Plant Biotechnology 24, 99–106 (2007)

Original Paper

Effects of the removal of cotyledons on endogenous gibberellin levels in hypocotyls of young cucumber and tomato seedlings

Masashi Asahina[1,2], Yukika Yamauchi[2,a], Atsushi Hanada[2], Yuji Kamiya[2], Hiroshi Kamada[1], Shinobu Satoh[1], Shinjiro Yamaguchi[2,*]

[1] Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan; [2] RIKEN Plant Science Center, Yokohama 230-0045, Japan

* E-mail: shinjiro@postman.riken.go.jp Tel: +81-45-503-9663 Fax: +81-45-503-9662

Received October 17, 2006; accepted November 2, 2006 (Edited by M. Katsumi)

Abstract We have previously shown that gibberellin (GA) is required for tissue-reunion in the cortex of cut hypocotyls in cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) seedlings, and that intact cotyledons are also necessary for this process. These results suggested that cotyledons might play an important role in controlling GA levels in the hypocotyl in these plant species. In this study, we found that a local application of a GA biosynthesis inhibitor uniconazole to cotyledons was effective to inhibit hypocotyl elongation, and that simultaneous application of GA canceled this inhibition. To study the role of cotyledons in GA content in the hypocotyl directly, cotyledons were removed from 7-days-old seedlings and endogenous GA levels in the hypocotyl were determined. Our results demonstrated that the cotyledon-removed seedlings contained lower levels of bioactive GAs and their precursors in the hypocotyls than did intact seedlings. Quantitative RT-PCR analysis indicated that transcript levels of *LeGA20ox1* and *LeGA3ox2* genes were elevated in the hypocotyl after the removal of cotyledons, suggesting that the reduced bioactive GA levels caused upregulation of these genes via the feedback regulation mechanism. Taken together, our results suggest that cotyledons are necessary for maintaining normal GA levels in young cucumber and tomato hypocotyls.

Key words: Cotyledon, GA20-oxidase and GA3-oxidase, gibberellin, hypocotyl.

In higher plants, the two daughter cells are attached to each other immediately after cell division, as a result of the formation of the cell plate. These cells then either maintain cell-to-cell attachment or separate from each other. Re-adhesion of separated cells, which is commonly seen in animal systems, is not typically observed in plants, but it does occur in certain processes including carpel fusion during gynoecium and tissue-reunion in cut tissues (Walker 1975; Siegel and Verbeke 1989; van der Schoot et al. 1995). Also, the ability of plant cells to re-adhere has been utilized to generate grafts in agriculture (Kollmann and Glockmann 1985; Wang and Kollmann 1996; Richardson et al. 1996).

In Japan, grafting of cucumber on squash root-stock is often performed to prevent damage from soil-borne diseases during cultivation (Satoh 1996). In this procedure, cotyledons of the scion and stock are preferentially left on the hypocotyl to improve grafting efficiency. Although the exact role of the cotyledon in the formation of the graft union is not understood, it is possible that the cotyledon produces compounds required for the formation of the graft union. Previously, dihydroconiferyl alcohol has been reported to act a factor that is produced in cotyledons and is required for the maximum activity of gibberellin (GA) on the hypocotyl elongation in cucumber (Katsumi et al. 1965a) and lettuce (Kamisaka 1973; Shibata et al. 1974) seedlings. These results suggest that cotyledons might produce key compounds that control the growth of hypocotyls in some dicotyledonous species.

We have previously performed morphological and physiological analyses of the tissue-reunion process in cut hypocotyls of cucumber and tomato seedlings (Asahina et al. 2002, 2006). In these systems, cell division and elongation began 3 days after cutting, and the cortex was nearly completely united within 7 days. Interestingly, our data suggested that GA, presumably produced in cotyledons, is required for cell division during tissue-reunion in the cortex of cut hypocotyls, based on the following observations (Asahina et al. 2002). (1) Cut hypocotyls failed to re-unite when cotyledons were mechanically removed. (2) GA-deficiency (caused either by a GA synthesis inhibitor uniconazole [Izumi et al. 1985] or the *gib-1* mutation of

Abbreviations: DW; distilled water, GA; gibberellin, GC-MS; gas chromatography-mass spectrometry, LC-MS; liquid chromatography-mass spectrometry, QRT-PCR; quantitative reverse transcriptase-polymerase chain reaction.

[a] Present address: National Institute of Agrobiological Science, Tsukuba 305-8602, Japan

tomato [Groot et al. 1987; Bensen and Zeevaart 1990; Rebers et al. 1999]), resulted in a failure in the tissue-reunion in cut hypocotyls. (3) Even after the removal of cotyledons, exogenous GA treatment can restore the ability of tissue-reunion in hypocotyls.

GAs are a class of diterpenoids, some of which act as hormones that control many aspects of plant growth and development. In the GA biosynthesis pathways (Figure 1), GA_1 and GA_4 act as bioactive hormones, while others are precursors for bioactive GAs or inactivated forms. Recently, significant progress has been made in understanding GA biosynthesis and signaling pathways, including identifications of genes encoding GA metabolism enzymes and components in the GA response pathway in such model species as Arabidopsis, rice, pea and tomato (Ross et al. 1997; Rebers et al. 1999; Yamaguchi and Kamiya 2000; Olszewski et al. 2002; Sun and Gubler 2004; Ueguchi-Tanaka et al. 2005). Nevertheless, our knowledge on transport of GA within the plant body is still limited. Previous studies using isotope labeled-GAs or grafting experiments with GA-deficient mutants suggested that GAs or GA precursors could move in the plant body (Rudich et al. 1976; Katsumi et al. 1983; Reid et al. 1983; Yang et al. 1995; Eriksson et al. 2006). However, the necessity and physiological significance of GA movement during plant development and in environmental responses still remain elusive. Also, it has not been well understood whether and how the GA status in one organ affects that in the others.

