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Tall or short? Slender or thick? A plant strategy for regulating elongation growth of roots by low concentrations of gibberellin

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• *Background* Since the plant hormone gibberellin (GA) was discovered as a fungal toxin that caused abnormal elongation of rice shoots, the physiological function of GA has mainly been investigated in relation to the regulation of plant height. However, an indispensable role for GA in root growth has been elucidated by using severely GA-depleted plants, either with a gene mutation in GA biosynthesis or which have been treated by an inhibitor of GA biosynthesis. The molecular sequence of GA signalling has also been studied to understand GA functions in root growth.
• *Scope* This review addresses research progress on the physiological functions of GA in root growth. Concentration-dependent stimulation of elongation growth by GA is important for the regulation of plant height and root length. Thus the endogenous level of GA and/or the GA sensitivity of shoots and roots plays a role in determining the shoot-to-root ratio of the plant body. Since the shoot-to-root ratio is an important parameter for agricultural production, control of GA production and GA sensitivity may provide a strategy for improving agricultural productivity. The sequence of GA signal transduction has recently been unveiled, and some component molecules are suggested as candidate *in planta* regulatory sites and as points for the artificial manipulation of GA-mediated growth control.
• *Conclusions* This paper reviews: (1) the breakthrough dose–response experiments that show that root growth is regulated by GA in a lower concentration range than is required for shoot growth; (2) research on the regulation of GA biosynthesis pathways that are known predominantly to control shoot growth; and (3) recent research on GA signalling pathways, including GA receptors, which have been suggested to participate in GA-mediated growth regulation. This provides useful information to suggest a possible strategy for the selective control of shoot and root growth, and to explain how GA plays a role in rosette and liana plants with tall or short, and slender or thick axial organs.

Key words: Ancymidol, GA receptors, GA sensitivity, gibberellin, GID1, *Lactuca sativa*, low concentration of GA, *Pisum sativum*, root elongation, root thickening, rosette form, shoot-to-root ratio.

INTRODUCTION

Plant growth is regulated by multiple environmental factors and nutritional supply; however, most of the regulatory events are mediated by plant hormones. Among these plant hormones, gibberellin (GA) has been known to control plant height ever since its discovery in studies of an abnormally elongating disease of rice seedlings caused by a fungal infection by *Gibberella fujikuroi* (Takahashi *et al.*, 1955, 1957, 1991).

Exogenously applied GA strongly promotes the elongation growth of shoots in many plants, as shown in Fig. 1, whereas it does not show such clear effects on roots, even in GA-sensitive pea plants (Fig. 2). Therefore, the question arises of whether GA is a plant hormone that regulates shoot growth but does not affect root growth. The answer to this question is a resounding 'no'. A GA requirement for root growth has been elucidated by GA depletion experiments in GA-deficient mutants and/or treatment with growth retardants. Some growth retardants were discovered to suppress shoot elongation by inhibiting endogenous production of GA (Rademacher, 1991). By using precise concentrations of such chemical inhibitors of GA biosynthesis in combination with GA-deficient mutants, GA has been shown to control root growth at a considerably lower concentration than is necessary for controlling shoot growth (Tanimoto, 1994). It is now recognized that GA plays an indispensable role in both shoots and roots in many plants. The next question is how shoot and root growth is quantitatively regulated by GA in one and the same plant body.

Since the shoot/root ratio is important for agricultural production, practical methods to change the shoot/root ratio have long been awaited, as demonstrated by 'the green revolution' where partial suppression of shoot growth resulted in more cereal production (Sasaki *et al.*, 2002; Hedden, 2003). Moreover, there are diverse plant shapes with different shoot/root ratios in nature. Extreme examples are liana (climbing) plants, such as pea and kudzu (*Pueraria lobata*), which contrast with rosette plants such as radish and dandelion (*Taraxacum officinale*), the latter having short stems but well-developed roots. It is an interesting physiological question as to how stem and root growth can be separately controlled in these plants.

Although the molecular mechanism of GA-mediated elongation growth itself is still under investigation, molecular events

FIG. 1. Typical effect of gibberellin in the enhancement of stem growth. Two cabbage plants (on the right) were treated with GA_3 for 5 months. Stem growth was strongly enhanced as compared with the control plant (to the left) to which no GA was applied.
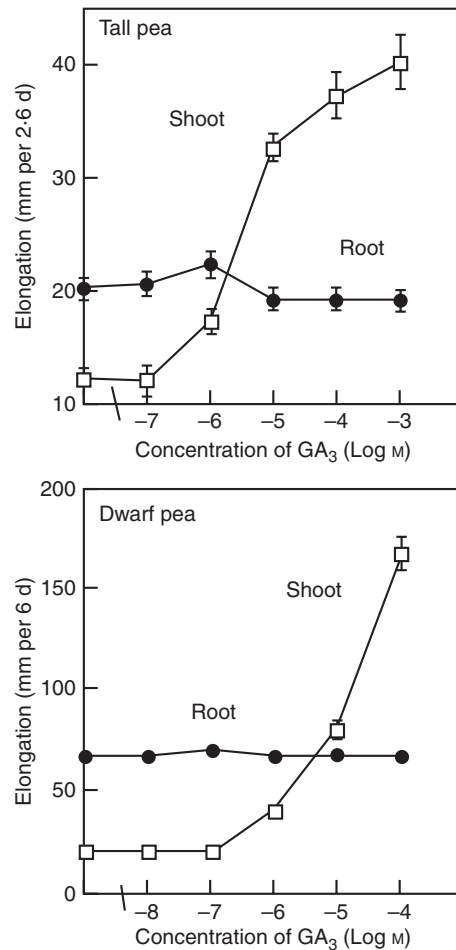

