J. Plant Biol. (2009) 52:268–274
DOI 10.1007/s12374-009-9032-z

ORIGINAL RESEARCH

Effects of Brassinolide and IAA on Ethylene Production and Elongation in Maize Primary Roots

Hye Ree Yun · Se-Hwan Joo · Chan Ho Park · Seong-Ki Kim · Soo Chul Chang · Soon Young Kim

Received: 26 March 2009 / Accepted: 6 April 2009 / Published online: 7 May 2009

Abstract We examined the effects of brassinolide (BL) and/or an auxin (indole-3-acetic acid) on ethylene production and elongation in the primary roots of maize (*Zea mays*). When these two hormones were applied exogenously, both increased ethylene production. Before the tenth hour after treatment began, the influence of IAA was more evident than that of BL; the reverse was found beyond 10 h. When these hormones were treated simultaneously, the increase in level of ethylene was greater than the sum of effects by each hormone. Such a positive interaction was also recorded for changes in the activity of ACC synthase and the expression of its gene. For ACC oxidase, however, the two hormones had no apparent influence. When applied separately, neither affected root elongation nor proton extrusion. However, when given in combination, both phenomena occurred. Our results suggest that BL interacts with IAA to promote ethylene biosynthesis and elongation in roots. Therefore, it is possible that brassinolide acts by inducing auxin, which then stimulates both ethylene production (at the early stage) and root development.

H. R. Yun · S. Y. Kim (✉)
Department of Biological Science, Andong National University, Andong 760-749, South Korea

S.-H. Joo · C. H. Park · S.-K. Kim
Department of Life Science, Chung-Ang University, Seoul 156-756, South Korea

S. C. Chang (✉)
University College, Yonsei University, Seoul 120-749, South Korea
e-mail: schang@yonsei.ac.kr

Present Address:
H. R. Yun
Division of Proteome Research and Development, Genomine Inc., Pohang, Kyungbuk, South Korea

Keywords Auxins · Brassinosteroids · Ethylene · Root responses · *Zea mays*

Abbreviations

ACC	1-aminocycloporpane-1-carboxylic acid
AdoMet	S-adenosylmethionine
IAA	indole-3-acetic acid

Auxins are primary plant hormones involved in various physiological and developmental processes. The mechanisms for auxin activity have been identified through research on their transport (Sieberer and Leyser 2006), signal transduction pathways (Leyser 2006), and associated molecules. These include auxin receptors (Dharmasiri et al. 2005) and transcription factors (Guilfoyle and Hagen 2007). Other studies have investigated the interactions of other hormones with auxin, especially brassinosteroids and ethylene (Stepanova et al. 2007; McSteen and Zhao 2008). However, these interactions should also be examined at the molecular, cellular, and individual levels. And associated many events remain to be explained in physiological and developmental terms.

Ethylene, a gaseous plant hormone, is active in various physiological events, e.g., seed germination, cell expansion and differentiation, fruit ripening, senescence of leaves and flowers, and abscission (Abeles et al. 1992; Joo and Kim 2007). Production of this stress hormone increases in response to mechanical or disease-related stimuli (Abeles et al. 1992). In its synthesis pathway, ethylene molecules are obtained from their precursors of 1-aminocyclopropane-1-carboxylic acid (ACC), which comes from S-adenosylmethionine (S-AdoMet). The former and the latter are catalyzed by ACC oxidase and ACC synthase, respectively. These two enzymes are regulated by various factors (Abeles et al. 1992), including

auxin, which promotes ethylene biosynthesis by increasing the expression level of ACC synthase genes (Zarembinski and Theologis 1994; Kim and Mulkey 1997a, b).

