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Interaction of Gibberellin A_3 and Ancymidol in the Growth and Cell-Wall Extensibility of Dwarf Pea Roots

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Effects of ancymidol (Anc) and gibberellin A_3 (GA_3) on root growth, osmotic concentration and cell-wall extensibility of the root were investigated in the gibberellin-sensitive cultivar of dwarf pea, Little Marvel. Anc strongly suppressed elongation of both shoots and roots in darkness. Although the elongation of shoots of this dwarf cultivar was severely retarded in the light, it was repressed still further by Anc. GA_3 promoted elongation of shoots both in the presence and in the absence of Anc, whereas it reversed suppression of root elongation by Anc. The concentration of GA_3 required for the recovery of root elongation was lower than that required for the promotion of shoot elongation. Treatment with Anc led to increased thickening of roots with increased numbers of cells per cross section and lateral expansion of cells in the cortex.

GA_3 had little effect on the osmotic concentration of cell sap obtained from root segments. Anc-treated roots did not respond to acid solutions by elongation, whereas GA_3-treated roots responded normally to such solutions. Anc suppressed but GA_3 enhanced the cell-wall extensibility of roots as measured in vivo and in vitro.

These results indicate that a low concentration of gibberellin plays a role in normal elongation of roots by maintaining the extensibility of the cell wall in this gibberellin-sensitive dwarf pea.

Key words: Cell-wall extensibility — Dwarf pea — Elongation growth — Gibberellin — *Pisum sativum* — Root growth.

The interaction of gibberellin A_3 (GA_3) and ancymidol (Anc) in the elongation of roots has been reported in a tall cultivar of pea, namely Alaska (Tanimoto 1988). GA_3 promotes root elongation in Alaska pea, while root growth is suppressed by Anc, a growth retardant that inhibits the biosynthesis of gibberellin (Coolbaugh et al. 1982). GA_3 has been shown to promote the elongation of both intact and excised stems of GA_3-sensitive dwarf pea (Brian and Hemming 1957, Tanimoto et al. 1967, Reid 1990), whereas it does not promote the elongation of roots of either tall (Tanimoto 1988) or dwarf (Tanimoto 1990) pea cultivars. However, GA_3 strongly enhances root elongation of these plants when root growth has been repressed by pretreatment with Anc (Tanimoto 1988, 1990). A dose-response analysis with Alaska pea (Tanimoto 1988) suggested that the concentration of gibberellin required for root elongation is lower than that required for stem elongation. Thus, it seems probable that the elongation of roots of gibberellin-sensitive dwarf peas such as cv. Little Marvel, would show the same dose-response to Anc and gibberellin as that of Alaska pea, in contrast to the different results for stem elongation. One of the purposes of this study was to examine this hypothesis in a gibberellin-sensitive dwarf pea by determining whether root elongation of the dwarf pea could be inhibited by Anc and promoted by a low concentration of gibberellin only in the presence of Anc.

Although GA_3 has been reported to increase the osmotic concentration within cells and cell-wall extensibility and such increases has been used as an explanation of the enhancement by gibberellin of stem elongation (Katsumi and Kazama 1978, Katsumi et al. 1980, Miyamoto and Kamisaka 1988a, b), no analogous results for roots have been reported.

The mechanical properties of cell walls have been intensively studied in relation to the auxin- and gibberellin-induced elongation of stems and coleoptiles (Cosgrove and Sovonick-Dunford 1989, Masuda 1990, Taiz 1984, Tanimoto and Masuda 1971, Yamamoto and Masuda 1971, Yamamoto et al. 1970). There is, however, little information about the mechanical properties of cell walls in

roots (Beusmans and Silk 1988, Silk and Beusmans 1988). Although the effects of gibberellin on the mechanical properties of cell walls have been studied in epicotyls of pea plants (Nakamura et al. 1975, Miyamoto et al. 1992), little is known about roots.

Since the growth response of stems of dwarf pea to gibberellin is influenced by the availability of light (Hashimoto et al. 1989, Reid 1983, 1988, 1990, Ross et al. 1992), the growth response of roots of dwarf pea to gibberellin might be expected to be similarly affected. Therefore, in the present study, the growth response of dwarf pea roots to GA_3 and Anc was examined in darkness and in the light. In addition, the effects are reported of GA_3 on the osmotic concentration in cells and the cell-wall extensibility of roots in a gibberellin-responsive variety of dwarf pea, cv. Little Marvel. Preliminary results of this study have appeared previously (Tanimoto 1992).

