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Effects of Chromosaponin I and Brassinolide on the Growth of Roots in Etiolated *Arabidopsis* Seedlings

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Summary

The stimulatory effects of chromosaponin I (CSI) on the growth of roots in etiolated *Arabidopsis thaliana* (ecotype Columbia) seedlings were compared with those of brassinolide (BR) bearing a structural similarity to CSI. The optimum concentrations of CSI and BR were 100 µmol/L and 1 nmol/L, respectively. The roots grew curved on wetted filter paper in the absence of CSI, but elongated straight in the presence of CSI. CSI increased the length of root cells and decreased the diameter of roots. However, BR did not increase the cell length although it decreased the root diameter. Neither CSI- nor BR-induced growth in roots was detected in ethylene-insensitive mutants *etr1-1* and *ein2-1*, supporting the idea that CSI and BR stimulate the growth of roots by interfering with the action of ethylene. However, the mechanism of CSI action is not the same as that of BR.

Key words: Arabidopsis thaliana, brassinolide, chromosaponin I, ethylene-insensitive mutants, root growth.

Abbreviations: AVG = L-α-(2-aminoethoxyvinyl)glycine; BR = brassinolide; CSI = chromosaponin I; NBD = 2,5-norbornadiene.

Introduction

Application of saponins to plants has been reported to influence some biological processes as reviewed by Geuns (1978). Saponins stimulated development of shoots and roots on *Begonia* leaves (Balansard and Pellissier, 1943 a) and growth of wheat embryos (Balansard and Pellissier, 1943 b) and pea embryos (Helmkamp and Bonner, 1953). Root growth was inhibited by various saponins in *Lepidium* and *Hordeum* seedlings (Von Euler, 1946) and in *Cucumis* seedlings (Rezk and Ferenczy, 1969), but was stimulated by chromosaponin I (CSI) in a variety of plants (Tsurumi and Tsujino, 1995; Tsurumi and Wada, 1995). Germination of fenugreek seeds was inhibited by endogenous saponin-like substances (Zambou et al., 1993). A glycosidic triterpenoid saponin was reported to be a specific inhibitor of diguanylate cyclase, the key regulatory enzyme of the cellulose synthesizing apparatus of *Acetobacter xylinum* (Ohana et al., 1998). However, no definite physiological role for saponins in regulating the growth and development of plants has been established.

CSI is a γ-pyronyl-triterpenoid saponin isolated from pea (Tsurumi et al., 1991, 1992) and characterized as an amphipathic natural reductant (Tsujino et al., 1995). In 7-day-old etiolated pea seedlings, hooks and root tips contained high concentrations of CSI, i.e. 2 to 3 mmol/L. This saponin was also isolated from soybean and other leguminous plants (Kudou et al., 1992, 1993; Massiot et al., 1992). The mechanism of CSI action in stimulating the growth of roots has been

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studied using lettuce roots; CSI enhanced the elongation of cortical root cells (Tsurumi and Wada, 1995) and increased the mechanical extensibility of cell walls (Tsurumi et al., 1996). Recently, Tsurumi and Ishizawa (1997) found that CSI reduces the response of lettuce roots to ethylene and proposed a mode of action of CSI; CSI reduces the ethylene signaling activity leading to growth inhibition, which in turn allows the longitudinal loosening of root cell walls and finally results in cell elongation. These facts raise the possibility that endogenous saponins play a role in growth of plants.

Arabidopsis is a very useful plant material for investigating the mechanism of actions of plant hormones. *Arabidopsis* seedlings show typical ethylene effects and some mutants related to ethylene are available (Ecker, 1995). Hence, we have been studying the effects of CSI on the growth of *Arabidopsis* seedlings to prove the involvement of ethylene in CSI action by using wild-type, two ethylene receptor mutants *etr1-1* and *etr1-3* (Chang et al., 1993) and an ethylene signaling mutant *ein2-1* (Guzmán and Ecker, 1990). In our preliminary experiments, CSI as well as BR stimulated the growth of the wild-type roots. Although effects of BR on the growth of roots have been reported to be mainly inhibitory, low concentrations of BR stimulated root growth in some plants (Takematsu et al., 1983; Roddick and Guan, 1991). Since CSI has a hydrophobic triterpenoid moiety bearing a structural similarity in part with BR, one might argue that CSI acts by a mechanism similar to that of BR. To test this possibility we compared the effects of two compounds on the growth of roots.

In the present paper we describe the involvement of ethylene in the actions of CSI and BR in stimulating the growth of roots in *Arabidopsis* seedlings and also report similarity and dissimilarity between the effects of CSI and BR on the growth of roots.

Materials and Methods

Plant material

Wild-type *Arabidopsis thaliana* (L.) Heynh., line Columbia, and its mutants with altered responses to ethylene, *etr1-1, etr1-3* (Chang et al., 1993), and *ein2-1* (Guzmán and Ecker, 1990), were used in this study. The ethylene response mutants were obtained from the *Arabidopsis* Biological Resource Center (Ohio, USA). Seeds of these lines were propagated as follows: seeds were sown on vermiculite fertilized with a Hyponex nutrient solution (Hyponex Corp., Ohio, USA), placed in a cold room at 4 °C for 4 days, and then allowed to grow at 23 °C under a 16 h photoperiodic cycle. The light source was two 40W-white fluorescent lamps (FLR40SW/M36, Hitachi, Tokyo, Japan) and the intensity at plant level was about 10 $W m^{-2}$. The nutrient solution was given every week and the ripened seeds were harvested in order of siliques into full maturity.