FIG. 2. Effect of GA_3 concentration on the elongation growth of pea seedlings. Shoot elongation was strongly enhanced by GA_3 supplied by hydroponics, whereas root elongation was not affected. Tall pea ('Alaska') and dwarf pea ('Little Marvel') varieties were used. (Adapted from Tanimoto, 1988, 2002.)

of GA signal transduction, including GA receptor molecules, have been revealed. Additionally, the GA biosynthesis pathway and its regulation have been investigated to show the local regulation of active GA production in a plant body, suggesting the separate regulation of GA production in roots and shoots.

Inhibitors of GA biosynthesis have been shown to cause swelling of onion bulb cells and to change their microtubule and microfibril orientation (Mita and Shibaoka, 1984; Shibaoka, 1994). In GA depletion experiments, either by inhibition of GA biosynthesis or using GA-deficient mutants, remarkable thickening of the root was observed in pea and lettuce, while slender roots were induced by GA treatment (Tanimoto, 1987, 1988, 1994).

This short review describes in detail the progress of research on GA-mediated regulation of root growth, and presents a recent hypothesis for 'GA sensitivity', as well as discussing possible methods to change the shoot/root ratio by regulating the GA concentration and GA sensitivity. Brief details of the materials and methods used in some of the experiments referred to in this review are given in the Appendix.
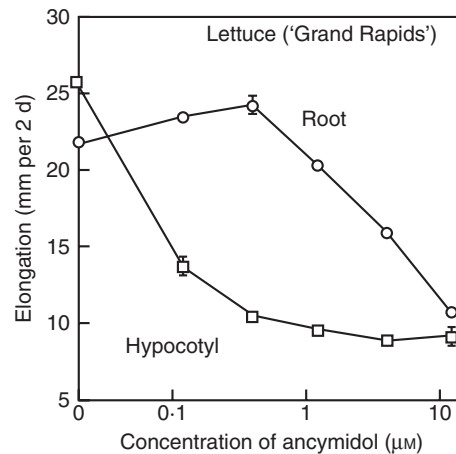

FIG. 3. Concentration-dependent inhibition by ancymidol of hypocotyl and root elongation in *Lactuca sativa*. (Adapted from Tanimoto, 1987.)

REGULATION OF ROOT ELONGATION AND THICKNESS BY GA DEPLETION AND GA SUPPLEMENTATION

Gibberellin and root elongation

An inhibitor of GA biosynthesis, ancymidol (Coolbaugh and Hamilton, 1976; Coolbaugh *et al.*, 1978), strongly suppresses hypocotyl elongation of lettuce seedlings at a relatively low concentration at which root growth is not correspondingly suppressed (Fig. 3; Tanimoto, 1987). Gibberellin shows clear stimulation of root elongation under growth-suppressed conditions induced by this inhibitor of GA biosynthesis in lettuce
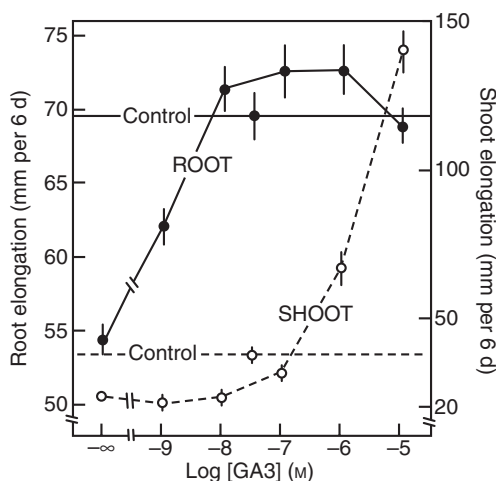
FIG. 4. Concentration-dependent stimulation of root and shoot elongation of dwarf pea seedlings by GA_3 applied to hydroponics in the presence of ancymidol. Ancymidol-suppressed root elongation was recovered by GA_3 at nanomolar concentration and saturated at a 10 nM concentration, whereas shoot elongation was enhanced at the micromolar level. (Adapted from Tanimoto, 1994).