In this study, we have analyzed endogenous GA content and transcript levels of GA biosynthesis genes in order to address the possible contribution of cotyledons to altering GA levels in hypocotyls. Our results suggest that intact cotyledons are necessary for maintaining normal GA levels in young cucumber and tomato hypocotyls.

Materials and methods

Plant materials and growth conditions

Seeds of cucumber (*Cucumis sativus* L., cv. Shimoshirazu-jibai) were obtained from Sakata Seed Co. (Kanagawa, Japan), and those of tomato (*Solanum lycopersicum*; formerly *Lycopersicon esculentum* Mill., cv. Moneymaker) were obtained from Sapporo-Nouen Co. (Sapporo, Japan). The seeds were germinated and grown in artificial soil (Kurehakagaku, Tokyo, Japan) under continuous white fluorescent light ($32\ \mu molm^{-2}\,s^{-1}$) at 26°C.

Treatment with GA_4 and uniconazole

GA_4 and uniconazole P (GA biosynthesis inhibitor: Izumi et al. 1985) were applied locally to cotyledons or hypocotyls as lanolin pastes. The lanolin pastes were prepared by adding anhydrous lanolin to an aqueous solution of the chemical or to distilled water (DW: as a control). Five days after the start of treatment, hypocotyl elongation was measured. Experiments were performed twice using independently grown seedlings with similar results.

Quantitative Reverse Transcription (QRT)–PCR

Total RNA was extracted from tomato seedlings using an RNAqueous RNA isolation kit with Plant RNA isolation aid (Ambion, Austin, TX). First-strand cDNA was synthesized from total RNA (1 μg) with QuantiTect Reverse Transcription Kit according to the manufacturer's instructions (Qiagen, Valencia, CA). QRT-PCR with Taq-Man technology (Holland et al. 1991) or SYBR Green I regents (Qiagen) was performed using the first-strand cDNA as a template on a sequence detector system (ABI PRISM 7000, Applied Biosystems, Foster, CA) as described in Yamauchi et al. (2004). Results were normalized using an 18S rRNA as the internal control. Following nucleotide sequences of gene-specific primers and Taq-Man probes were used:

for *LeGA20ox 1*, primer; 5′- CAA CTA CTA TCC ACC ATG CCA GAA-3′ and 5′- TGA TGT TGG ATC ACA ATG AGG C-3′, Taq-Man probe; 5′-FAM- CCG GAG CTC GCC TTA GGA ACG G-TAMRA-3′, for *LeGA3ox 2*, primer; 5′-CCG GAC GAG ATT TTC CCT-3′ and 5′-CCA GCG ATG CCA TCG G-3′, Taq-Man probe; 5′-FAM- CCG GAG CTC GCC TTA GGA ACG G -TAMRA-3′.

SYBR Green primer for *LeGA20ox 2*, 5′-ACC AGA TCT TGC GTT AGG AAC TG -3′and 5′-CCT GAG ACG TTG TCT TGA TGG A-3′; *LeGA20ox 3*, 5′-GGA CAG GGC CTC ATT GTG AT -3′ and 5′-AAA CTT GAA GCC CAC CAA CAC T-3′; *LeGA3ox 1*, 5′-CAG ACC ACA TGA GCT TCG AGA A -3′ and 5′-CCT GAT GGT GTC ACT GGC TAT G-3′.

Endogenous GA Measurements

Cotyledons were eliminated from 7-days-old cucumber or tomato seedlings, and the plants were grown for an additional 1day. Intact seedlings were grown in an identical growth condition and were used as a control. About 30 g of fresh hypocotyls of cucumber seedlings were used for each GA measurement by gas chromatography-selected ion monitoring (GC-SIM). Quantitative analysis of GA was performed using ^{2}H-labeled GAs as internal standards as described previously (Gawronska et al. 1995), using a mass spectrometer (Automass Sun, JEOL, Tokyo, Japan) equipped with a GC (6890N, Agilent Technologies, Palo Alto, CA, USA) and a capillary column (DB-1, Agilent Technologies). GA measurements were repeated three times using independently prepared plant materials. GA analysis in tomato hypocotyls was carried out using approximately 2 g (fresh weight) of plant materials. GA-containing fractions were subjected to LC-ESI-MS/MS analysis on a mass spectrometer (Q-Tof Premier, Waters) equipped with an Acquity Ultra Performance LC (Waters) using a reverse-phase column (Acquity UPLC BEH-C18, Waters). Experiments were repeated twice using independently prepared plant materials. Authentic GA samples and ^{2}H-labeled internal standards were purchased from Professor Lewis Mander (Australian National University, Canberra).

Results

The effect of local application of uniconazole on hypocotyl elongation of cucumber seedlings

Our previous observations of the tissue-reunion process in cut hypocotyls suggested that normal GA biosynthesis in cotyledons is necessary for the maintenance of GA levels in the hypocotyl (Asahina et al. 2002). In this study, to further investigate this hypothesis, we measured elongation of hypocotyls after a local application of uniconazole (a GA biosynthesis inhibitor) to cotyledons, because hypocotyls elongation is known to be a GA-dependent process in cucumber (Katsumi et al. 1965b; Moore 1967; Sandhu et al. 1974; Taylor and Cosgrove 1989; Lopez-Juez et al. 1995). It was previously reported that radio labeled uniconazole applied to a cucumber leaf did not move to other organs significantly (Izumi et al. 1988). Therefore, a local application of this chemical to cotyledons would allow us to test the effect of local inhibition of GA biosynthesis in cotyledons.