Since brassinolide (BL) was first isolated from rape pollen (Grove et al. 1979), more than 50 brassinosteroids (BRs) have been identified. BRs are ubiquitous in plants; their functioning at lower-than-micromolar concentrations suggests that they comprise a sixth class of plant hormones (Sakurai and Fujioka 1993; Yokota 1997). Their roles have been well characterized in various events during plant growth and regulation (Yokota 1997; Altman 1999; Li and Chory 1999; Choe 2007). The effects of BRs were previously considered to be a result of auxin activity because plant responses to either were similar. In fact, many of the interactions between these two hormones, and some auxin-induced responses as well, are either synergistic or additively enhanced by BRs (Kim et al. 1990; Fujii et al. 1991; Fujioka et al. 1998; Sasse 1999; Holliday 2004). Several studies have been focused at the molecular level in an attempt to unravel the nature of those close associations (Goda et al. 2002; Nemhauser et al. 2004; Guilfoyle and Hagen 2007; Vert et al. 2008). For example, BRs can affect ethylene biosynthesis by increasing ACC synthase activity (Mandava 1988; Sakurai and Fujioka 1993).

We previously examined some hormonal interactions in corn and *Arabidopsis* plants. Lim et al. (2002) have shown that BL increases ethylene production in corn roots. Based on those data and other results, it has been suggested that BL and indole-3-acetic acid (IAA) interact during the synthesis of ethylene. Chang et al. (2004) have reported that BL exert its role via ethylene production at least partially by promoting a gravitropic response in corn roots. Because aminoethoxyvinylglycine, an inhibitor of ACC synthase, reduces the influence of BL on that response, one can speculate that BL acts on ACC synthase. Likewise, in *Arabidopsis* roots, BL and IAA seemed to interact in regulating gravitropism (Kim et al. 2007). Therefore, the objective of our current research was to better understand the nature of these hormone interactions. Here, we analyzed the response patterns of ethylene production and the elongation of maize roots in the presence or absence of BL and/or IAA. Our results indicate that BL may interact with IAA for ethylene production and elongation of roots. Also implicated is that BL exerts its role at early stage of ethylene production and in elongation by modulating IAA action in maize roots.

Materials and Methods

Plant Materials and Chemicals

Plants of maize (*Zea mays* L. cv "Golden Cross Bantam") were grown as described by Chang et al. (2004), with slight modification. Seeds were washed several times with tap water and soaked in distilled water for 10 h in the dark at 27 ±1°C. Afterward, they were placed on trays (27×20× 2.5 cm) covered with damp paper towels. The trays were positioned vertically for 42 h in the dark at 27±1°C and 70% relative humidity. Following germination, seedlings with 1.5- to 2.0-cm-long straight-grown primary roots were selected and used for experiments on root elongation and proton extrusion.

All chemicals used here were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise indicated.

Measurement of Ethylene Content

To measure ethylene content, we used a slightly modified version of the method from Kelly and Bradford (1986). Root tissues (1 cm long and including the tips) were harvested and incubated with or without growth hormones. Vials containing 40 roots and potassium phosphate buffer (50 mM, pH 6.8)±IAA and/or brassinolide were incubated at 27±1°C in the dark with shaking (170 rpm). Air samples (1 ml) from these vials were withdrawn with a syringe and injected into a gas chromatograph equipped with a column containing alumina (HP5890 Series II; Hewlett-Packard, USA).

Activity Assays for ACC Synthase and ACC oxidase

Activity of ACC synthase was assayed as described by Woeste et al. (1999). Eighty root segments were ground on ice plus sea sand in a solution containing 250 mM potassium phosphate buffer (pH 8.0), 10 μM pyridoxal phosphate, 1 mM ethylenediaminetetraacetic acid, 2 mM phenylmethylsulfonyl fluoride, and 5 mM dithiothreitol. The homogenate was kept for 4 min on ice and centrifuged for 15 min at 15,000×*g*. Afterward, 1 mL of the supernatant was incubated for 1 h in a solution containing 5 mM S-AdoMet (0.1 mL) at 22±1°C with shaking (170 rpm). The solution was then placed for 10 min in ice with a solution containing 0.1 mL of 20 mM $HgCl_2$ and 0.1 mL of a 1:1 NaOH–NaOCl mixture. Ethylene content was measured from 1-ml samples of gas withdrawn from each vial, and the data were used for determining ACC synthase activity (Lizada and Yang 1979).