(Tanimoto, 1987), a tall variety of pea (Tanimoto, 1988) and in dwarf pea (Fig. 4; Tanimoto, 1994). When the endogenous GA level is decreased by the GA biosynthesis inhibitor or by mutation of a GA biosynthesis gene, root growth is stunted and abnormal thickening is induced. These phenomena are completely reversed by the exogenous application of GA_3 at a rather low nanomolar concentration (Fig. 4), which is insufficient to promote shoot elongation. The phenomenon has been further confirmed by time course experiments using a rhizometer, in which lettuce seedlings are dipped in hydroponics containing growth regulators for 2 min at 30 min intervals during a 4 d experiment (Fig. 5; Tanimoto, 2002). It was shown that GA_3 enhances root elongation but not that of the hypocotyl at 1 nM, whereas hypocotyl elongation is stimulated at 1 μM GA_3.

By these dose–response examinations, the growth-regulating function of GA in roots has been elucidated and the interesting hypothesis has been advanced that root elongation occurs at a lower GA concentration than stem growth, as shown by Fig. 6, in which dose–response curves of GA-regulated root and stem growth are indicated with reference to those of indole-3-acetic acid (IAA)-regulated growth.

Since a specific transporter of GA has not been found, there may be no regulatory system to distribute GA molecules to specific tissues in a plant body. How, then, is root elongation enhanced without causing stem elongation in rosette plants? The first strategy may involve a local production of GA in roots at a precisely controlled concentration. The second strategy may be to provide roots with a high sensitivity to GA to enable root elongation at very low GA concentrations that are not sufficient for stem growth. These two methods are compatible with each other, but the first may be hazardous for plants, because overproduction of GA in roots may cause stem elongation under an unfavourable climate. However, it must be noted that the GA_1 and GA_4 inactivation system may well have been developed to prevent an oversupply of
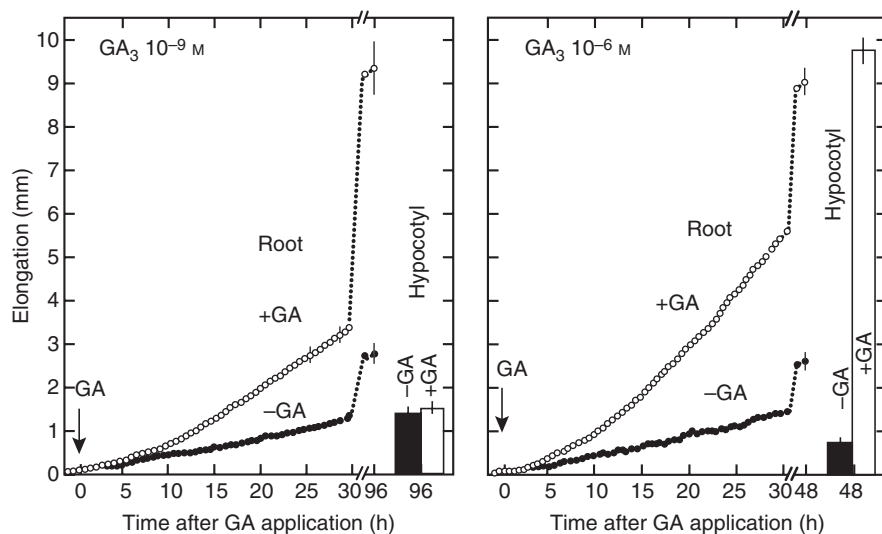
FIG. 5. Time course of GA-induced root elongation and final hypocotyl length of lettuce seedlings treated with 1 nM (left) and 1 μM (right) GA_3 in the presence of ancymidol. Root lengths of 14 seedlings were measured every 30 min in a rhizometer (Tanimoto and Watanabe, 1986). Whole seedlings were dipped for 2 min in hydroponic solution containing growth regulators at 30 min intevals. Root elongation was enhanced by 1 nM GA_3, whereas hypocotyl growth was not enhanced. GA_3 at 1 μM strongly enhanced both root and hypocotyl elongation. (Adapted from Tanimoto, 2002.)

active GAs, and that externally applied GA_3, which is often used for dose–response experiments, is relatively more stable than GA_1 and GA_4. The *in planta* stability of GA_1, GA_4 and GA_3 remains a question for further quantitative investigation.