Figure 2A shows that an application of uniconazole to cotyledons resulted in a decrease in the hypocotyl length, and a simultaneous application of GA_4 canceled the inhibitory effect of uniconazole on hypocotyl elongation. Application of uniconazole to one of the cotyledons showed a weaker inhibitory effect on hypocotyl elongation (data not shown). Uniconazole-treatment on cotyledons was likely to cause GA-deficiency in the hypocotyl, because the inhibitory effect was canceled when GA_4 was applied locally to the hypocotyls as well (Figure 2B).

GA levels in cucumber hypocotyls are reduced after the removal cotyledons

Our previous observations (Asahina et al. 2002) and the results in Figure 2 suggest that the maintenance of GA levels in hypocotyls is dependent on the presence of cotyledons in cucumber seedlings. To directly test this possibility, we determined endogenous levels of precursor and bioactive GAs in hypocotyls of intact and cotyledon-removed seedlings. In cucumber, GA_4 in the non-13-hydroxylation pathway (Figure 1) acts as the major bioactive form (Smith et al. 1991). Figure 3 shows that hypocotyls after the removal of cotyledons contained a reduced amount of bioactive GA_4 relative to those of intact seedlings (almost 50% reduction), and that a more drastic reduction in the level of GA_{12} was evident in the cotyledon-removed seedlings (almost 25% of the intact plants). These results indicate that cotyledons are necessary for maintaining the levels of both bioactive and precursor GAs in cucumber hypocotyls. These data are consistent with our notion that the removal of cotyledons causes a failure in the tissue reunion of cut hypocotyls (Asahina et al. 2002) and a reduction in the length of hypocotyls (Figure 2).

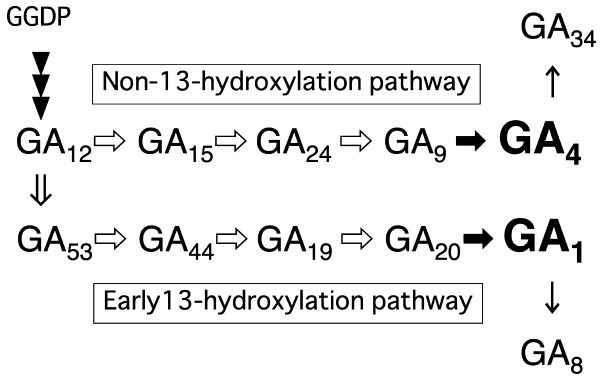

Figure 1. The major GA biosynthesis pathways in plants. Open arrows and bold arrows indicate reactions catalyzed by GA 20-oxidase and GA 3-oxidase, respectively. GA_1 and GA_4 are biologically active forms. GA_{34} and GA_8 are inactive GAs. Effects of the application of uniconazole and GA to the cotyledon on the hypocotyl elongation of cucumber.

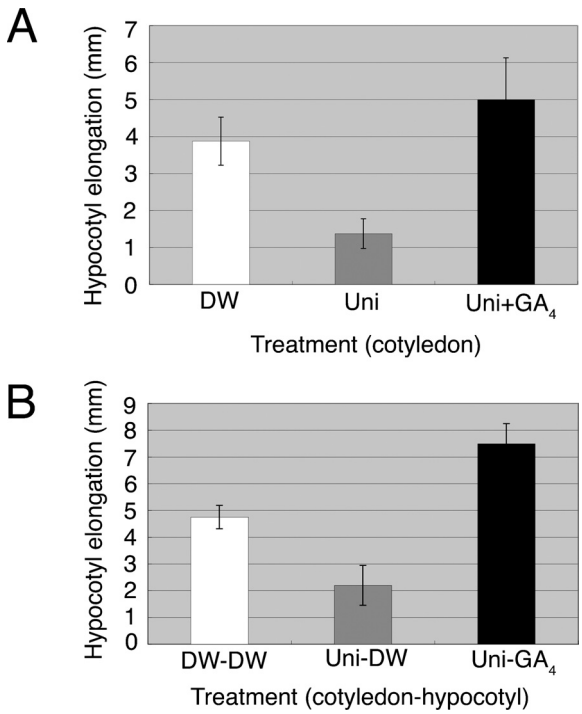

Figure 2. Effects of local applications of uniconazole and GA_4 on hypocotyl elongation of cucumber seedlings. (A) Chemicals were applied as a lanoline paste to cotyledons at a final concentration of 10 μM. (B) Lanoline pastes containing the chemicals (10 μM) were applied locally to both cotyledons and hypocotyls as indicated. Hypocotyl elongation was measured 3 days after the chemical treatment. Uni; uniconazole, DW; distilled water as a control. Data are means from 8 measurements with standard errors.