An assay for ACC oxidase activity was performed in vivo according to the method of Wang and Woodson (1989). Here, 40 root segments were incubated in a 50 mM potassium buffer (pH 6.8) containing 0.1 mM aminoethoxyvinylglycine (AVG), with or without BL and/or IAA. Afterward, they were washed with distilled water, then infiltrated for 2 h with 1 mM ACC at 27±1°C in the dark. Values for ACC oxidase activity were based on the amount of ethylene produced from primary root segments that had been placed for 1 h in distilled water without ACC.

Measurement of Maize Primary Roots

Straight-grown root segments were positioned vertically for 2 h in a buffer solution (5 mM potassium phosphate, pH6.8) containing various concentrations of BL and/or IAA, with aeration at 27±1°C. In order to retain their moisture, the segments were placed in a lucent Plexiglas™ container where relative humidity was maintained at 95%. Root lengths were measured with a closed circuit digital camera (SAC-410NDX; Samsung Aerospace, Korea) and Image-Pro Plus software (Yongma, Seoul, Korea). Segment images were recorded for 2 h by a time-lapse video cassette recorder (STLU-36D; Samsung, Korea). They were magnified ten times on a monitor and lengths were measured after evaluation with that computer program.

Extraction of Total RNA and RT-PCR

Total RNA was extracted by a modified phenol sodium dodecyl sulfate (SDS) method (Sambrook and Russell 2001). Samples were ground to a powder with a mortar and pestle under liquid nitrogen. This powder was resuspended with an RNA extraction buffer [0.1 M Tris–Cl (pH9.0), 0.1 M NaCl, and 1%SDS]. A 25:24:1 volume of phenol–chloroform–isoamylalcohol equal to the buffer was added and vigorously mixed. After centrifugation at 13,000 rpm for 30 min at 4°C, the supernatant was transferred to a new tube and incubated with 2.5 M LiCl for 1 h at 4°C. Following 15 min of centrifugation at 13,000 rpm, the pellet was washed with 70% EtOH and dissolved in DEPC-treated water. The concentration of total RNA was measured by spectrophotometer.

Purified total RNA (2 μg) was used for first-strand complementary DNA synthesis with M-MLV reverse transcriptase (RT; Promega, Madison, WI, USA). Polymerase chain reaction (PCR) conditions included 30 cycles of denaturing at 95°C for 5 min, annealing at 55°C for 20 s, and extension at 72°C for 5 min; then, a final elongation step at 72°C for 10 min. The gene-specific primers for this reaction are listed in Table 1. *Actin5* served as an internal control, with 24 cycles of denaturing at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 1 min.

Determining Content of Protons Extruded from Root Segments

After treatment with BL or IAA, 40 root segments were positioned in a small vial containing a fine mesh divided horizontally and held in place with a magnetic bar below. The segments were immersed in distilled water and all vials were set above a magnetic stirrer. After the tissues were treated with hormones for 2 h, that solution was replaced with distilled water. An electrode (3-mm diameter) was inserted and pH was measured for up to 2 h.

Statistical Analysis

All experiments were performed at least three times, with no fewer than 40 primary roots each. To test for significance at p values of <0.05, the data mean values were calculated according to Student t tests.

Results and Discussion

BL and IAA Treatments Improve Ethylene Production in Maize Roots

We previously showed that brassinolide application increases ethylene production in the primary roots of maize (Lim et al. 2002; Chang et al. 2004). Based on those results and other preliminary data, we opted to treat at levels of 10^{-7}M BL and 10^{-5}M IAA for all experiments unless otherwise mentioned. Time courses were run for ethylene production in the presence or absence of BL and/or IAA. Up to 8 h after treatment, both BL and IAA increased ethylene production, with the effect of the latter hormone being more pronounced (Fig. 1). However, from 10 h posttreatment, BL had a greater influence. In the presence of BL and IAA, both exerted interactive positive effects throughout the experimental