Chemicals

CSI was extracted from 7-day-old etiolated pea seedlings (*Pisum sativum* L. cv. Alaska) with aqueous methanol and purified by HPLC as described previously (Tsurumi et al., 1992). The purified CSI was dried to white powder and kept under N_2 at −80 °C. BR was purchased from Fuji Chemical Industries, Ltd. (Toyama, Japan).

Growth test

Test solutions were prepared by dissolving chemicals in 20 mmol/L MOPS buffer (pH 6.6). The buffer was made of 5 mmol/L KNO_3, 2 mmol/L $Ca(NO_3)_2$, 2 mmol/L $MgSO_4$, 1 mmol/L KH_2PO_4 and

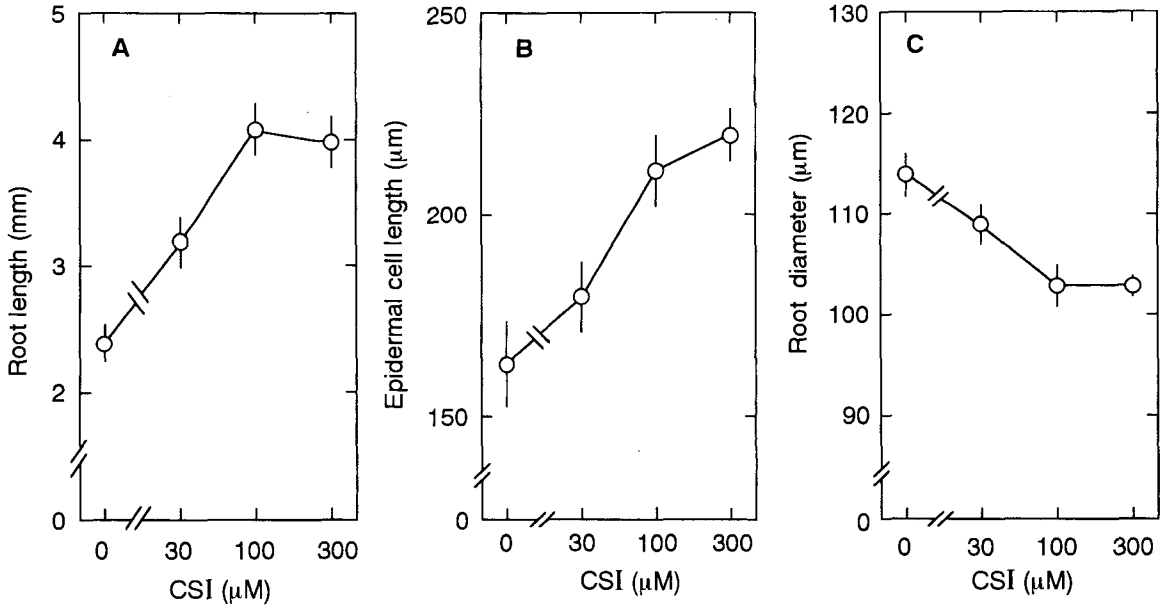

Fig. 1: Effects of CSI on the root elongation (A), the length of mature epidermal cells (B), and the root diameter (C) in wild-type *Arabidopsis*. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) with CSI (0, 30, 100 and 300 μmol/L) for 4 days in the dark. The length of mature epidermal cells and the diameter of roots were measured at the longitudinal midpoint of the root. The length of epidermal cells for an individual seedling was the average from 10 cells. Data are the averages from 10 to 15 seedlings (± SE).

20 mmol/L MOPS. The pH of the buffer was adjusted with KOH. Fifteen *Arabidopsis* seeds were placed in a 2.6 cm Petri dish on filter paper (Advantec No. 2; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) wetted with 300 µL of the buffer. Two or 4 days after cold treatment at 4 °C under nearly saturating humidity in the dark, the dishes were incubated at 23 °C in the dark for 1 day and then irradiated for 4 h with white fluorescent tubes (FL 20SS-BRN/18, Toshiba, Tokyo, Japan) at an irradiance of about 1.6 W m^{-2}. The irradiated seeds were transferred with forceps to another 2.6 cm Petri dish on filter paper wetted with 300 µL of a test solution and allowed to germinate under nearly saturating humidity in the dark at 23 °C. For ethylene treatment, the dishes containing irradiated seeds were placed in a sealed 140-mL plastic cylinder. Ethylene was injected with a syringe into each cylinder through a small side hole as described previously (Tsurumi and Ishizawa, 1997). Four days after incubating the seedlings in the dark at 23 °C, the length and width of roots were measured under a microscope, and the mean (± SE) for 10 to 15 seedlings was calculated. The diameter of roots and the length of epidermal cells were measured at the longitudinal midpoint of roots. Each assay was repeated two or three times.

