CycB1;1*, *Orysa;CycB2;2*, *Orysa;CycD1;1*, *Orysa;CycD2;2*, *Orysa;CycD4;1*, *Orysa;-CycD4;2*, *Orysa;CycD5;1*, *Orysa;CycL1;1*, *Orysa;CycT1;1*, *Orysa;CycT1;3*, *Orysa;CycT1;4*, *Orysa;CycT1;6*, *Orysa;-CycU4;3*, *Orysa;CDKC;1*, *Orysa;CDKC;3*, *Orysa;CDKE;1*, *Orysa;CDKF;1*, *Orysa;CDKL2*, *Orysa;CDKL9*, *Orysa;CKL10*, *Orysa;CKS1*, *OrysaE2F3*, *Orysa;KRP5*, and *Orysa;Rb2*, and expression of most of these genes was detected in root tips of rice seedlings. On the other hand, four cell cycle genes (*Orysa;CycD5;3*, *Orysa;CycU3;1*, *Orysa;CDKA;2*, and *Orysa;CDKD;1*) were downregulated by auxin treatment. These results indicate that auxin can regulate cell division by regulating the expression of various cyclin genes in rice root tips.

Auxin promotes cell elongation by making the cell wall ductile or plastic via the induction of dextranase (α-1,6-D-glucan 6-glucanohydrolase, EC 3.2.1.11) (Heyn 1981), which breaks down certain cell wall components. The molecular linkages broken in the process are probably the arabinogalactan crosslinks of the hemicellulose matrix. The breaking of these crosslinks provides the necessary plasticity to the wall for cell extension to occur. In addition, auxin can promote cell elongation by inducing the expression of expansin genes (Caderas and others 2000).

However, above the threshold level, auxin inhibits cell division and cell elongation. Auxins have been divided into two classes based on their effects on cell division, elongation, and actin organization (Rahman and others 2007). Rahman and others (2007) reported that IAA and NAA inhibited root growth primarily through reducing the length of the growth zone rather than affecting the maximal rate of elemental elongation, and they did not reduce cell production rate. These two auxins have little effect on the extent of filamentous actin but tend to increase actin bundling. In contrast, 2,4-dichlorophenoxy-acetic acid (2,4-D) can remove actin, slow down cytoplasmic streaming, and inhibit root growth, primarily by reducing the cell production rate via the regulation of actin-dependent processes.

Effects of Ethylene on Root Growth

Ethylene is generally considered a growth inhibitor. Chadwick and Burg (1970) reported that ethylene inhibited the growth of roots and that the ethylene concentration was inversely correlated with growth rate. However, evidence has also accumulated that ethylene can also promote growth (Pierik and others 2006). Promotion of root growth by ethylene has been reported in deep-water rice (Lorbiecke and Sauter 1999) and in *Arabidopsis* under phosphorus stress (Ma and others 2003).

Our results indicate that in the presence of high concentrations of auxin, a certain threshold level of endogenous ethylene is required to maintain seminal root growth (Table 2). Furthermore, Fig. 2a, b shows that Ag^+ (≥0.4 μM) or AVG (≥1 μM) significantly inhibited seminal root growth, suggesting that a certain threshold level of endogenous ethylene is required for the rapid growth of rice seminal roots. However, treatment with low concentrations of Ag^+ (0.016-0.08 μM) or AVG (0.0025-0.05 μM) promoted seminal root growth (Fig. 2a, b), and treatment with ACC or ethephon inhibited seminal root growth (Table 1). These results led to the suggestion that endogenous ethylene levels were already supraoptimal for rapid growth of rice seminal roots; thus, low concentrations of Ag^+ or AVG might promote rice seminal root growth by reducing ethylene action or ethylene levels down to optimal levels, respectively. Taken together, we conclude that in wild-type control plants, endogenous ethylene is required, but is supraoptimal, for rapid growth of rice seminal roots; thus, treatment with ACC or ethephon might inhibit rice seminal root growth through the promotion of ethylene production. Our conclusion is consistent with previous findings that the roots of the ethylene-insensitive mutants *etr1-3* and *ein2-1* (Bleecker and others 1988; Guzmán and Ecker 1990; Roman and others 1995) are longer and the roots of the constitutive ethylene triple response mutant *ctr1-1* are shorter than those of wild-type plants (Kieber and others 1993), and that the shorter roots of the *ctr1-1* mutant can be phenocopied by subjecting wild-type plants to ACC levels above 5 μM (Kieber and others 1993).