Table 1 Gene-specific primers used for RT-PCR experiments

Gene (accession number)	Sequence (5' to 3')	
ZmACS2 (AY359569)	F	ATCGCGTACAGCCTCTCCAAGGA
	R	GATAGTCTTTTGTCAACCATCCCATAGA
ZmACO15 (AY359572)	F	CTCGTCTTCGATCAATTCCCAAGT
	R	TACATTATCATTATTTCTCCGGCTGT
ZmACO35 (AY359576)	F	CTCATCCTGCTGCTCCAGGACGAC
	R	ACACACATAACTGTGCCACTATAAGCA
Actin5	F	CTCAAGAAGTTCTCAGCAGTA
	R	TCACCTTCTTCATCCGCAGTT

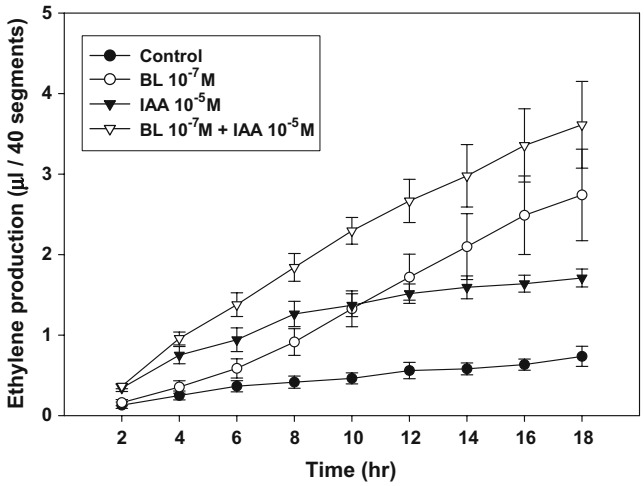

Fig. 1 Time course for ethylene production in the presence or absence of brassinolide and/or auxin. Root segments were incubated for various periods in solution containing potassium phosphate buffer (50 mM, pH6.8), with or without IAA and/or BL. Afterward, 1 mL of air was withdrawn for measuring ethylene content. *Symbols* denote mean values±SE of five independent experiments

period. For example, at 6 h after treatment, the 266% increase in ethylene production from this combined application was greater than the sum from individual treatments, i.e., 53% for BL or 158% for IAA. These results supported those of Lim et al. (2002) who had also reported a synergy for these two.

The BL-induced enhancement of ethylene production in the presence of IAA became greater over time, whereas the IAA-induced increase in titers remained constant from 6 h after the start of treatment. Two different patterns of hormonal actions were apparent in the early and late experimental stages, depending upon which hormone was more effective. This suggests that BL and IAA interact in different manners at the beginning and end of the period. For example, after 4 h of treatment, the influence of BL was almost negligible compared with the more evident IAA effect. We can conclude that, when both are combined, BL plays its role mainly via IAA action, i.e., by activating IAA capacity to promote ethylene production. However, this was not true in the late stage, when auxin, instead, affected BL action.

The interactive physiological events between BL and IAA include a role in the gravitropic response (Kim et al. 2000). In corn roots, auxins seem to be essential to the brassinolide-induced response, and BL apparently interacts with IAA, possibly by increasing root sensitivity to auxin. One possible explanation is that brassinolide causes changes in that sensitivity for ethylene production during the early stage. Additionally, BL may affect auxin biosynthesis (Kim et al. 2007). Although a mechanism exists for BL action that depends on root sensitivity to IAA alone, no experimental data have been reported for this type of auxin biosynthesis.

Effects of BL and IAA on ACC Synthase and ACC Oxidase

Auxins increase ACC activity at the gene level (Zarembinski and Theologis 1994). Lim et al. (2002) have shown that BL and/or IAA participate in ethylene production by increasing the activities of ACC synthase and ACC oxidase. To locate the functional site of our two hormones and explore their possible patterns for interaction, we measured the activities of ACC synthase and ACC oxidase in the presence or absence of brassinolide and/or auxin. At 8 h after treatment, the increase in ACC synthase activity was less from BL alone (22%) than from IAA alone (40%; Fig. 2A). However, when those two were combined, this activity rose by 77%. This suggests a synergistic interaction. Such a positive influence was also found at 16 h posttreatment (data not shown).