Materials and Methods

Plant material—Pea roots from *Pisum sativum* L. cv. Little Marvel were prepared as described elsewhere (Tanimoto 1988). Seeds were surface-sterilized and soaked in running tap water for 20 h at 23°C. Seeds with swollen radicles were placed in notches on the top edge of a frame of acrylic resin which was designed for preparation of straight roots (Tanimoto 1988). The frames were placed in air-tight boxes ($20 \times 30 \times 10$ cm) that contained sufficient distilled water to reach the bottom edge of the filter paper in the frame. The boxes were incubated at 23°C in darkness for 2 days to obtain straight roots of 20 ± 5 mm in length for use in this study.

Hydroponic culture and measurements of growth—Twenty seedlings were supported on a stainless-steel net (5-mm mesh), fixed in a polystyrene cup (150 ml). A standard growth solution containing inorganic nutrients was used, as described elsewhere (Tanimoto 1988). The roots were dipped in a solution of GA_3 and/or Anc that contained inorganic nutrients (Fig. 1A). The elongation growth of root and shoots was measured after incubation under the laboratory fluorescent lights at 23°C unless otherwise indicated.

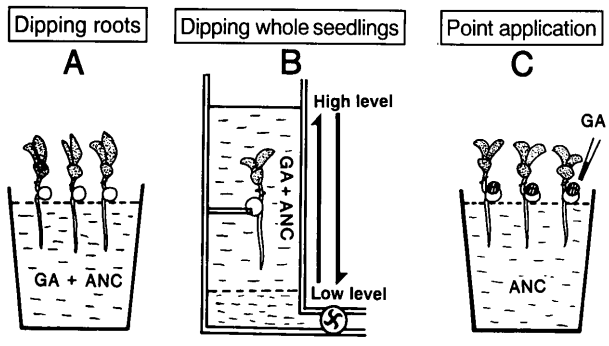

Fig. 1 The three methods used for application of GA_3. (A) GA_3 was applied to roots by dipping roots in a hydroponic solution. (B) GA_3 was applied to whole seedlings by dipping each entire seedling in hydroponic solution. (C) GA_3 was applied to a point between cotyledons at which a piece of absorbent cotton had been inserted.

Application of GA_3 by the dipping method—Roots of seedlings were pretreated for 3 days with 10 μM Anc by hydroponic culture to decrease the endogenous growth and then they were transferred to the measuring boxes of the rhizometer as shown in Figure 1B (Tanimoto 1988, Tanimoto and Watanabe 1986). The initial lengths of roots and shoots were recorded and the seedlings were held vertically in the box with a continuous supply of humid air. Influx into the box of the standard growth solution, which contained 3 μM Anc with or without GA_3, was controlled by a microcomputer-linked pump such that whole seedlings were submerged. After bathing the seedlings, with aeration, for 15 min the solution was drained. It took 5 min for influx and draining of the growth solution. The seedlings were then kept in a continuous stream of humid air for 10 min. This cycle was repeated every 30 min for 4 days by a computer-controlled system designed specifically for the rhizometer so that each entire seedling was dipped in a solution of GA_3 192 times during a 4-day treatment.

Application of GA_3 to cotyledons—The initial lengths of seedling roots were recorded and a small ball of absorbent cotton was inserted between cotyledons after the seed coat had been partially removed (Fig. 1C). Roots were dipped in the standard growth solution with or without 30 μM Anc. Fifty microliters of an aqueous solution of GA_3 were applied daily to the cotton ball that had been placed between cotyledons. Elongation of roots and shoots was measured after 9 days.

Measurement of osmotic concentration—Osmotic concentrations of root segments and buds of young seedlings were measured by a vapor pressure method (Miyamoto and Kamisaka 1988b). Groups of thirty root segments and buds were excised from seedlings that had been pretreated with 10 μM Anc with or without 0.1 μM GA_3. Root segments were cut at 4-mm intervals with 1-mm root tips removed. Separate parts of root segments and buds were collected in air-tight vials and frozen at −25°C. Frozen segments were transferred to a funnel with a stainless-steel net (140 mesh) fixed to the bottom of the funnel. The funnel was centrifuged in a vial at 4°C for 10 min at $1,000 \times g$. Eight-microliter portions of the filtrate were subjected to analysis in a vapor pressure osmometer (model 5100 C ; Wescor; Logan, UT, U.S.A.). The measurement was repeated 3–5 times for each filtrate and means were calculated. Free-space liquid was collected directly from unfrozen fresh sections by the same centrifugation procedure. The volume of free-space liquid in each part of the root was less than 5% of the total liquid obtained from frozen and thawed seg-

ments. Therefore, the osmotic concentration of the total liquid obtained from frozen and thawed segments was taken as the osmotic concentration in root cells.