Transcript levels of GA biosynthesis genes in tomato seedlings

Our previous work using a GA-deficient mutant *gib-1* (Koornneef et al. 1990) has shown that, like in cucumber seedlings, GA is required for tissue-reunion in cut hypocotyls of tomato seedlings (Asahina et al. 2002). Also, the removal of cotyledons from wild-type tomato

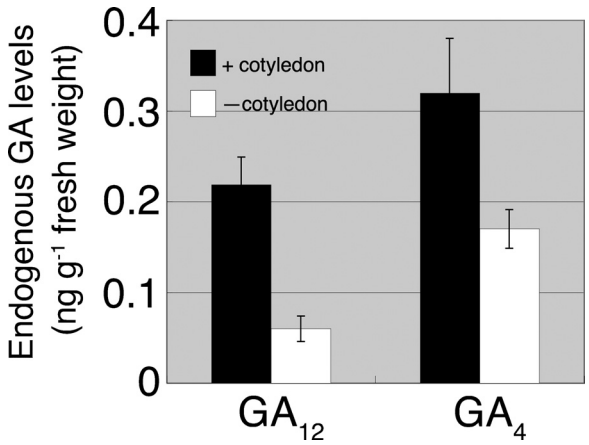

Figure 3. Quantitative analysis of endogenous GAs in cucumber hypocotyls. –cotyledon; cotyledons were removed from 7-days-old cucumber seedlings and the cotyledon-removed seedlings were grown for an additional 1 day before harvesting. +cotyledon; 7-days-old intact seedlings were grown for an additional 1 day under the same condition. GA measurements were repeated three times using independently grown plant materials. Means with standard errors are shown.

seedlings resulted in inhibition of tissue-reunion in cut hypocotyls, as observed in cucumber seedlings (Asahina et al. 2002). To study the possible role of cotyledons in the maintenance of GA levels in hypocotyls in more detail, we took advantage of the tomato system, in which GA biosynthesis genes have previously been identified and characterized (Rebers et al. 1999). GA 20-oxidase and GA 3-oxidase catalyze conversions of $GA_{12/53}$ to $GA_{9/20}$ and of $GA_{9/20}$ to $GA_{4/1}$, respectively (Figure 1), and are each encoded by a multi-gene family in many plant species (Lange et al. 1994; Chiang et al. 1995; Lester et al. 1997; Martin et al. 1997; Yamaguchi et al. 1998; Itoh et al. 1999; Rebers et al. 1999). In an attempt to assess the major family members expressed at the seedling stage, we carried out RT-PCR analysis using RNA samples prepared from aerial part of tomato seedlings and degenerate primers designed from conserved amino acid sequences of each enzyme in *Arabidopsis*, rice, and tobacco. Sequence analysis of the PCR products revealed that the majority of the clones isolated from the seedling sample corresponded to *LeGA20ox1* for GA 20-oxdase and *LeGA3ox2* for GA 3-oxidase, suggesting that these genes would represent the major GA 20-oxidase and GA 3-oxidase activities, respectively, at the seedling stage.

We determined transcript levels of *LeGA20ox1* and *LeGA3ox2* genes in each organ during seedling growth by QRT-PCR (Figure 4). Expression of both of these GA biosynthesis genes was detectable in every organ that we examined, but the *LeGA3ox2* transcript was relatively more abundant in cotyledons than in hypocotyls at all time points (Figure 4). At stage 1 (before the visible expansion of true leaves, Figure 4), the level of *LeGA20ox1* transcript was higher in cotyledons than in

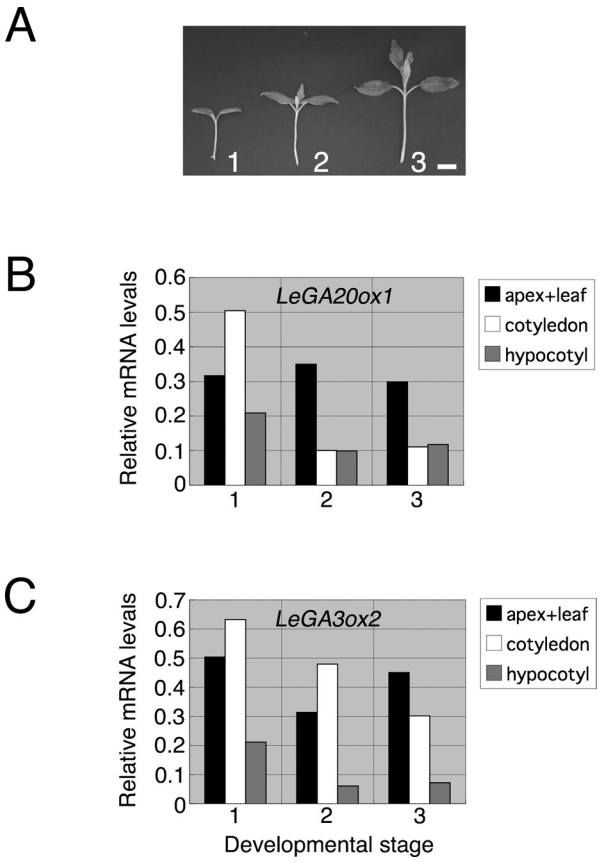

Figure 4. Transcript levels of GA biosynthesis genes in each organ of tomato seedlings. (A) Tomato seedlings at various developmental stages were used in this experiment. Stage 1; 7-days-old, Stage 2; 10 -days-old, Stage 3; 14-days-old. Scale bar indicates 1 cm. (B, C) Relative transcript levels of *LeGA20ox1* (B) and *LeGA3ox2* (C) as determined by quantitative RT-PCR. Experiments were performed twice using independent plant materials with similar results. Results from one of the replicates are shown.

hypocotyls. These observations support the idea that GAs are produced in all organs including cotyledons in tomato seedlings.