Ethylene can regulate root growth by affecting cell division or cell elongation. Lorbiecke and Sauter (1999) reported that at the third node of deep-water rice, ACC or

ethephon treatment promoted adventitious root growth, whereas the growth-promoting effect was inhibited by 2,5-norbornadiene (an ethylene action inhibitor). They confirmed that ethylene action was necessary for adventitious root growth because of the necessity for mitotic cyclin induction. However, treatment with ACC or ethephon does not promote but inhibits rice seminal root growth (Table 1). The inhibitory effects of exogenously supplied ethylene or ethylene precursors on seminal (primary) root growth have also been reported in other plant species such as *Arabidopsis* (Růžička and others 2007), pea (*Pisum sativum* L.) (Eliasson and others 1989), and maize (Karahara and others 2008). Ethylene inhibits root growth by hampering cell elongation rather than by interfering with cell-cycle regulation (Růžička and others 2007; Swarup and others 2007). The inhibition of root growth by ethylene is associated with a change in the directional orientation of microfibrils and microtubules (Dolan 1997). In normally elongating root cells, microtubules are in a transverse orientation and ethylene treatment changes them into a longitudinal or random orientation (Le and others 2004).

Effects of Auxin–Ethylene Interactions on Root Growth

Auxin can induce ethylene biosynthesis by upregulation of ACC synthase, the key enzyme in ethylene production (Abel and others 1995). Furthermore, the typical response of intact roots toward exogenous auxin, as well as ethylene, is growth inhibition. It was hypothesized that the inhibition of root growth by auxin may be due to ethylene induced by auxin (Chadwick and Burg 1967; Steen and Chadwick 1973; Mulkey and others 1982b). Recently, Zhao and Hasenstein (2009) reported that in flax (*Linum usitatissimum*) roots, exogenous IAA not only upregulates the transcription levels of ACC synthases (*Lu-ACS1*, *Lu-ACS2*, *Lu-ACS3*), it also upregulates the transcription levels of ACC oxidases (*Lu-ACO1* and *Lu-ACO2*). Their results also revealed that the auxin antagonists 4,4,4-trifluoro-3-(indole-3-)-butyric acid (TFIBA) and PCIB can downregulate the transcription of *Lu-ACS1*, *Lu-ACS2*, *Lu-ACS3*, and *Lu-ACO3*. Moreover, TFIBA can downregulate the transcription levels of *Lu-ACO1* and *Lu-ACO2*, suggesting that root growth promotion by TFIBA or PCIB may be related to the inhibition of ethylene biosynthesis (Zhao and Hasenstein 2009). However, in *Lactuca sativa* L. (cv. Baijianye), the results of Zhang and Hasenstein (2002) indicated that the inhibition of ethylene biosynthesis by TFIBA was not the cause of root elongation, and TFIBA-stimulated root elongation was not related to the inhibition of ethylene biosynthesis. In addition, our results indicated that the alleviating effects of PCIB on auxin inhibition of seminal root growth should result from decreasing the action of auxin rather than the inhibition of ethylene biosynthesis caused by PCIB, because endogenous ethylene is required and may antagonize the inhibition of rice seminal root growth by IAA in the presence of exogenous IAA.

In this study, to determine whether auxin inhibition of rice seminal root growth was caused by ethylene, the ethylene action inhibitor Ag^+ and the biosynthetic inhibitor AVG were applied in the presence of exogenous IAA. Our results revealed that in rice, AVG or Ag^+ did not weaken but strengthened the inhibition of seminal root growth by exogenous IAA (Table 2), although levels of Ag^+ in the range of 0.016–0.4 μM can weaken the inhibition of seminal root growth by exogenous ACC or ethephon (Table 1), suggesting that the level of endogenous ethylene is not supraoptimal for maintaining rice seminal root growth in the presence of high concentrations of exogenous IAA, although we cannot exclude the possibility that levels of endogenous ethylene may be increased by exogenous IAA. Thus, auxin inhibition of rice seminal root growth is apparently not caused by ethylene; indeed, endogenous ethylene is required and may antagonize the inhibition of rice seminal root growth by IAA in the presence of exogenous IAA. Taken together, it is inappropriate to conclude that auxin inhibition of root growth is caused by ethylene based only on the phenomenon that exogenous auxin can promote ethylene biosynthesis.