Gibberellin and root thickness

Ancymidol-enhanced and GA-suppressed thickening of roots is usually observed in the elongation zone of the root (Fig. 7) and is mainly caused by the expansion of cortical cells (Fig. 8) (Tanimoto, 1987, 1988, 1994). Ancymidol-induced thickening of root is also observed in woody roots of tea, *Camellia sinensis* (Fig. 7, right). As reported for onion bulbs (Mita and Shibaoka, 1984; Shibaoka, 1994), GA has been shown to limit cell expansion by controlling microtubule orientation and to facilitate the cellulose–microfibril orientation perpendicular to the longitudinal axis of the root (Hogetsu and Oshima, 1986), resulting in the elongation of slender roots. Expansion of cortical cells in the elongation zone of the root may be caused by a disorder of
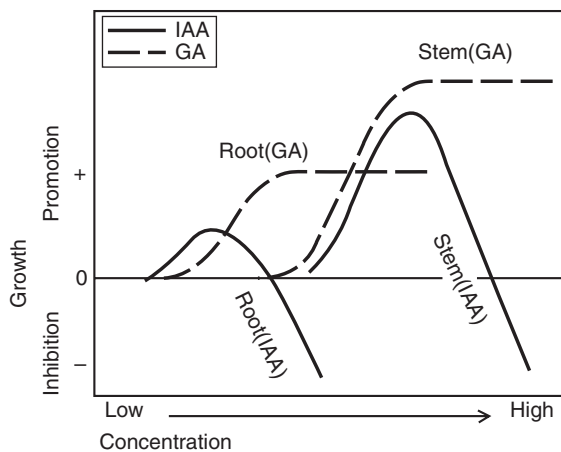
FIG. 6. Concentration-dependent promotion or inhibition of root and stem elongation by GA as compared with auxin (IAA). (Adapted from Tanimoto, 2005.)

GA-mediated microtubule and microfibril orientation due to GA depletion and/or lack of GA signalling. A similar phenomenon was also reported in maize roots (Baluška *et al.*, 1993). Thickening of root cells by GA depletion is suggested to occur in radish and other rosette plants.

REGULATION OF THE ACTIVE GA LEVEL AND GA BIOSYNTHESIS PATHWAY

Gibberellin-sensitive dwarf plants have been investigated in order to elucidate the biosynthetic pathway and metabolism of GA. Many dwarfing genes were identified as controlling the enzyme activity of each biochemical step of the pathway, some of which were also suppressed by growth retardants (Hedden and Kamiya, 1997; Tanimoto, 2002; Yamaguchi, 2008). The morphological appearance of dwarf plants affected by these genes is somewhat complicated, particularly in roots, where GA is saturated at a low level. The reason for a variable degree of dwarfism may be ascribed to the redundancy and/or leaky functions of these genes. For example, the *le* mutant of dwarf pea has considerably stunted stems but their roots grow normally, as shown in Fig. 9. This mutant produces one-tenth of the normal GA content in the shoot, but the amount of GA in the roots was comparable with that of the wild type (Yaxley *et al.*, 2001). However, when the dwarf pea bearing the mutant *na* gene (cultivar 'Little Marvel') was treated with ancymidol, roots were severely stunted and thickened, and stems were also further shortened (Tanimoto, 1994). Ancymidol-suppressed root growth was recovered at a lower concentration of GA_3 than the stem growth (Fig. 4). Thus, these dwarf genes controlling GA biosynthesis may be leaky, and a combination of these genes with the GA biosynthesis inhibitor might cause a more severe syndrome of GA depletion, i.e. short stems, dark-green leaves and short and thickened roots.

Gibberellin biosynthesis is controlled by feedback and feed-forward systems (Hedden and Kamiya, 1997; Hedden, 1999; Olszewski *et al.*, 2002; Yamaguchi, 2008), and is also regulated by another plant hormone, auxin (Ross *et al.*, 2000, 2002). The major native auxin, IAA, which is transported from the apical meristem, increases the active GA_1 level in
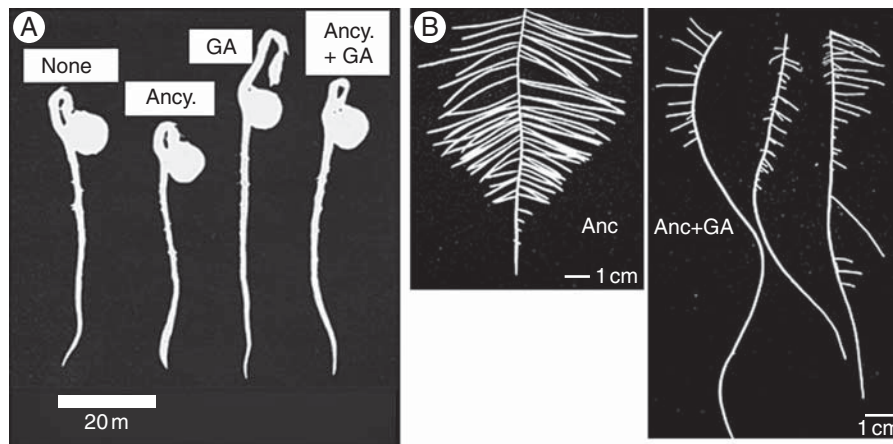
FIG. 7. Thickening of roots caused by ancymidol and its recovery promoted by GA_3. (A) Pea seedlings treated with ancymidol (Ancy.) and/or GA_3 (adapted from Tanimoto, 1988). (B) Tea roots treated by ancymidol with or without GA_3, grown in hydroponics.

stems by two mechanisms (Fig. 9). IAA stimulates the expression of GA 3-oxidase which converts GA_{20} to bioactive GA_1, and suppresses the GA 2-oxidase which decreases the GA_{20} level. Although IAA regulation of the GA_1 level has not been demonstrated in roots, it may be highly possible that it also participates in the regulation of low GA production in roots. The role of IAA and GA in root growth was reviewed by Tanimoto (2005), and cross-talk between IAA and GA has been demonstrated in the signalling system (Fu and Harberd, 2003; Gou *et al.*, 2010; Heo *et al.*, 2011). The IAA–GA cross-talk for lateral root formation has also been briefly reviewed (Farquharson, 2010).