GA levels in tomato hypocotyls are reduced after the removal cotyledons

To examine whether the removal of cotyledons affects GA levels in the hypocotyl of tomato seedlings as observed in cucumber seedlings (Figure 3), we measured endogenous levels of precursor, bioactive and inactive GAs in the hypocotyls of intact and cotyledon-removed plants. Unlike in cucumber seedlings, both the non-13-hydroxylation and the early-13-hydroxylation pathways (Figure 1) appear to operate in tomato seedlings (Koornneef et al. 1990; Rebers et al. 1999). Figure 5 shows that levels of all GAs that could be reliably quantified were reduced in the cotyledon-removed plants relative to those in intact seedlings reproducibly in duplicated experiments. The amounts of bioactive GA_4 and GA_1 after the removal of cotyledons were about 10% and 50% of those in intact seedlings, respectively.

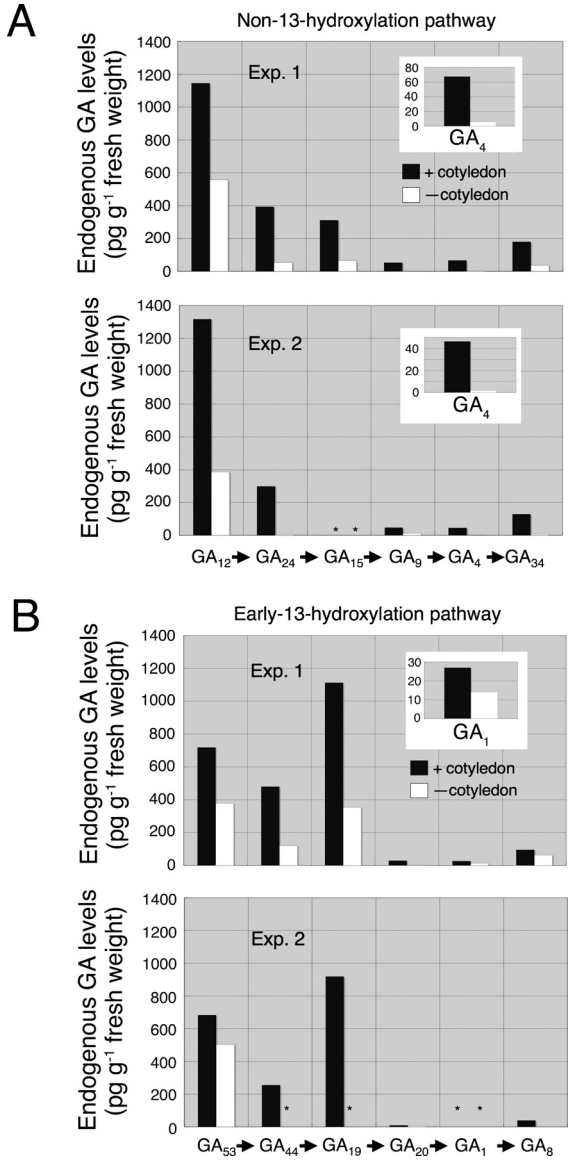

Figure 5. The Qquantitative analysis of endogenous GAs in the tomato hypocotyls by LC-MS.(A) GAs in the non-13-hydroxylation pathway. (B) GAs in the early-13-hydroxylation pathway. –cotyledon; cotyledons were removed from 7-days-old tomato seedlings and the cotyledon-removed seedlings were grown for an additional 1 day before harvesting. +cotyledon; 7-days-old intact seedlings were grown for an additional 1 day under the same condition. GA measurements were carried out twice using independently grown plant materials and the results from each replicate (Exp.1 and Exp. 2) are presented. Data for bioactive GA_4 and GA_1 are shown in insets for clarity. Asterisk indicates that these GAs could not be quantified reliably due to low abundance or co-migration of impurities.

Some GA biosynthesis genes are upregulated in the hypocotyl of cotyledon-removed tomato seedlings

To better understand the status of the GA biosynthesis pathway in tomato hypocotyls after the elimination of cotyledons, we determined relative transcript levels of GA biosynthesis genes by QRT-PCR (Figure 6). We found that the level of *LeGA20ox2* transcript was highly elevated in hypocotyls after the removal of cotyledons, while expression of the other two *LeGA20ox* genes was not drastically altered under the same conditions. The *LeGA3ox1* and *LeGA3ox2* genes, both of which encode GA 3-oxidase, were expressed at higher levels in hypocotyls of the cotyledon-removed seedlings than in those of the intact ones. It has been shown in many plant species that some GA 20-oxidase and GA 3-oxidase genes are negatively regulated by GA activity (Martin et al. 1996; Hedden and Kamiya 1997; Elliot et al. 2001), which would function as part of the homeostasis mechanism in the GA biosynthesis and response pathways. Therefore, the observed upregulation of *LeGA20ox* and *LeGA3ox* genes in hypocotyls of the cotyledon-removed seedlings might be a consequence of reduced levels of bioactive GAs in this organ.

Discussion

Several lines of evidence presented in this study suggest that cotyledons play an important role in maintaining GA levels in hypocotyls of cucumber and tomato seedlings. We have demonstrated that GA levels in hypocotyls are decreased significantly when cotyledons are mechanically removed (Figures 3, 5). This was observed commonly in cucumber and tomato seedlings. These results are consistent with our previous findings that tissue-reunion of cut hypocotyls did not occur after the removal of cotyledons, while exogenous GA treatment to the cotyledon-removed seedlings was effective to restore the tissue-reunion (Asahina et al. 2002). Therefore, our current data strongly support the hypothesis that GA-deficiency in hypocotyls is responsible for the failure in tissue-reunion in the cotyledon-eliminated seedlings.