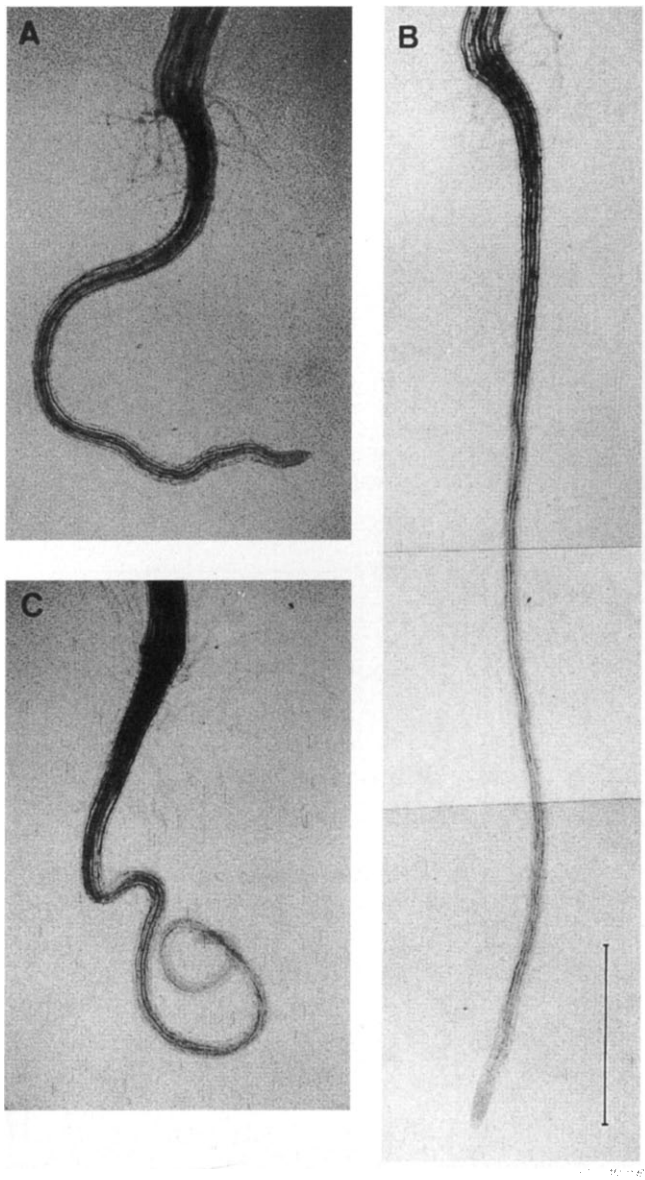

Fig. 2: Effects of CSI and BR on the growth of wild-type *Arabidopsis* roots. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) without additives (A) or with 100 µmol/L CSI (B) and 1 nmol/L BR (C) for 4 days in the dark. The length of a bar is 1 mm.

Results

Effects of CSI on the growth of Arabidopsis roots

Application of CSI stimulated the growth of roots in wild-type *Arabidopsis* seedlings as shown in Fig. 1 A. CSI accelerated cell elongation in the epidermis (Fig. 1 B) and reduced the root diameter (Fig. 1 C). These effects of CSI on *Arabidopsis* roots are quite similar to those on lettuce roots (Tsurumi and Ishizawa, 1997), but the optimum concentration of CSI was lower in *Arabidopsis* than in lettuce: 100 µmol/L in the former and 4.4 mmol/L in the latter, respectively. This sensitivity to CSI of *Arabidopsis* roots is highest among the plants that we have tested (Tsurumi and Wada, 1995). Figure 2 shows typical morphological changes in *Arabidopsis* roots treated with CSI. Control roots showed a curved growth and CSI-treated roots provided a straight growth. Time courses of the root elongation and the decrease in root diameter are shown in Fig. 3 A and 3 B, respectively. No effect of CSI on growth was observed until the second day of incubation, suggesting that CSI does not affect the seed germination. The effects of CSI on elongation and lateral expansion appeared in the later incubation period.

Effects of BR on the growth of roots in the presence and absence of CSI

The elongation of Arabidopsis roots was stimulated by treating with 1 nmol/L BR ($0.005 < p < 0.01$) but inhibited at concentrations higher than 100 nmol/L (Fig. 4 A). This promotion in the elongation of *Arabidopsis* roots treated with BR at the narrow range of concentrations had been observed by Clouse et al. (1996), but they did not mention it. The lateral expansion of roots was suppressed by BR in the range of concentrations of 1 to 10 nmol/L ($p < 0.001$) (Fig. 4 B). To clarify the interaction between BR and CSI, effects of BR were investigated in the presence of 100 µmol/L CSI. The stimulatory effects of CSI on root elongation were not affected by 1 nmol/L BR, which provided promotive effects by itself, but suppressed by BR higher than 10 nmol/L (Fig. 4 A). The reduction in the diameter caused by CSI was slightly enhanced in the presence of 1 nmol/L BR ($0.02 < p < 0.05$) and 10 nmol/L BR ($0.05 < p < 0.1$) (Fig. 4 B).

To investigate the effects of BR on cell elongation in roots, the length of epidermal cells was measured at the longitudinal midpoint of roots. BR at 1 nmol/L, which stimulated the growth of roots, had no effect on cell elongation (Fig. 5), suggesting a possible increase in cell number. Moreover, 1 nmol/L BR interfered with the promotive effects of 100 µmol/L CSI on cell elongation. In contrast to CSI, the BR-treated roots provided a curved growth as shown in Fig. 2 C. These results clearly show that the effects of BR are different from those of CSI.