Local production and translocation of GA are also important factors for the regulation of shoot and root growth. Unlike IAA

FIG. 8. Control of root thickness by ancymidol and GA. Cross-sections of the elongation zone of lettuce roots treated with ancymidol and/or GA_3 for 2 d. (Adapted from Tanimoto, 1987.)

transport, GA has been shown to move relatively freely from roots to shoots, and vice versa (Prochazka, 1981; Matthysse and Scott, 1984), although no specific transporter of GA has been found. For example, shoot- or cotyledon-applied GA_3 was found to enhance root elongation in pea plants (Tanimoto, 1994), and shoot-applied GA_4 enhanced root growth in arabidopsis (Bidadi *et al.*, 2010). Using grafting experiments between the wild type and either the *le* mutant or the *nana* mutant of pea, the ability of a wild-type rootstock to restore stem elongation of a GA-deficient scion was shown to be dependent on the position of the lesion in the GA biosynthesis pathway (Dodd, 2005), i.e. wild-type root stocks restored stem elongation of *nana* scions, which are deficient in an early step of the pathway (the conversion of ent-kaurenoic acid to GA_{12} aldehyde) (Proebsting *et al.*, 1992). In contrast, wild-type rootstocks did not restore stem elongation of *le* scions, which are deficient in the conversion of inactive GA_{20} to active GA_1 (Reid *et al.*, 1983).

Gibberellin also regulates auxin transport by modulating the turnover of the auxin efflux facilitator, PIN, and thus affects root gravitropism (Willige *et al.*, 2011). In GA-deficient plants, auxin transport is reduced in *Arabidopsis thaliana*, and this is correlated with a reduction of PIN abundance in GA-deficient plants, which is restored to the wild-type level by external GA application. However, since the endogenous level of auxin is thought to be supraoptimal for many roots, GA may inhibit root elongation when GA increases the endogenous auxin level (Kuraishi and Muir, 1962) and/or when GA increases the tissue sensitivity to auxin, as previously suggested for stems (Ockerse and Galston, 1967; Tanimoto *et al.*, 1967).
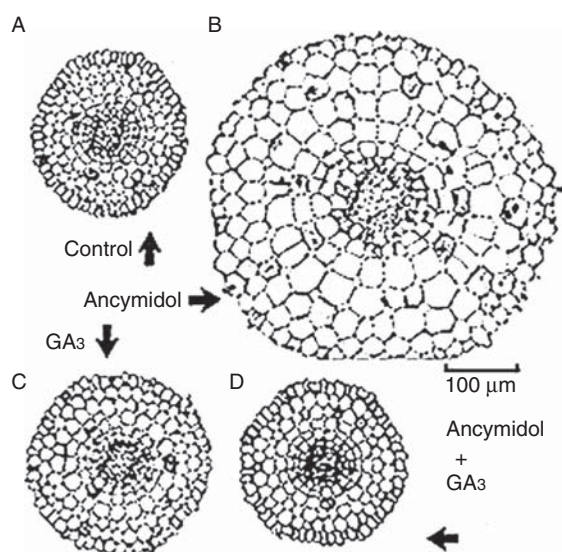
GA SIGNALLING PATHWAY AND GA RECEPTORS

The signalling pathway of GA action has been uncovered in the last decade (Ueguchi-Tanaka *et al.*, 2005, 2007; Hirano *et al.*, 2008; Murase *et al.*, 2008; Sun, 2010). The pathway is
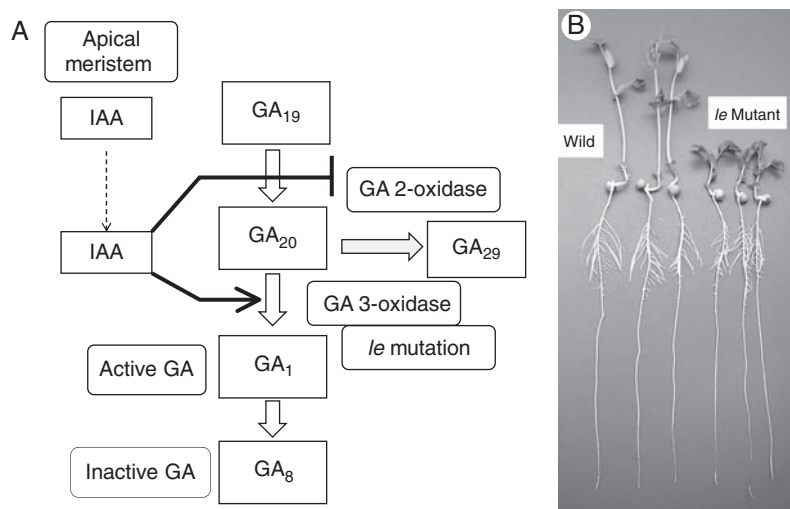