There is some evidence that suggests the capability of GA precursors and/or bioactive GAs to move across different cell types and between organs (Rudich et al. 1976; Katsumi et al. 1983; Reid et al. 1983; Yang et al. 1995). Therefore, the reduced GA levels in hypocotyls after the removal of cotyledons (Figures 3, 5) might be explained by the movement of GA(s) from cotyledons to the hypocotyl in intact seedlings. This hypothesis is supported by the fact that the effect of local inhibition of GA biosynthesis in cotyledons (based on the immobility of uniconazole applied to cucumber leaves, Izumi et al, 1988) on hypocotyl elongation was canceled by a local application of GA_4 to cotyledons (Figure 2). A similar observation was reported using a local application of AMO1618 to cucumber cotyledons (Katsumi et al. 1965b), although the mobility of this inhibitor in plant is unknown. The hypothetical movement of GAs from cotyledons to the hypocotyl in cucumber and tomato seedlings should be demonstrated experimentally to confirm this model, because it is still possible that non-GA factor(s) produced in cotyledons is involved in the maintenance of hypocotyl GA levels. Recently, auxin

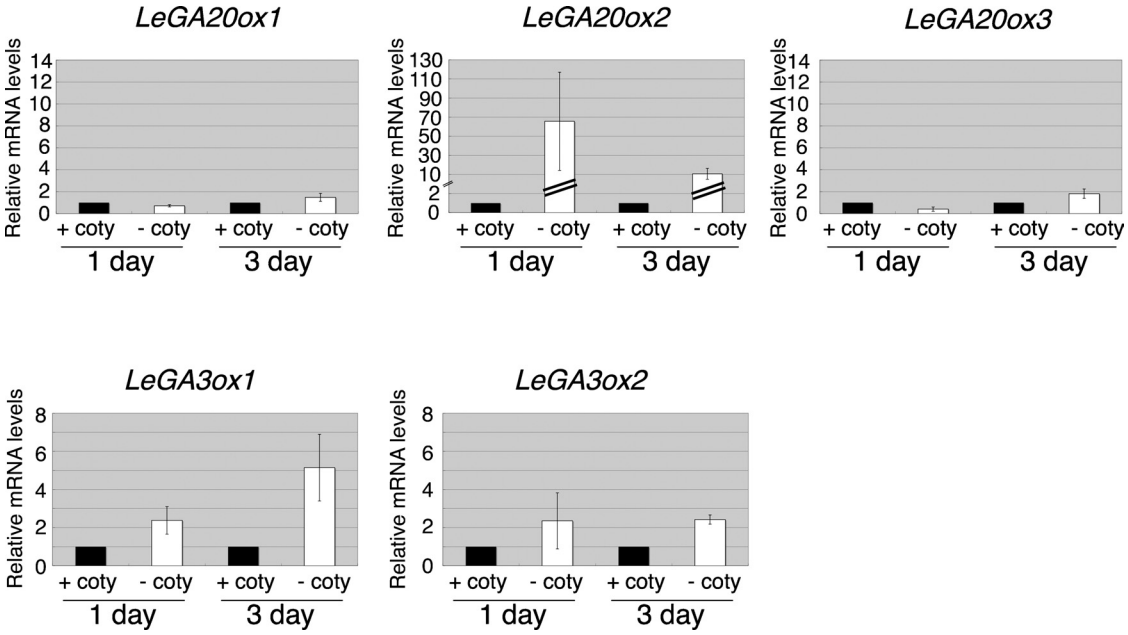

Figure 6. Transcript levels of GA biosynthesis genes in hypocotyls of intact and cotyledon-removed tomato seedlings. –coty; cotyledons were removed from 7-days-old tomato seedlings and the cotyledon-removed seedlings were grown for additional 1 or 3 days before harvesting. +coty; 7-days-old intact seedlings were grown for additional 1 or 3 days under the same condition. Relative transcript levels were determined by quantitative RT-PCR. Values for +coty (1 day) were arbitrarily set as 1. Values are means with standard errors from triplicates using independently prepared plant materials.

(indole-3-acetic acid) has been shown to play an essential role in maintaining normal GA levels in pea internodes (Ross and O'Neill 2001; O'Neill and Ross 2002). Application of indole-3-acetic acid alone, however, could not restore the inhibition of tissue-reunion in the cotyledon-eliminated cucumber seedlings (Asahina et al. 2002).

It is interesting to note that the removal cotyledons resulted in reductions in the levels of not only bioactive GAs but also their precursors (Figures 3, 5). At the same time, upregulation of some GA 20-oxidase and GA 3-oxidase genes was observed after the elimination of cotyledons in tomato seedlings (Figure 6). There are at least two possible mechanisms to explain these changes in GA levels and expression of GA biosynthesis genes after the removal of cotyledons. As the first model, we hypothesize that bioactive GA(s) synthesized in cotyledons move to the hypocotyl in intact seedlings. In this model, after the removal of cotyledons, the amount of bioactive GAs would be decreased in the hypocotyl due to their reduced supply from cotyledons, which in turn causes upregulation of GA 20-oxidase and GA 3-oxidase genes. Precursor GAs would not accumulate at high levels in the hypocotyl because they should be quickly metabolized by elevated GA 20-oxidase and GA 3-oxidase activities. The second hypothesis involves a movement of precursor(s) for bioactive GAs (such as GA_{12} or an earlier intermediate) from cotyledons to the hypocotyl. Once the supply of this GA precursor to the hypocotyl is blocked by the removal of cotyledons, the levels of both precursor and bioactive GAs would be decreased, which should then cause elevated expression of GA 20-oxidase and GA 3-oxidase genes via the feedback regulation. These two models are not mutually exclusive, and it is possible that the movement of both GA precursor(s) and bioactive GA(s) play a role in the maintenance of GA levels in the hypocotyl in intact cucumber and tomato seedlings.