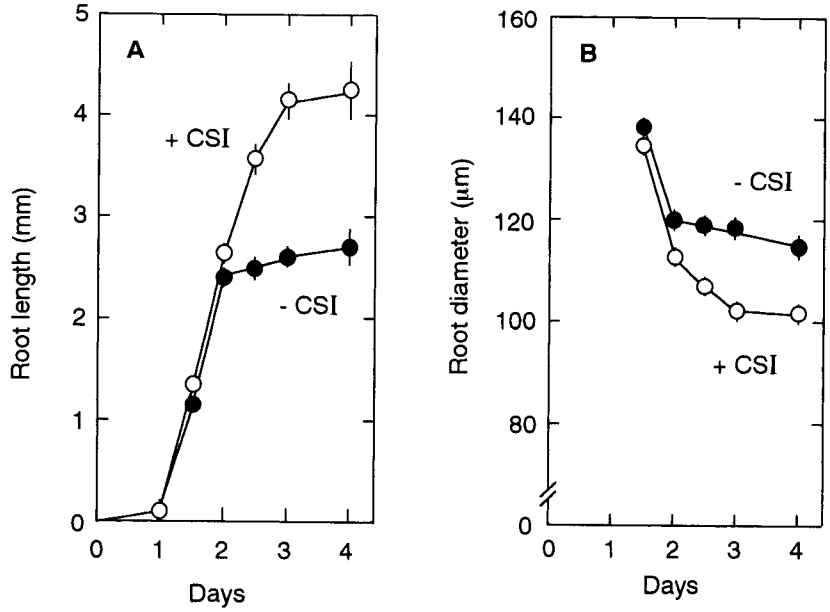

Fig. 3: Time course of the elongation of wild-type *Arabidopsis* roots (A) and the change in the root diameter (B) in the presence and absence of CSI. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) with (○) or without (●) 100 μmol/L CSI in the dark. Other explanation as in Fig. 1.

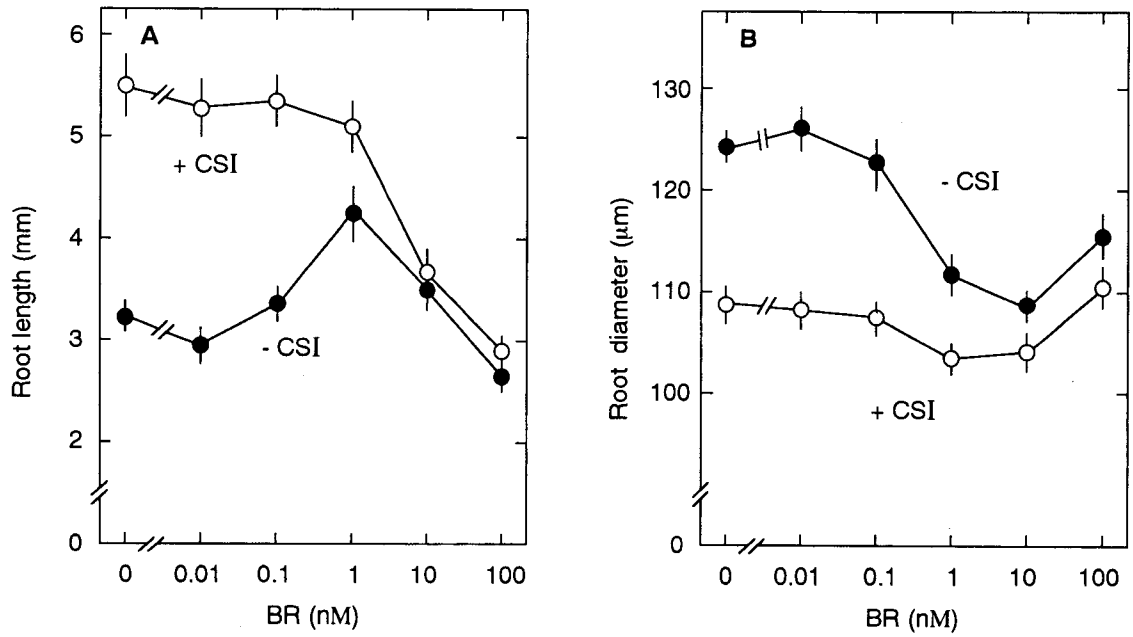

Fig. 4: Effects of BR on the root length (A) and the root diameter (B) in wild-type *Arabidopsis* in the presence and absence of CSI. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) with various concentrations of BR (0, 0.01, 0.1, 1, 10, 100 nmol/L) in the presence (○) or absence (●) of 100 μmol/L CSI for 4 days in the dark. Other explanation as in Fig. 1.

Involvement of ethylene in the actions of CSI and BR

To examine possible involvement of ethylene in the actions of CSI and BR, ethylene-insensitive mutants of *Arabidopsis, etr1-1* and *etr1-3* (Chang et al., 1993; Schaller and Bleecker, 1995), and *ein2-1* (Guzmán and Ecker, 1990), were introduced as experimental materials. Since germination of these mutants was delayed as compared with wild-type, we expressed the effects of chemicals on root growth as percent of control in respective mutants. As shown in Fig. 6, the roots of two insensitive mutants, *etr1-1* and *ein2-1,* did not respond to 10 μL L^{-1} ethylene. In the mutant *etr1-3,* however, treatment with ethylene distinctly increased the diameter of roots and slightly inhibited root elongation. The effects of ethylene on the growth of *etr1-3* roots were weaker than in wild-type, indicating that *etr1-3* weakly responds to ethylene as described by Bleecker and Schaller (1996).

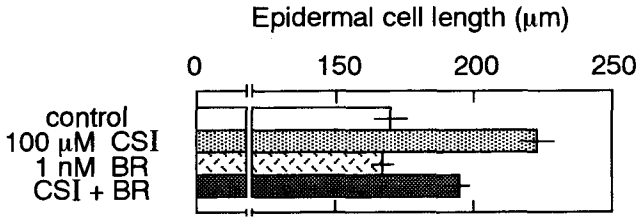

Fig. 5: Effects of CSI and BR on the length of mature epidermal cells of wild-type *Arabidopsis* roots. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) without additives (control) or with 100 μmol/L CSI, 1 nmol/L BR, and 100 μmol/L CSI + 1 nmol/L BR for 4 days in the dark. Other explanation as in Fig. 1.