FIG. 9. (A) Final steps of GA_1 biosynthesis and its inactivation. IAA supplied by the apical meristem controls the active GA_1 level through the regulation of enzyme-encoding genes participating in the production of GA_1. (B) Pea seedlings of the *le* mutant (the three seedlings on the right) which has a defective gene for GA 3-oxidase, as indicated in A, and its wild-type counterpart (the three seedlings on the left). (Adapted from Tanimoto, 2002.)
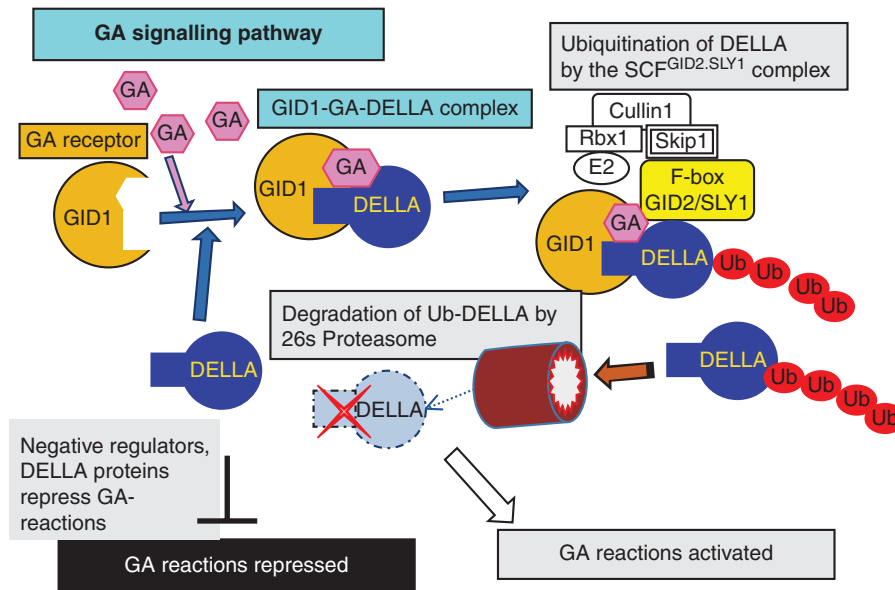
FIG. 10. Model for the activation of GA reactions. GA signalling is initiated by the interaction between the GA molecule and the GA receptor protein GID1 to form a complex with DELLA proteins, negative regulators of GA reactions. The GA–GID1–DELLA complex is recognized by the SCF$^{GID2/SLY1}$ complex and DELLA proteins are polyubiquitinated, resulting in the degradation of DELLA proteins by the 26S proteasome machinery. By GA-initiated degradation of DELLA proteins, through the ubiquitin–proteasome system, the GA-related genes are expressed and GA reactions are activated.
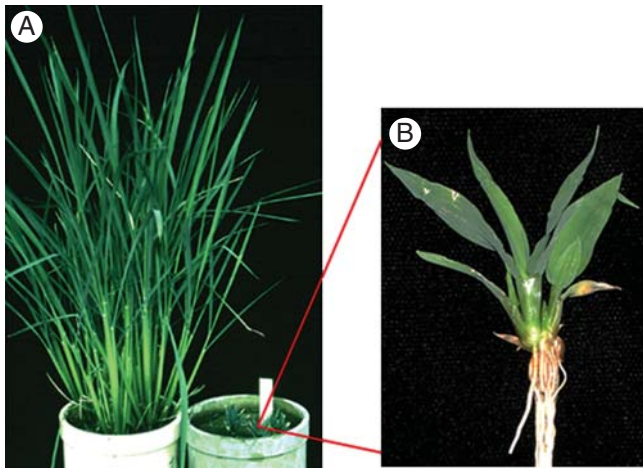

FIG. 11. (A) GA-insensitive dwarf1 mutant of rice in the right-hand pot (magnified in B) and its wild-type counterpart (left-hand pot). The GA receptor GID1 was discovered in the study of the gibberellin-insensitive dwarf1 (*gid1*) mutant, GIBBERELLIN INSENSITIVE DWARF1, which encodes a nuclear-localized soluble receptor for GA. (Courtesy of Dr M. Ueguchi-Tanaka.)
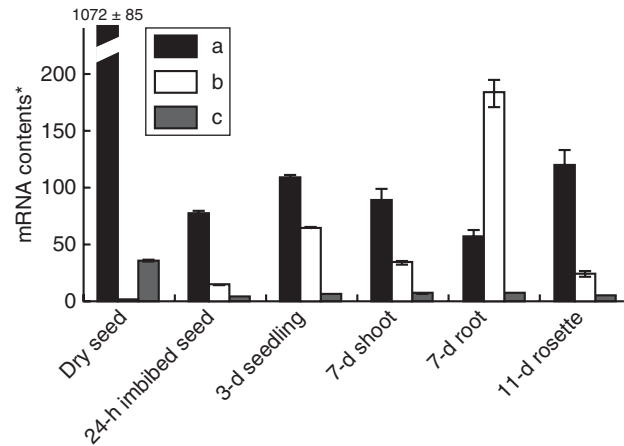