Another key enzyme in ethylene production is ACC oxidase. At 8 h after treatment, BL was associated with a 0.84-fold

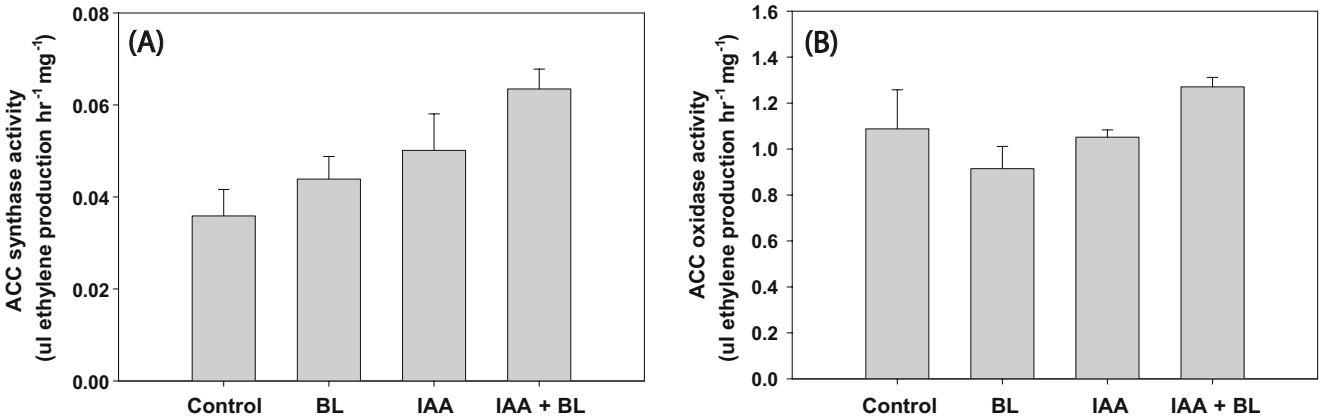

Fig. 2 Effect of BL and/or IAA on ACC synthase (**A**) and ACC oxidase (**B**). Activity of ACC synthase was based on estimation of ACC content, using crude extract from root segments incubated for 8 h in presence or absence of BL and/or IAA. ACC oxidase activity was assayed with intact root segments incubated for 8 h in solution containing 0.1 mM AVG, in the presence or absence of BL and/or IAA. *Symbols* denote mean values±SE of nine (**A**) or five (**B**) independent experiments

improvement in enzyme activity over that of the control (Fig. 2B) compared with a 0.97-fold increase due to IAA. However, when combined, these two hormones prompted a 17% rise in activity. Nevertheless, such synergy was not evident when corn roots were treated for 18 h, thereby implying that ACC oxidase activity may not directly reflect those BL- and/or IAA-induced changes in ethylene production.

Both ACC synthase and ACC oxidase have gene families in *Arabidopsis* and corn (De Paepe and Van der Straeten 2005). Among the seven genes tested here, only one for ACC synthase (*ZmACS2*) and three for ACC oxidase (*ZmACO15*, *ZmACO20*, and *ZmACO35*) were expressed in the roots (data not shown). For *ZmACS2*, RT-PCR showed that its expression level was enhanced by BL or IAA (Fig. 3). When the two hormones were combined, the 16.4% increase in transcript was much greater than that gained with the individual hormones (4.16%+5.28%). Again, this demonstrates a synergistic interaction, which suggests that this influence on ACC synthase is exerted at the transcription level, resulting in more pronounced production of ethylene. Therefore, ACC synthase could be the site for this synergy between auxin and BRs. In contrast, the expression levels of ACC oxidase genes did not exhibit any evident pattern of correlation between hormones and changes in ACC oxidase activities (Fig. 3).

BL Enhances Root Elongation Only in the Presence of IAA

To describe the interaction between BL and IAA in corn roots, we investigated their effects on root elongation and proton extrusion, beginning at 2 h after treatment. During this early period, untreated roots grew 2.3±0.4 mm. BL application resulted in a 3% increase in root length, which was within the error range (Fig. 4). In contrast, IAA was associated with shorter roots, but this was also within the error range. Unexpectedly, simultaneous treatment caused a 74% increase in root lengths compared with the control. Therefore, we could suggest two scenarios; either BL improved root development by activating IAA or else auxin had a positive effect on brassinolide action.