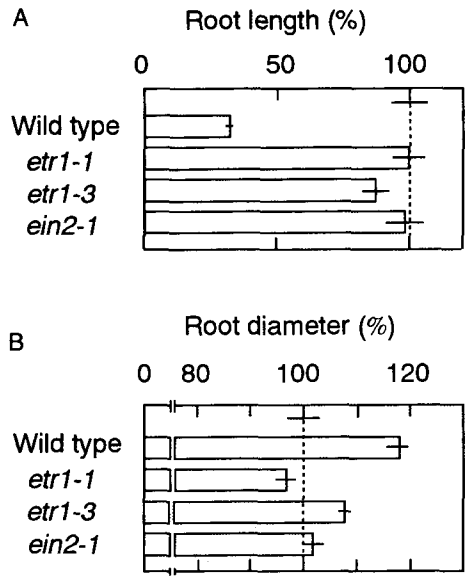

Fig. 6: Effects of 10 μL L^{-1} ethylene on the root length (A) and the root diameter (B) in ethylene-insensitive *Arabidopsis* mutants. Mutant and wild-type seedlings were incubated in the buffer (pH 6.6) without (control) or with ethylene for 4 days in the dark. Data were expressed as percent of control. Length and diameter of control roots were 3.08 mm and 126 μm (wt), 3.82 mm and 114 μm (*etr1-1*), 2.40 mm and 115 μm (*etr1-3*) and 3.07 mm and 111 μm (*ein2-1*), respectively. A short bar on the upper end of a dotted line shows SE of wild-type control. Other explanation as in Fig. 1.

Neither application of 100 μmol/L CSI nor of 1 nmol/L BR to the mutants *etr1-1* and *ein2-1* resulted in any effects on root growth and root diameter (Fig. 7). In the weak mutant *etr1-3*, CSI promoted root elongation and suppressed lateral expansion, and BR had no effect on the growth of roots. These results suggest that the actions of CSI and BR require the ethylene signaling systems.

Recovery from the effects of ethylene by CSI and BR

The idea that CSI and BR block ethylene action was tested by investigating the activities of two compounds to alleviate the effects of ethylene on the elongation and lateral expansion of roots. Treatment with 1,000 μL L^{-1} ethylene provided a curved thick root (Fig. 8 A) and the root length was about 30 % of the control without exogenous ethylene. This inhibition of the elongation was partially recovered by 100 μmol/L CSI ($p < 0.001$) and 1 nmol/L BR ($p = 0.05$) (Figs. 8 B, 8 C, 9 A). The treatment with ethylene also increased the diameter of roots by a factor of 1.2 and the ethylene-induced lateral expansion was partially suppressed by CSI ($p < 0.001$) and BR ($0.002 < p < 0.005$) (Fig. 9 B). Although the extent of recovery by the two compounds was only a portion of the effects caused by ethylene, the recovery was significant and consistent. Thus, both CSI and BR suppressed ethylene action, but each compound differently affected the morphology of roots: CSI made the shape of roots straight (Fig. 8 B) but not BR (Fig. 8 C). These results suggested that the CSI action is different from the BR action.

Discussion

Tsurumi and Ishizawa (1997) proposed that CSI stimulates the growth of lettuce roots by interfering with the action of

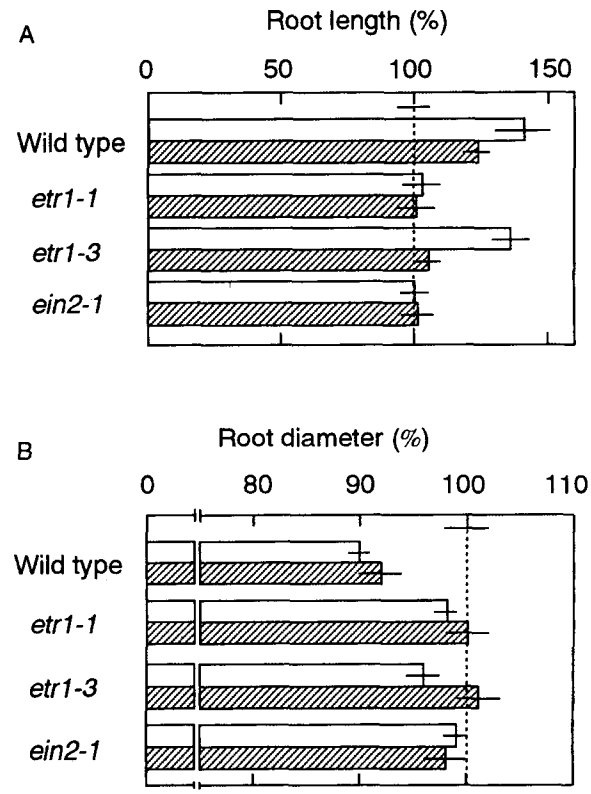

Fig. 7: Effects of CSI and BR on the elongation of roots (A) and the root diameter (B) in ethylene-insensitive *Arabidopsis* mutants. Mutant and wild-type seedlings were incubated in the buffer (pH 6.6) without additives (control) or with 100 μmol/L CSI (blank bar) and 1 nmol/L BR (shaded bar) for 4 days in the dark. Data were expressed as percent of control. Length and diameter of control roots were 3.64 mm and 119 μm (wt), 3.23 mm and 116 μm (*etr1-1*), 2.65 mm and 114 μm (*etr1-3*) and 3.22 mm and 118 μm (*ein2-1*), respectively. A short bar on the upper end of a dotted line shows SE of wild-type control. Other explanation as in Fig. 1.