FIG. 12. Different mRNA contents of GID1a, b and c in seed, seedling, shoot, root and rosette leaves of *Arabidopsis thaliana*. *The mRNA contents are shown as relative values to that of GID1b in dry seed, which is set at 1. The root contains the highest amount of GID1b mRNA. (Adapted from Griffiths *et al.*, 2006.)

initiated by the interaction of GA and the GA receptor protein, GID1 (Fig. 10). The GA–GID1 conjugate binds to the DELLA and TVHYNP motifs of the DELLA proteins, a repressor of GA reactions in the absence of GA. The GA–GID1–DELLA protein complex is recognized by a polyubiquitination system, and polyubiquitinated DELLA protein is degraded by the 26S proteasome machinery. The disappearance of DELLA protein results in the expression of GA-related genes, leading to the activation of GA reactions. One of the GA reactions is the regulation of shoot growth through the functional regulation of DELLA protein, as demonstrated in the 'green revolution' in which semi-dwarf cereals were used (Sasaki *et al.*,

2002; Hedden, 2003). Participation of DELLA proteins in the growth regulation of roots was also elucidated (Fu and Harberd, 2003; Ubeda-Tomas *et al.*, 2008). Although it is not yet clear to what extent such DELLA regulation is working in root growth, a quantitative regulation of the GA signalling process might be a possible site of artificial control of root and shoot growth in addition to the regulation of active GA content *in situ*, as described above.

Evidence for the participation of GA signalling in root growth and development is accumulating; GA regulates meristem size and growth of roots (Ubeda-Tomás *et al.*, 2008, 2009) and lateral root development (Zimmermann *et al.*, 2010).
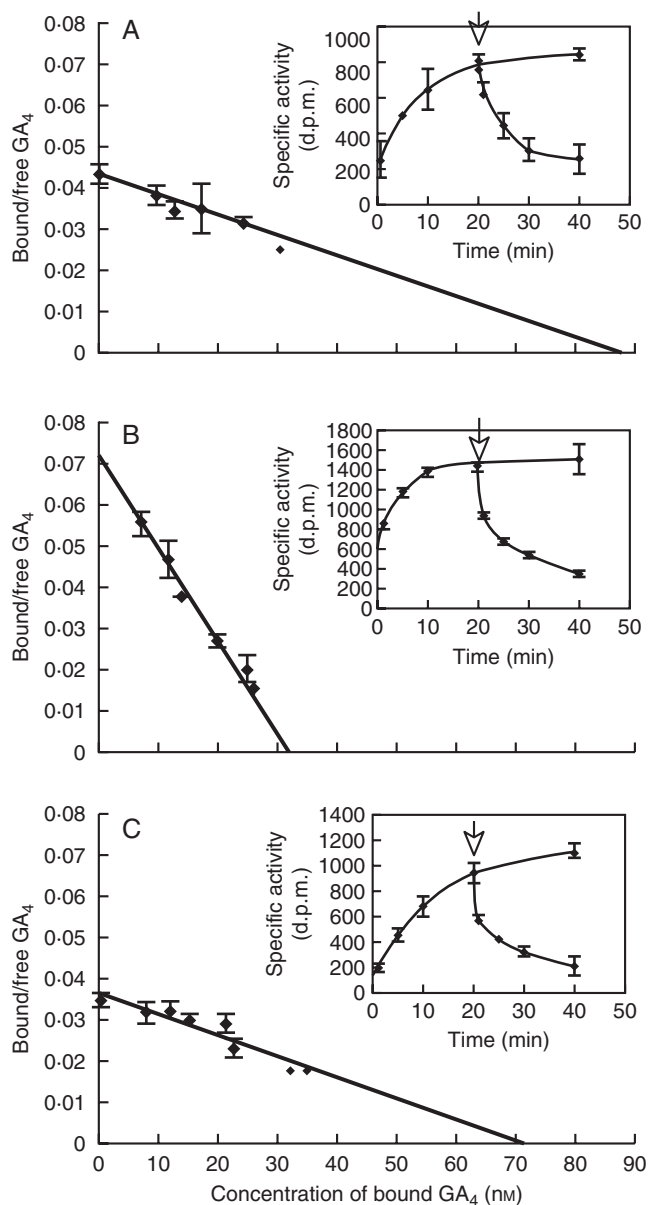
FIG. 13. Association and dissociation kinetics of (A) AtGID1a, (B) AtGID1b and (C) AtGID1c. Dissociation constants of GID1a, b and c were calculated from GA binding kinetics of each AtGID1. K_d values are: (A) $K_d(H2\text{-}GA4)$:AtGID1a $= 2\cdot0 \times 10^{-6}$ M, (B) $K_d(H_2\text{-}GA_4)$:AtGID1b $= 4\cdot8 \times 10^{-7}$ M, (C) $K_d(H_2\text{-}GA_4)$:AtGID1c $= 1\cdot9 \times 10^{-6}$ M. AtGID1b shows a much lower K_d value than AtGID1a or AtGID1c. Insets show the time course characteristics for GA binding and replacement of AtGID1s with an excess amount of unlabelled GA$_4$ (0·125 mM) (arrow). (Adapted from Nakajima *et al.*, 2006.)