Interestingly, recent reports have revealed that in *Arabidopsis*, Ag^+ can increase auxin efflux independently of effects on ethylene response; 5 μM Ag^+ dramatically decreased the inhibition of primary root growth by exogenous IAA (Strader and others 2009). AVG also has anti-auxin activity, and 30 μM AVG can partially decrease IAA levels in rice roots (Soeno and others 2010). In the present study, however, Ag^+ did not decrease the inhibition of seminal root growth in rice by exogenous IAA. Moreover, under our experimental conditions, although we cannot exclude the possibility that AVG may partially decrease auxin activity, the AVG-induced decrease of auxin activity apparently does not play a key role in the inhibition of rice seminal root growth by exogenous auxin because the inhibition caused by exogenous IAA should be weakened rather than strengthened by AVG.

On the other hand, ethylene can induce auxin biosynthesis in roots by regulating the expression of two *WEAK ETHYLENE INSENSITIVE* (*WEI2* and *WEI7*) genes that encode subunits of anthranilate synthase, the rate-limiting enzyme in tryptophan biosynthesis (Stepanova and others 2005). Ethylene can also modulate the transcription of several components of the auxin transport machinery, and stimulate basipetal auxin transport toward the elongation zone, where it activates a local auxin response leading to inhibition of cell elongation (Růžička and others 2007). Furthermore, in mutants impaired in auxin perception or

basipetal auxin transport, ethylene can neither activate the auxin response nor regulate root growth; thus, it has been suggested that the effect of ethylene on root growth is largely mediated by the regulation of auxin biosynthesis and transport-dependent local auxin distribution (Růžička and others 2007).

Auxin and Ethylene Can Regulate Root Growth Through Different Mechanisms

Auxin and ethylene can also inhibit root growth and interact during the regulation process. However, the pathways of auxin- and ethylene-mediated root inhibition are not identical (Stepanova and others 2007; Swarup and others 2007; Yoo and others 2009). Our results revealed that the I_{50} caused by ACC or ethephon on seminal root growth was weakened by Ag^+ concentrations ranging from 0.016 to 0.4 μM (Table 1), whereas the I_{50} caused by auxin was strengthened by Ag^+ or AVG and weakened by PCIB concentrations ranging from 0.5 to 50 μM (Table 2). Thus, we concluded that the inhibitory mechanisms of auxin and ethylene on seminal root growth differ and that auxin inhibition on seminal root growth is not caused by ethylene. Our conclusion is consistent with a recent report that auxin-mediated root growth inhibition can also occur in ethylene-insensitive mutants such as *etr1-3* and *ein2* (Růžička and others 2007). In addition, it has been reported that the root cap is essential for the ethylene-induced regulation of root growth, and exogenous ethylene is no longer able to inhibit root growth when the root cap has been surgically removed prior to hormone treatment, whereas removal of the root cap does not per se affect growth inhibition by auxin (Hahn and others 2008). Thus, auxin and ethylene regulate root growth through different mechanisms.

Effects of Other Plant Hormones on Root Growth

Gibberellin (GA) plays an important role in plant growth and development. In rice, a typical phenotype of GA-deficient mutants is dwarfism, and normal growth is restored by the application of exogenous GA (Sakamoto and others 2004). In *Arabidopsis*, primary roots of the GA-deficient *ga1-3* mutant are shorter than those of the wild-type, whereas GA-treated *ga1-3* roots are of a similar length to those of the wild-type (Fu and Harberd 2003). GA promotes the growth of plants by opposing the effects of nuclear DELLA protein growth repressors (Richards and others 2001). GA opposes the action of several DELLA proteins by destabilizing them, reducing both the concentration of detectable DELLA proteins and their growth-restraining effects (Itoh and others 2002). Fu and Harberd (2003) reported that auxin was necessary for GA-mediated control of root growth, and that attenuation of auxin transport or signaling delays the GA-induced disappearance of RGA (a DELLA protein) from root cell nuclei. These observations indicate that auxin controls root growth by modulating GA response. In addition, our previous results showed that auxin can promote rice internode elongation by the promotion of GA biosynthesis (Yin and others 2007). However, in rice, the role of auxin–GA interaction in the regulation of root growth is less clear and awaits further study.

Cytokinins exert a widespread influence on plant growth and development and usually act as negative regulators of root growth and development. Transgenic plants that overexpress cytokinin oxidase/dehydrogenase (*CKX*) genes have lower cytokinin levels and display a variety of phenotypes, including enlarged root meristem, increased root branching, and the promotion of adventitious root formation (Werner and others 2003). Cytokinins inhibit lateral root initiation by blocking the cycling of pericycle founder cells at the G2-to-M phase transition (Li and others 2006), and reduce meristem size by promoting cell differentiation in plant roots by repressing both auxin transport and responses to auxin at the boundary between the meristem and the root elongation zone (Chapman and Estelle 2009).

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