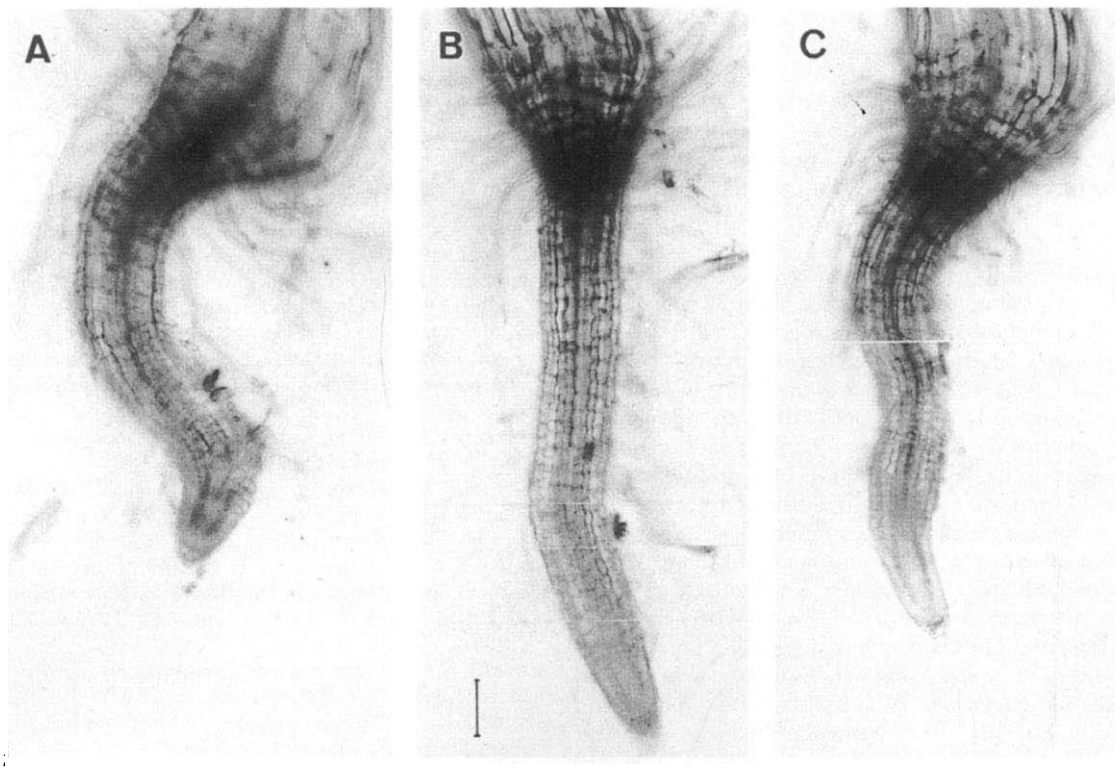

Fig. 8: Effects of CSI and BR on the growth of wild-type *Arabidopsis* roots treated with 1,000 µL L^{-1} ethylene. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) without additives (A) or with 100 µmol/L CSI (B) and 1 nmol/L BR (C) in the presence of ethylene for 4 days in the dark. The length of a bar is 100 µm.

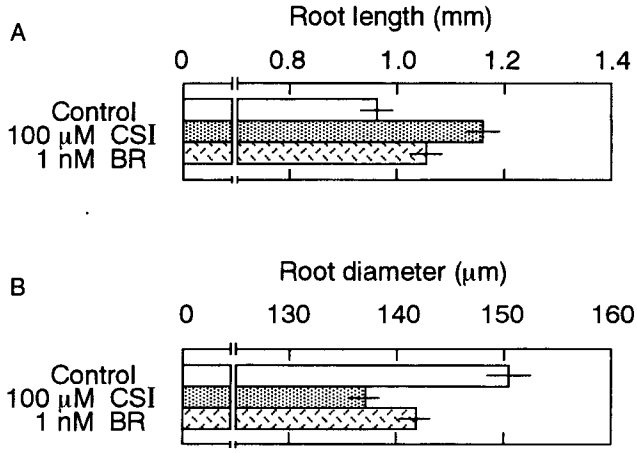

Fig. 9: Effects of CSI and BR on the root length (A) and the root diameter (B) in wild-type *Arabidopsis* roots treated with 1,000 µL L^{-1} ethylene. *Arabidopsis* seedlings were incubated in the buffer (pH 6.6) without additives (control) or with 100 µmol/L CSI and 1 nmol/L BR in the presence of ethylene for 4 days in the dark. Other explanation as in Fig. 1.

ethylene. In the present paper the CSI-induced stimulation in the growth of *Arabidopsis* roots was not observed in the ethylene-insensitive mutants *etr1-1* and *ein2-1,* but observed partially in *etr1-3.* The latter was only partly insensitive in the response to ethylene as reported by Bleecker and Schaller (1996). It has been proposed that ETR1 acts as an ethylene receptor protein and that the ethylene-binding site is contained within the amino-terminal hydrophobic domain (Schaller and Bleecker, 1995). The difference in the responsiveness to ethylene between *etr1-1* and *etr1-3* is due to the difference in a single amino acid in the hydrophobic domain (Chang et al., 1993; Bleecker and Schaller, 1996). Both the lack of response to CSI in the ethylene-insensitive mutants and the partial response in the weak mutant support our idea that CSI induces the growth of roots by reducing the ethylene signaling activity.