Growth response of root segments to acid solution—Apical 10-mm segments were excised from roots of seedlings that had been pretreated with 10 μM Anc with or without 0.1 μM GA_3. Sixteen segments, with 2-mm apical tips removed, were set on the measuring box of the rhizometer and elongation was monitored at 10-min intervals in growth solutions with different pH values, as described elsewhere (Tanimoto and Watanabe 1986, Tanimoto et al. 1989).

Measurement of cell-wall extensibility in vivo—Apical 10-mm segments of roots, with 2-mm apical tips removed, were excised from seedlings that had been pretreated with 10 μM Anc with or without 0.1 μM GA_3 for 24 h. These segments were set in the rhizometer and dipped twenty times either in standard growth solution or in 0.4 M mannitol in standard growth solution at pH 7. For each round of treatment, segments were dipped either in hypertonic or hypotonic solution for 5 min and kept in humid air for 1 min. It took 4 min to drain and load the solution. The increase or the decrease in root length was recorded at 10-min intervals by a microcomputer system linked to the rhizometer.

Measurements of cell-wall extensibility with a tensile tester—Apical 10-mm segments of roots were excised from seedlings that had been pretreated with 10 μM Anc with or without 0.1 μM GA_3 for 24 h. The thickness of Anc-treated roots was not significantly different from that of roots treated with GA_3 plus Anc after 24 h. The segments were killed in methanol at 70°C for 5 min, washed twice with methanol and kept in fresh methanol prior to measurements. They were then rehydrated with distilled water and subjected to analysis with a Tensiron RTM-25 tester, an Instron-type tensile tester (Toyo Baldwin; Tokyo, Japan). Root segments were secured between two clamps, leaving a 5-mm distance for extension. Mechanical properties of the cell walls of roots were analyzed by two methods; stress-relaxation analysis after rapid extension at 20 mm min^{-1} (Masuda 1990, Yamamoto et al. 1970); and load-extension analysis with slow extension (0.5 mm min^{-1}) or rapid extension (10 mm min^{-1}). In stress-relaxation analysis, two parameters, T_0 and B, were obtained by the methods described by Yamamoto et al. (1970). In load-extension analysis, extension and shrinkage of the root segments were recorded during loading and releasing of an 8 g load. The typical load-extension curve is shown in Figure 2. Three parameters were obtained from each curve: (A) extension during initial loading from 0.8 g to 8 g; (B) extension during loading with 8 g for 18 s; and (C) shrinkage during reduction of the load from 8 g to 0.8 g. In order to minimize the fluctuation in the initial extension due to slackening of root specimens, the initial extension was read from a 0.8 g load (10% of the full load).

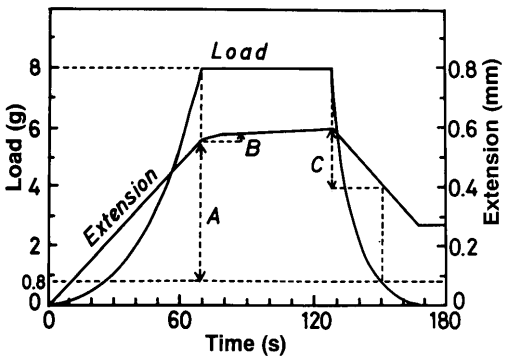

Fig. 2 Typical load-extension curve obtained by slow extension at 0.5 mm min^{-1}. When the load reached 8 g, the extension speed was adjusted to hold the 8 g load for 60 s, and then the root segment was allowed to shrink with the release of the load. Three parameters were determined: A, extension during loading from 0.8 to 8.0 g; B, extension during loading with 8 g for 18 s; C, shrinkage during reduction of the load from 8.0 to 0.8 g.