To determine which scenario was more probable, we measured the amount of proton that was extruded, theorizing that IAA would enhance cell elongation by

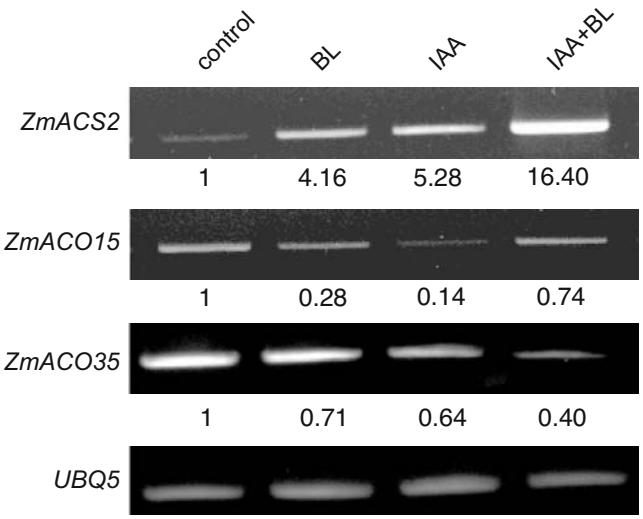

Fig. 3 Effect of BL and/or IAA on changes in gene expression levels for ACC synthase and ACC oxidase. Root segments were incubated for 8 h in solution containing potassium phosphate buffer (50 mM, pH 6.8), with or without IAA and/or BL. Afterward, total RNAs were extracted and used for RT-PCR. Gel photo shows results with consistent patterns from four independent experiments

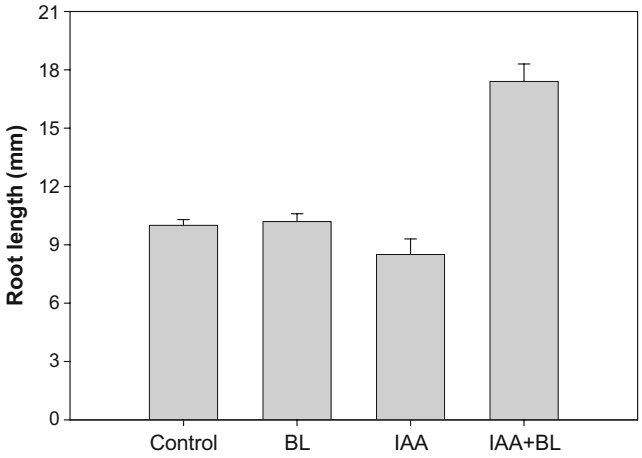

Fig. 4 Effect of BL and/or IAA on root elongation. Root segments were incubated for 2 h in solution containing potassium phosphate buffer (50 mM, pH 6.8), with or without IAA and/or BL. Segments were then measured for elongation. *Bars* in graph denote SE for four independent experiments

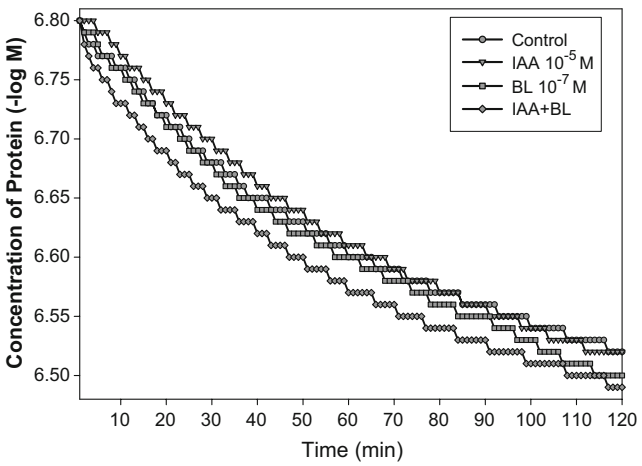

Fig. 5 Effect of BL and/or IAA on proton extrusion from corn. Root segments were incubated for 2 h in solution containing potassium phosphate buffer (50 mM, pH6.8), with or without IAA and/or BL. After hormones were removed, segments were incubated in vials containing distilled water. During that period, levels of protons extruded from 40 root segments to 50 μL of water were measured with microelectrode. *Graph* shows results with consistent patterns from four independent experiments