In conclusion, we have investigated the status of the GA biosynthesis pathway in hypocotyls of cucumber and tomato seedlings after the removal cotyledons. Our results indicate that cotyledons play an essential role in the maintenance of GA levels in hypocotyls in these species.

Acknowledgements

This work was supported in part by the Program for Promotion of Basic Research Activities for Innovative Biosciences (PROBRAIN). It was also supported by Research Fellowship from Japan Society for the Promotion of Science for Young Scientists (to M. Asahina).

References

Asahina M, Gocho Y, Kamada H, Satoh S (2006) Involvement of inorganic elements in tissue reunion in the hypocotyl cortex of *Cucumis sativus*. *J Plant Res* 119: 337–342

Asahina M, Iwai H, Kikuchi A, Yamaguchi S, Kamiya Y, Kamada H, Satoh S (2002) Gibberellin produced in the cotyledon is required for cell division during tissue-reunion in the cortex of

cut cucumber and tomato hypocotyls. *Plant Physiol* 129: 201–210

Bensen RJ, Zeevaart JAD (1990) Comparison of *ent*-kaurene synthase A and B activities in cell-free extracts from young tomato fruits of wild-type and *gib-1*, *gib-2*, and *gib-3* tomato plant. *J Plant Growth Regul* 9: 237–242

Eriksson S, Bohlenius H, Moritz T, Nilsson O (2006) GA_4 is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. *Plant Cell* 18: 2172–2181

Elliot RC, Ross JJ, Smith JJ, Lester DR (2001) Feed-forward regulation of gibberellin deactivation in pea. *J Plant Growth Regul* 20: 87–94

Gawronska H, Yang YY, Furukawa K, Kendrick RE, Takahashi N, Kamiya Y (1995) Effects of low irradiance stress on gibberellin levels in pea seedlings. *Plant Cell Physiol* 36: 1361–1367

Groot SPC, Bruinsma J, Karssen CM (1987) The role of endogenous gibberellin in seed and fruit development of tomato: Studies with a gibberellin-deficient mutant. *Physiol Plant* 71: 184–190

Hedden P, Kamiya Y (1997) Gibberellin biosynthesis: Enzymes, genes and their regulation. *Annu Rev Plant Physiol Plant Mol Biol* 48: 431–460

Holland PM, Abramson RD, Watson R, Gelfand DH (1991) Detection of specific polymerase chain reaction product by utilizing the 5′-3′ exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proc Natl Acad Sci USA* 88: 7276–7280

Itoh H, Ueguchi-Tanaka M, Kawaide H, Chen X, Kamiya Y, Matsuoka M (1999) The gene encoding tobacco gibberellin 3β-hydroxylase is expressed at the site of GA action during stem elongation and flower organ development. *Plant J* 20: 15–24

Itoh H, Ueguchi-Tanaka M, Sentoku N, Kitano H, Matsuoka M, Kobayashi M (2001) Cloning and functional analysis of two gibberellin 3β-hydroxylase genes that are differently expressed during the growth of rice. *Proc Natl Acad Sci USA* 98: 8909–8914

Izumi K, Iwai T, Oshio H (1988) Growth retarding effects of uniconazole on plant. *Chem Regul Plants* (Syokubutsu Kagaku Chousetsu) 24: 142–146 (in Japanese)

Izumi K, Kamiya Y, Sakurai A, Oshio H, Takahashi N (1985) Studies of sites of action of a new plant growth retardant (E)-1-(4-chlorophenyl)-4,4-dimethyl-2- (1, 2, 4-triazol-1-yl)-1-penten-3-ol (S-3307) and comparative effects of its stereoisomers in a cell-free system from *Cucurbita maxima*. *Plant Cell Physiol* 26: 821–827

Katsumi M, Foard DE, Phinney BO (1983) Evidence for the translocation of gibberellin A_3 and gibberellin like substances in graft between normal, $dwarf_1$ and $dwarf_5$ seedling of *Zea mays L.* *Plant Cell Physiol* 24: 379–388

Katsumi M, Purves WK, Phinney BO, Kato J (1965a) The role of the cotyledons in gibberellin- and auxin-induced elongation of the cucumber hypocotyl. *Physiol Plant* 18: 550–556.

Katsumi M, Phinney BO, Purves WK (1965b) The roles of gibberellin and auxin in cucumber hypocotyl growth. *Physiol Plant* 18: 462–473

Kollmann R, Glockmann C (1985) Studies on graft unions. I. Plasmodesmata between cells of plants belonging to different unrelated taxa. *Protoplasma* 124: 224–235

Kamisaka S (1973) Requirement of cotyledons for gibberellic acid-induced hypocotyl elongation in lettuce seedlings. Isolation of the cotyledon factor active in enhancing the effect of gibberellic acid. *Plant Cell Physiol* 14: 747–755

Koornneef M, Bosma TDG, Hanhart CJ, van der Veen JH, Zeevaart JAD (1990) The isolation and characterization of gibberellin-deficient mutants in tomato. *Theor Appl Genet* 80: 852–857

Lange T, Hedden P, Graebe JE (1994) Expression cloning of a gibberellin 20-oxidase, a multifunctional enzyme involved in gibberellin biosynthesis. *Proc Natl Acad Sci USA* 91: 8552–8556

Lester DR, Ross JJ, Davies PJ, Reid JB (1997) Mendel's stem length gene (*Le*) encodes a gibberellin 3β -hydroxylase. *Plant Cell* 9: 1443–1453