Observations of root cells by scanning electron microscopy (SEM)—Morphological observations were made by SEM of the apical parts of roots that had been treated with 10 μM Anc with or without 0.1 μM GA_3 for 6 days. Root segments were cut consecutively at intervals of 1 or 2 mm from the tip to the base of the roots. Excised segments were fixed with glutaraldehyde (3.5% in 50 mM phosphate buffer, pH 7.0), post-fixed with osmium tetroxide (1% in the same buffer) and dehydrated in an ethanol series. Ethanol was replaced by isoamylalcohol and specimens were dried by replacement with liquid CO_2 in a critical point dryer (CP-2; Hitachikouki; Katsuta, Ibaraki, Japan). Specimens were coated with gold and observed under a scanning electron microscope (JSM-T100; JOEL Ltd., Tokyo, Japan). The number of cortical cells and the diameters of root and stele were measured on cross sections of root segments.

Results

Regulation of growth by Anc and GA_3—The effects of Anc and GA_3 on the elongation growth of roots and shoots were compared in darkness and in the light. Elongation of roots was significantly reduced by Anc both in the light (Fig. 3A) and in darkness (Fig. 3C). GA_3 at two concentrations (10 nM and 10 μM) prevented the Anc-induced reduction in root elongation when the concentration of Anc was below 30 μM. Shoot elongation was slightly reduced by Anc in the light (Fig. 3B) both in the presence of 10 nM GA_3 and in its absence. By contrast, in the presence of 10 μM GA_3 elongation was greatly enhanced as compared to that of control plants that had not been exposed to any other growth regulator. The inhibition of shoot elongation by Anc was more significant in darkness (Fig. 3D) and the inhibition was not prevented by 10 nM GA_3. GA_3 at 10