Treatment with BR at 1 nmol/L not only stimulated the elongation of *Arabidopsis* roots but also reduced the diameter of roots as observed in CSI (Fig. 4). These effects of the two compounds were not detected in the ethylene-insensitive mutants, *etr1-1* and *ein2-1* (Fig. 7). Furthermore, ethylene-induced inhibition in growth as well as thickening in diameter, which are typical effects of ethylene (Abeles et al., 1992), were reduced in the presence of these compounds (Figs. 8, 9). These results suggest that both BR and CSI act as inhibitors of ethylene actions. However, with regard to cell elongation the effects of two compounds were different. Treatment with CSI increased the length of the mature epidermal cells but not with BR (Fig. 5). The stimulatory effect of CSI on cell elongation was inhibited in the presence of 1 nmol/L BR, although root growth was stimulated in the case of the single treatment. The inability of BR to enhance cell elongation in

Arabidopsis roots contrasts with its activity in stems (Mandava, 1988; Mayumi and Shibaoka, 1995; Clouse et al., 1996). The most striking difference between BR and CSI is the morphological appearance of roots as shown in Fig. 2. The treatment with BR made the roots curved, but CSI-treated roots provided a straight growth. These results indicate that the mechanism of CSI action is not the same as that of BR, although ethylene seems to be involved in both actions.

The effects of BR on the growth of roots are variable depending on its concentration and the plant (Roddick and Guan, 1991). In *Arabidopsis*, the elongation of roots was stimulated by 1 nmol/L BR but inhibited at concentrations higher than 100 nmol/L (Fig. 4). In contrast to the effects of 1 nmol/L BR, the inhibitory effects of 1 µmol/L BR were detected in the ethylene-insensitive mutants *etr1-1* and *ein2-1* (data not shown), indicating that the mechanism of promotive action with lower concentrations of BR is different from that for inhibitory action with higher concentrations.

In the case of lettuce roots (Tsurumi and Ishizawa, 1997), the effects of CSI disappeared when ethylene actions were nullified by the treatments with AVG, an ethylene synthesis inhibitor (Yang and Hoffman, 1984), and NBD, an ethylene action inhibitor (Sisler and Yang, 1984). Initially we expected to obtain the same effects of AVG and NBD on *Arabidopsis* roots. Actually AVG and NBD promoted the growth of *Arabidopsis* roots and reduced the diameter of roots, but the stimulation in growth caused by the two compounds was smaller than that with CSI (data not shown). It is not yet clear whether or not the weak activities of AVG and NBD on *Arabidopsis* are due to incomplete removal of the effects of endogenous ethylene.

In lettuce and *Arabidopsis* roots, CSI made the root cells narrow in diameter as well as longer, suggesting that CSI may modify the polarity of cell growth (Tsurumi and Ishizawa, 1997). It is noteworthy that the effects of CSI on the shape of root cells are similar to those of GA reported in stems (Shibaoka, 1994). We investigated the effect of GA_3 on the growth of roots in lettuce and *Arabidopsis* but no significant effect was obtained (data not shown). The apparent lack of GA response may be due to a saturation level of endogenous GA, because the stimulatory effects of exogenous GA on root growth were often observed when the endogenous level of GA was reduced by GA-synthesis inhibitors (Tanimoto, 1987, 1988; Barlow et al., 1991; Baluska et al., 1993). In contrast to GA, CSI counteracted the action of ethylene, which is the endogenous regulator in roots and a natural component of the soil atmosphere (Abeles et al., 1992). Although the physiological importance of saponin is not yet established, the concentration of endogenous CSI in the root tips of pea seedlings was found to be about 2 mmol/L (Tsurumi et al., 1992). Further studies are required to prove the endogenous roles of CSI in roots and to clarify the relationship between actions of CSI and GA on the polarity of cell growth.

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References

Abeles, F.B., P. W. Morgan, and M. E. Saltveit Jr.: Ethylene in Plant Biology, 2nd Ed. pp. 120–181. Academic Press, London (1992).

Balansard, J. and F. Pellissier: Action de diverses saponines sur le bouturage épiphylle de quelques Bégoniacées. Compt. Rend. Soc. Biol. *137*, 454 (1943a).

– – Action sur les germes de Blé isolés de la saponine du Quillaya. Compt. Rend. Soc. Biol. *137*, 461–462 (1943b).

Baluska, F., J. S. Parker, and P. W. Barlow: A role for gibberellic acid in orienting microtubules and regulating cell growth polarity in the maize root cortex. Planta *191*, 149–157 (1993).

Barlow, P. W., P. Brain, and J. S. Parker: Cellular growth in roots of a gibberellin-deficient mutant of tomato (*Lycopersicon esculentum* Mill.) and its wild-type. J. Exp. Bot. *42*, 339–351 (1991).

Bleecker, A. B. and G. E. Schaller: The mechanism of ethylene perception. Plant Physiol. *111*, 653–660 (1996).

Chang, C., S. F. Kwok, A. B. Bleecker, and E. M. Meyerowitz: *Arabidopsis* ethylene-response gene *ETR1*: Similarity of product to two-component regulators. Science *262*, 539–544 (1993).

Clouse, S. D., M. Langford, and T. C. McMorris: A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiol. *111*, 671–678 (1996).