Lopez-Juez E, Kobayashi M, Sakurai A, Kamiya Y, Kendrick RE (1995) Phytochrome, gibberellins, and hypocotyl growth (A study using the cucumber (*Cucumis sativus* L.) long hypocotyl mutant). *Plant Physiol* 107: 131–140

Martin D, Proebsting W, Parks T, Dougherty W, Lange T, Lewis M, Gaskin P, Hedden P (1996) Feed-back regulation of gibberellin biosynthesis and gene expression in *Pisum sativum L.* *Planta* 200: 159–166

Martin D, Proebsting WM, Hedden P (1997) Mendel's dwarfing genes: cDNA from the *Le* alleles and function of the expressed proteins. *Proc Natl Acad Sci USA* 94: 8907–8911

Moore TC (1967) Kinetics of growth retardant and hormone interactions in affecting cucumber hypocotyl elongation. *Plant Physiol* 42: 677–684

Olszewski NE, Sun TP, Gubler F (2002) Gibberellin signaling: Biosynthesis, catabolism, and response pathways. *Plant Cell* (suppl) 14: 61–80

O'Neill DP, Ross JJ (2002) Auxin regulation of the gibberellin pathway in pea. *Plant Physiol* 130: 1974–1982

Rebers M, Kaneta T, Kawaide H, Yamaguchi S, Yang YY, Imai R, Sekimoto H, Kamiya Y (1999) Regulation of gibberellin biosynthesis genes during flower and early fruit development of *tomato.* *Plant J* 17: 241–250

Reid, JB, Murfet IC, Potts WC (1983) Internode length in *Pisum.* II. Additional information on the relationship and action of loci *Le, La, Cry, Na,* and *Lm.* *J Exp Bot* 34: 349–364

Richardson FVM, Saoir SMA, Harvey BMR (1996) A study of the graft union in *in vitro* micrografted apple explants. *Plant Growth Regul* 20: 17–23

Ross JJ, Murfet IC, Reid JB (1997) Distribution of gibberellins in *Lathyrus odoratus* L. and their role in leaf growth. *Plant Physiol* 102: 603–608

Ross JJ, O'Neill DP (2001) New interactions between classical plant hormones. *Trends Plant Sci* 6: 2–4

Rudich J, Sell HM, Baker LR (1976) Transport and metabolism of ^{3}H-gibberellin A_1 in dioecious cucumber seedlings. *Plant Physiol* 57: 734–737

Sandhu MS, Lockard RG, Kasperbauer MJ (1974) Localization of stem elongation control in *Cucumis sativus* L. *Plant Physiol* 53: 942–43

Satoh S (1996) Inhibition of flowering of cucumber grafted on rooted squash stock. *Physiol Plant* 97: 440–444

Shibata K, Kubota T, Kamisaka S (1974) Isolation and chemical identification of a lettuce cotyledon factor, a synergist of the gibberellin action in inducing lettuce hypocotyl elongation. *Plant Cell Physiol* 15: 191–194

Siegel BA, Verbeke JA (1989) Diffusible factors essential for epidermal cell redifferentiation in *Catharanthus roseus.* *Science* 244: 580–582

Smith VA, Sponsel VM, Knatt C, Gaskin P, MacMillan J (1991) Immunochromatographic purification of gibberellins from vegetative tissue of *Cucumis sativus* L: separation and identification of 13-hydroxy and 13-deoxy gibberellins. *Planta*

185: 583–586

Sun TP, Gubler F (2004) Molecular mechanism of gibberellin signaling in plant. *Ann Rev Plant Biol* 55: 197–223

Sun TP, Kamiya Y (1994) The *Arabidopsis* GA1 locus encodes the cyclase *ent*-kaurene synthetase A of gibberellin biosynthesis. *Plant Cell* 6: 1509–1518

Taylor A, Cosgrove DJ (1989) Gibberellic acid stimulation of cucumber hypocotyl elongation: Effects on growth, turgor, osmotic pressure, and cell wall properties. *Plant Physiol* 90: 1335–1340

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YC, Kitano H, Yamaguchi I, Matsuoka M (2005) Gibberellin insensitive dwarf encodes a soluble receptor for gibberellin. *Nature* 437: 693–698

van der Schoot C, Dietrich MA, Storms M, Verbeke JA, Lucas WJ (1995) Establishment of a cell-to-cell communication pathway between separate carpels during gynoecium development. *Planta* 195: 450–455

Walker DB (1975) Postgenital carpel fusion in *Catharanthus roseus* (Apocynaceae). I. Light and scanning electron microscopic study of gynoecial ontogeny. *Am J Bot* 62: 457–467

Wang Y, Kollmann R (1996) Vascular differentiation in the graft union of *in-vitro* grafts with different compatibility. Structural and functional aspects. *J Plant Physiol* 147: 521–533

Yamaguchi S, Kamiya Y (2000) Gibberellin biosynthesis: its regulation by endogenous and environmental signals. *Plant Cell Physiol* 41: 251–257

Yamaguchi S, Smith MW, Brown RGS, Kamiya Y, Sun TP (1998) Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating *Arabidopsis* seeds. *Plant Cell* 10: 2115–2126

Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S (2003) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. *Plant Cell* 16: 367–378

Yang YY, Yamaguchi I, Takeno-Wada K, Suzuki Y, Murofushi N (1995) Metabolism and translocation of gibberellins in seedling of *Pharbitis nil.* (I) Effect of photoperiod on stem elongation and endogenous gibberellins in cotyledon and their phloem exudates. *Plant Cell Physiol* 36: 221–227