In addition to the control of DELLA protein itself, receptor molecules of GA are also candidates for the regulation of GA reactions. The GID1 receptor molecule was discovered in a study of a GA-insensitive extreme dwarf of rice (Fig. 11; Ueguchi-Tanaka *et al.*, 2005). The receptor molecule was named after gibberellin-insensitive dwarf rice 1. The mutant rice shows the typical GA-deficient symptoms of dark-green leaves and short and thick stems. Roots look slightly thickened but not very stunted. The reason for this is not still clear.

The molecular mechanism of high GA sensitivity of roots is not clear, but an interesting mechanism has been suggested recently in the author's research group with Drs Miyako Ueguchi-Tanaka and Makoto Matsuoka (Nagoya University). There are three GA receptors in *A. thaliana*: AtGID1a, AtGID1b and AtGID1c (Ueguchi-Tanaka *et al.*, 2007). One of them, GID1b, has a higher GA affinity, i.e. a lower dissociation constant for, the GA molecule (Fig. 13; Nakajima *et al.*, 2006). The knockout strain of AtGID1b became more sensitive to the GA inhibitor biosynthesis than the other knockout strains, i.e. the root was stunted more significantly at a low level of growth retardant application. Furthermore, another laboratory found that AtGID1b is rather strongly expressed in roots (Griffiths *et al.*, 2006), and AtGID1b overproduction resulted in a slight promotion of root growth (Ariizumi *et al.*, 2008).

Genetic manipulation of GID1 molecules has been carried out and its biochemical and physiological function can be changed by a partial displacement of the peptide sequence in the GID1 molecule (Yamamoto *et al.*, 2010). At some point in the displacement manipulation, the GID1 molecule became active in GA signalling even in the absence of a GA molecule. Such a molecular manipulation technique provides a strategy for GA-mediated growth regulation of roots and shoots.

CONCLUSIONS

By reviewing research on GA function in roots during the last three decades, the role of GA in root elongation and thickening has become clear, as follows. (1) Root elongation is regulated by a significantly lower concentration of GA than stem elongation. (2) Higher GA sensitivity of roots in comparison with shoots may provide an advantage for rosette plants to have long roots and short stems when there is a limited production of GA, in addition to the local regulation of GA production. Although the molecular mechanism of higher GA sensitivity is not fully understood, this mechanism may endow plants with diverse shapes, tall or short, and slender or thick. (3) Knowledge of the molecular mechanism of higher sensitivity of roots to GA may also provide a new strategy for regulating the shoot/root ratio for agricultural purposes.

Possible strategies for selective control of shoot and root growth are expected to emerge from more precise understandings of the molecular mechanism of GA signal transduction including the GA receptor and DELLA proteins in roots.

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APPENDIX: PLANT MATERIALS AND METHODS FOR ROOT GROWTH EXPERIMENTS

Pea seedlings

Pea seeds were sterilized by dipping in diluted sodium hypochlorite solution for 30 min, and germinated on wet filter paper or a cotton bed in an air-tight box. After selecting germinated seedlings by root length, seedlings were put on a stainless steel mesh fixed on a 100–200 mL cup containing hydroponic solution and growth regulators. Hydroponically cultured seedlings, ten seedlings per cup, are convenient for measuring root growth over a period of a few weeks.

Lettuce seedlings

Lettuce seeds are layed in rows on a double layer of wet filter paper after sterilization (dipped in diluted sodium hypochlorite solution for a few minutes after rinsing with ethanol). The doubled filter paper was vertically attached inside a 100–200 mL cup containing hydroponic solution. Roots grow vertically downwards in the hydroponic solution containing growth regulators.

Arabidopsis seedlings

Sterilized seeds were layed on a 1 % agar plate in a square Petri dish (100 × 140 × 12 mm) and the plate was placed vertically to allow vertical root elongation on the agar surface. The Petri dish must be taped with surgical mending tape to maintain the required level of humidity and aeration.

Growth quantification of roots.

The length of the main root was measured either using a ruler or by image analysis of photographs (Qui *et al.*, 2007). For arabidopsis roots, the lengths of the longest roots, usually the main roots, were measured.

Continuous recording.

For continuous recording of root elongation, image analysis of photographs, or a rhizometer (TRZ-16) with minimum mechanical contact with the root tip, are both useful (Tanimoto and Watanabe, 1986).