Ecker, J. R.: The ethylene signal transduction pathway in plants. Science *268*, 667–675 (1995).

Geuns, J. M. C.: Steroid hormones and plant growth and development. Phytochemistry *17*, 1–14 (1978).

Guzmán, P. and J. R. Ecker: Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell *2*, 513–523 (1990).

Helmkamp, G. and J. Bonner: Some relationships of sterols to plant growth. Plant Physiol. *28*, 428–436 (1953).

Kudou, S., M. Tonomura, C. Tsukamoto, M. Shimoyamada, T. Uchida, and K. Okubo: Isolation and structural elucidation of the major genuine soybean saponin. Biosci. Biotech. Biochem. *56*, 142–143 (1992).

Kudou, S., M. Tonomura, C. Tsukamoto, T. Uchida, T. Sakabe, N. Tamura, and K. Okubo: Isolation and structural elucidation of DDMP-conjugated soyasaponins as genuine saponins from soybean seeds. Biosci. Biotech. Biochem. *57*, 546–550 (1993).

Mandava, N. B.: Plant growth-promoting brassinosteroids. Annu. Rev. Plant Physiol. Plant Mol. Biol. *39*, 23–52 (1988).

Massiot, G., C. Lavaud, M. Benkhaled, and L. Le Men-Olivier: Soyasaponin VI, a new maltol conjugate from alfalfa and soybean. J. Nat. Prod. *55*, 1339–1342 (1992).

Mayumi, K. and H. Shibaoka: A possible double role for brassinolide in the reorientation of cortical microtubules in the epidermal cells of azuki bean epicotyls. Plant Cell Physiol. *36*, 173–181 (1995).

Ohana, P., D. P. Delmer, G. Volman, and M. Benziman: Glycosylated triterpenoid saponin: a specific inhibitor of diguanylate cyclase from *Acetobacter xylinum*. Biological activity and distribution. Plant Cell Physiol. *39*, 153–159 (1998).

Rezk, M. R. and L. Ferenczy: Growth inhibiting activity of some steroid glycoalkaloids on higher plants. Acta Biol. Szeged *15*, 71–78 (1969).

Roddick, J. G. and M. Guan: Brassinosteroids and root development. In: Cutler, H. G., T. Yokota, and G. Adam (eds.): Brassinosteroids; Chemistry, Bioactivity and Applications, pp. 231–245. ACS symposium series 474. American Chemical Society, Washington DC (1991).

Schaller, G. E. and A. B. Bleecker: Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. Science *270*, 1809–1811 (1995).

SHIBAOKA, H.: Plant hormone-induced changes in the orientation of cortical microtubules: Alterations in the cross-linking between microtubules and the plasma membrane. Annu. Rev. Plant Physiol. Plant Mol. Biol. *45*, 527–544 (1994).

SISLER, E. C. and S. F. YANG: Anti-ethylene effects of *cis*-2-butene and cyclic olefins. Phytochemistry *23*, 2765–2768 (1984).

TAKEMATSU, T., Y. TAKEUCHI, and M. KOGUCHI: Bioassay methods and physiology of brassinolides. Chem. Regul. Plants *18*, 38–54 (1983).

TANIMOTO, E.: Gibberellin-dependent root elongation in *Lactuca sativa:* Recovery from growth retardant-suppressed elongation with thickening by low concentration of GA_3. Plant Cell Physiol. *28*, 963–973 (1987).

– Gibberellin regulation of root growth with change in galactose content of cell walls in *Pisum sativum*. Plant Cell Physiol. *29*, 269–280 (1988).

TSUJINO, Y., S. TSURUMI, T. AMAKAWA, and T. OSAKAI: Redox properties of a γ-pyronyl-triterpenoid saponin (chromosaponin I). J. Nat. Prod. *58*, 1829–1839 (1995).

TSURUMI, S. and K. ISHIZAWA: Involvement of ethylene in chromosaponin-induced stimulation of growth in lettuce roots. Plant Cell Physiol. *38*, 668–675 (1997).

TSURUMI, S. and Y. TSUJINO: Chromosaponin I stimulates the growth of lettuce roots. Physiol. Plant. *93*, 785–789 (1995).

TSURUMI, S. and S. WADA: Chromosaponin I stimulates the elongation of cortical cells in lettuce roots. Plant Cell Physiol. *36*, 925–929 (1995).

TSURUMI, S., T. TAKAGI, and T. HASHIMOTO: A new UV-B-absorbing substance in pea seedlings. Abstracts of Fourth Congress of the European Society for Photobiology, Amsterdam, p. 113 (1991).

– – – A γ-pyronyl-triterpenoid saponin from *Pisum sativum*. Phytochemistry *31*, 2435–2438 (1992).

TSURUMI, S., R. WATANABE, and S. KAMISAKA: Chromosaponin I increases mechanical extensibility of lettuce root cell walls. Physiol. Plant. *97*, 740–746 (1996).

VON EULER, H.: Einwirkung von Saponinen und Digitalis-Glukosiden auf Samenkeimung und Mitose. Arkiv Kemi Mineral. Geol. *22A*(14), 1–10 (1946).

YANG, S. F. and N. E. HOFFMAN: Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. *35*, 155–189 (1984).

ZAMBOU, K., C. G. SPYROPOULOS, I. CHINOU, and F. KONTOS: Saponin-like substances inhibit α-galactosidase production in the endosperm of fenugreek seeds. A possible regulatory role in endosperm galactomannan degradation. Planta *189*, 207–212 (1993).