increasing the level of protons apart from the influence of BL. If, however, IAA was correlated with longer roots as a result of BL activation, then we expected that proton would not increase. Our experiments showed that the combination of both hormones led to greater proton extrusion for the first 2 h of treatment whereas BL or IAA alone did not (Fig. 5). Therefore, brassinolide seemed to affect this IAA-induced phenomenon, at least in the early stage.

Many routes are possible for this interaction between IAA and BL. For example, BES1, a BR-regulated transcription factor, is involved in the expression of BL- and auxin-regulated genes (Nemhauser et al. 2004). BRs seem to promote auxin transport in lateral root formation (Bao et al. 2004) and in tropism (Li et al. 2005). Auxin signaling and BR biosynthesis also are connected (Mouchel et al. 2006). Finally, BIN2 interacts with ARF2, an auxin-related transcription factor, causing a synergistic effect on transcription (Vert et al. 2008).

When we monitored the early stage of ethylene production in the presence of BL and IAA, we concluded that the former exerts its role via action by the latter. This was also true for root elongation. One possible explanation is that BL influences an auxin-related transcription factor, causing greater ethylene production and longer roots. In contrast, auxin signaling may affect BL biosynthesis in the later stage (see also Mouchel et al. 2006). Moreover, changes in activity and gene expression imply that ACC synthase is the interaction site for these two hormones during ethylene production.

Acknowledgements This work was supported by a grant to SYK from the 2008 Research Fund of Andong National University.

References

Abeles FB, Morgan PW, Saltveit ME Jr (1992) Ethylene in Plant Biology, 2nd edn. Academic, San Diego

Altman T (1999) Molecular physiology of brassinosteroids revealed by the analysis of mutants. Planta 208:1–11

Bao F, Shen J, Brady SR, Muday GK, Asami T, Yang Z (2004) Brassinosteroids interact with auxin to promote lateral root development in *Arabidopsis*. Plant Physiol 134:1624–1631

Chang SC, Kim YS, Lee JY, Kaufman PB, Kirakosyan A, Yun HS, Kim T-W, Kim SY, Cho MH, Lee JS, Kim S-K (2004) Brassinolide interacts with auxin and ethylene in the root gravitropic response of maize (*Zea mays*). Physiol Plant 121:666–673

Choe S (2007) Signal-transduction pathways toward the regulation of brassinosteroid biosynthesis. J Plant Biol 50:225–229

De Paepe A, Van der Straeten D (2005) Ethylene biosynthesis and signaling: an overview. Vitam Horm 72:399–430

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445

Fujii S, Hirai K, Saka H (1991) Growth-regulating action of brassinolide in rice plant. In: Cutler HG, Yokota T, Adam G (eds) Brassinosteroids, chemistry, bioactivity, and application. ACS symposium series, vol 474. American Chemical Society, Washington DC, pp 306–311

Fujioka S, Noguchi T, Takatsuto S, Yoshida S (1998) Activity of brassinosteroids in the dwarf rice lamina inclination bioassay. Phytochem 49:1841–1848

Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S (2002) Microarray analysis of brassinosteroid-regulated genes in *Arabidopsis*. Plant Physiol 130:1319–1334

Grove MD, Spencer FG, Rohwededer WK, Mandava NB, Worley JF, Warthen JD Jr, Steffens GL, Flippen-Anderson JL, Cook JC Jr (1979) Brassinolide, a plant growth hormone promoting steroid isolated from *Brassica napus* pollen. Nature 281:216–217

Guilfoyle TJ, Hagen G (2007) Auxin response factors. Curr Opin Plant Biol 10:453–460

Holliday KJ (2004) Plant hormones: the interplay of brassinosteroids and auxin. Curr Biol 14:R1008–R1010

Joo S, Kim WT (2007) A gaseous hormone ethylene: the signaling pathway. J Plant Biol 50:109–116

Kelly M, Bradford KJ (1986) Sensitivity of the *diageotropica* tomato mutant to auxin. Plant Physiol 82:713–717

Kim S-K, Abe H, Little CHA, Pharis RP (1990) Identification of two brassinosteroids from cambial region of Scots pine (*Pinus sylvestris*) by gas chromatography-mass spectrometry, after detection using a dwarf rice lamina inclination bioassay. Plant Physiol 94:1709–1713

Kim S-K, Chang SC, Lee EJ, Chung W-S, Kim Y-S, Hwang S, Lee JS (2000) Involvement of brassinosteroids in the gravitropic response of primary root of maize. Plant Physiol 123:997–1004

Kim SY, Mulkey TJ (1997a) Effect of ethylene antagonists on auxin-induced of intact primary root elongation in maize (*Zea mays* L.). J Plant Biol 40:256–260

Kim SY, Mulkey TJ (1997b) Effect of auxin and ethylene on elongation of intact primary roots of maize (*Zea mays* L.). J Plant Biol 40:249–255

Kim T-W, Lee SM, Joo S-H, Yun HS, Lee Y, Kaufman PB, Kirakosyan A, Kim S-H, Nam KH, Lee JS, Chang SC, Kim S-K (2007) Elongation and gravitropic response of *Arabidopsis* roots are regulated by brassinolide and IAA. Plant Cell Environ 30:679–689

Leyser O (2006) Dynamic integration of auxin transport and signaling. Curr Biol 16:R424–R433

Li J, Chory J (1999) Brassinosteroid actions in plants. J Exp Bot 50:332–340

Li L, Xu J, Xu ZH, Xue HW (2005) Brassinosteroids stimulate plant tropisms through modulation of polar auxin transport in *Brassica* and *Arabidopsis*. Plant Cell 17:2738–2753

Lim SH, Chang SC, Lee JS, Kim S-K, Kim SY (2002) Brassinosteroids affects ethylene production in the primary roots of maize (*Zea mays* L.). J Plant Biol 45:148–153

Lizada C, Yang SF (1979) A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Ann Biochem 100:140–145

Mandava BN (1988) Plant growth promoting brassinosteroids. Annu Rev Plant Physiol Plant Mol Biol 39:23–52

McSteen P, Zhao Y (2008) Plant hormones and signaling: common themes and new developments. Dev Cell 14:467–473

Mouchel CF, Osmont KS, Hardtke CS (2006) BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. Nature 443:458–461

Nemhauser JL, Mockler TC, Chory J (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. PLoS Biol 2: E258

Sakurai A, Fujioka S (1993) The current status of physiology and biochemistry of brassinosteroids. Plant Growth Regul 13:147–159

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Press, Cold Spring Harbor

Sasse JM (1999) Physiological actions of brassinosteroids. In: Sakurai A, Yokota T, Clouse SD (eds) Brassinosteroids: steroidal plant hormone. Springer, Tokyo, pp 306–311

Sieberer T, Leyser O (2006) Auxin transport, but in which direction? Science 312:858–860

Stepanova AN, Yun J, Likhacheva AV, Alonso JM (2007) Multilevel interactions between ethylene and auxin in *Arabidopsis* roots. Plant Cell 19:2169–2185

Vert G, Walcher CL, Chory J, Nemhauser JL (2008) Integration of auxin and brassinosteroid pathways by auxin response factor 2. Proc Natl Acad Sci U S A 105:9829–9834

Wang H, Woodson WR (1989) Reversible inhibition of ethylene action and interruption of petal senescence on carnation flowers by norbornadiene. Plant Physiol 89:434–438

Woeste KE, Ye C, Kieber JJ (1999) Two *Arabidopsis* mutants that overproduce ethylene are affected in the posttranscriptional regulation of 1-aminocyclopropane-1-carboxylic acid synthase. Plant Physiol 119:521–529

Yokota T (1997) The structure, biosynthesis and function of brassinosteroids. Trends Plant Sci 2:137–143

Zarembinski TI, Theologis A (1994) Ethylene biosynthesis and action: a case of conservation. Plant Mol Biol 26:1579–1597