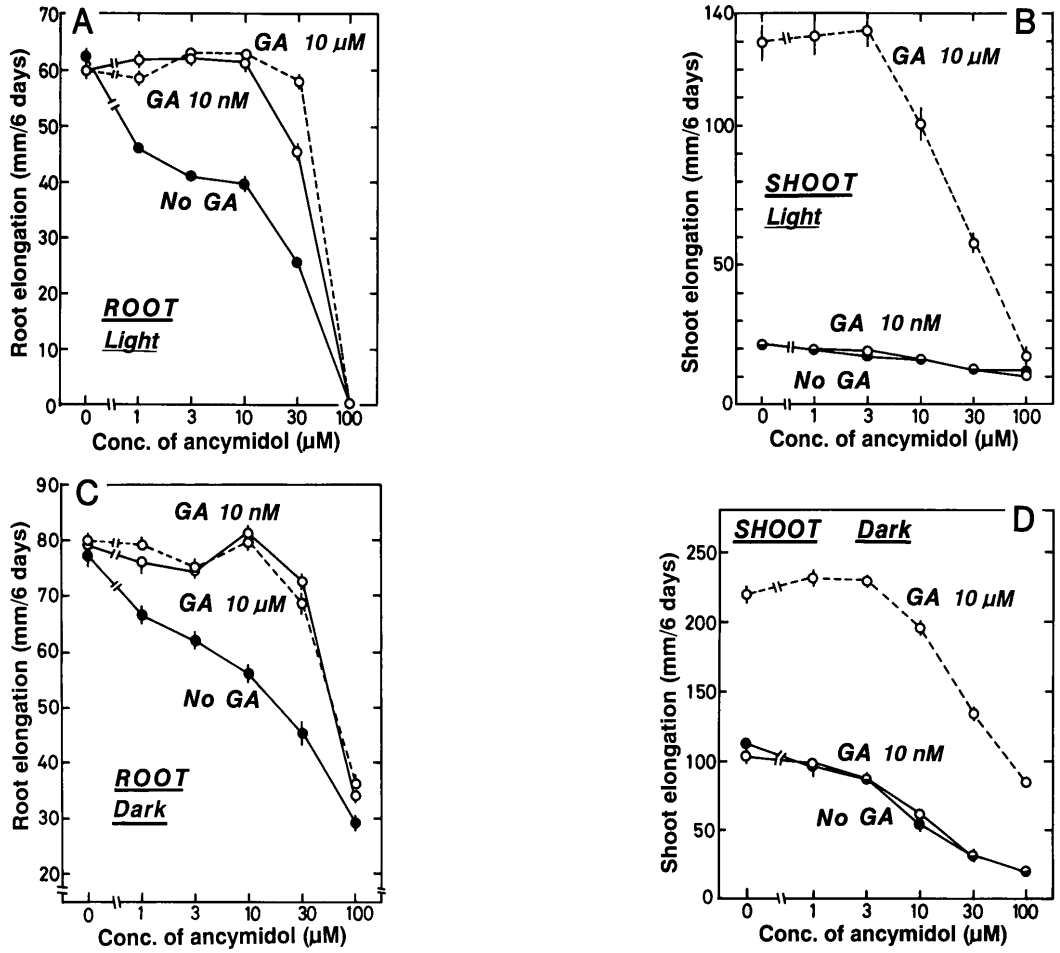

Fig. 3 Concentration-dependent inhibition by Anc and promotion by GA_3 of elongation of roots (A and C) and shoots (B and D) in the light (A and B) and in darkness (C and D). The growth regulators were applied to roots by dipping roots in hydroponic solution. Means of 20–30 seedlings with standard errors (vertical bars) are shown.

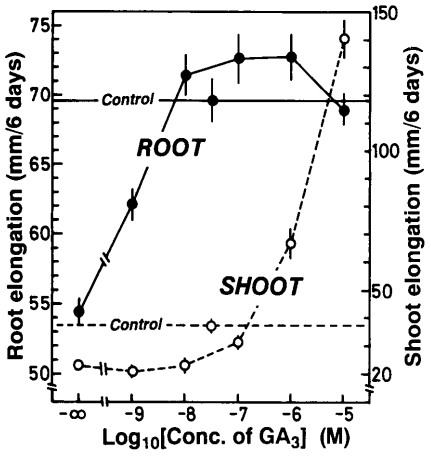

Fig. 4 Concentration-dependent promotion of elongation of roots and shoots by GA_3. GA_3 was applied to roots together with 10 μM Anc in hydroponic solution. Means of 20–30 seedlings with standard errors (vertical bars) are shown.

μM strongly promoted shoot elongation in darkness also (Fig. 3D).

The concentration-dependent promotion of growth by GA_3 is illustrated in Figure 4. The growth of roots and shoots of control plants without application of Anc or GA_3 are indicated by a horizontal line in each case. The inhibition by Anc of root elongation was completely overcome by 10 nM GA_3 and growth was maximal at 100 nM GA_3. By contrast, shoot elongation was only promoted by GA_3 at higher concentrations. These results suggested that the roots required less GA_3 than the shoots for normal elongation growth. However, these results may have been due to another mechanism whereby, at low concentrations, GA_3 was preferentially consumed by the root and did not reach the shoot when GA_3 was applied to the root. The following results indicate that this possibility can be ignored.

Table 1 shows the effects of GA_3 at two concentrations when it was applied equally to roots and shoots by dipping whole seedlings into a solution of GA_3 plus 3 μM Anc in

Table 1 Effects of the concentration of GA_3 on the elongation of roots and shoots, as determined by the dipping method

Concentration of GA_3 (μM)	Elongation after 4 days (mm)	
	Roots	Shoots
0	17.2±1.5	10.8±0.7
0.01	33.6±2.0	11.2±0.5
1.0	33.1±2.6	28.1±1.8

Seedling roots were pretreated with 10 μM Anc for 2 days; root and shoot lengths were recorded and then whole seedlings were repeatedly dipped in a solution of GA_3 that contained 3 μM Anc for 4 days. Means of 10–12 plants with standard errors are shown.

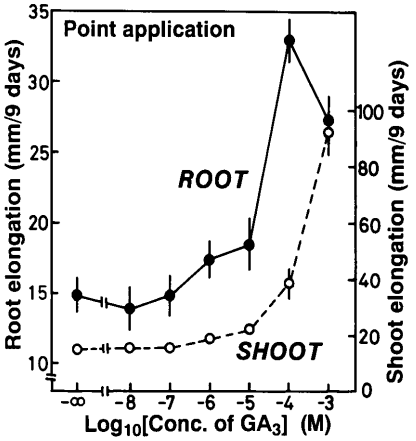

Fig. 5 Dose-response curves for elongation of roots and shoots promoted by cotyledon-applied GA_3. Fifty microliters of a solution of GA_3 were applied daily to a piece of absorbent cotton inserted between cotyledons, while 10 μM Anc was applied to roots in hydroponic solution. Means of 15–20 seedlings with standard errors (vertical bars) are shown.

the rhizometer. At a low concentration (10 nM), GA_3 restored the root elongation that had been suppressed by Anc but failed to overcome the latter's effect on shoot elongation even after dipping 192-times in a solution of GA_3 during a 4-day treatment. At a higher concentration (1 μM), GA_3 reversed the suppression of both root and shoot elongation by Anc.

In the next experiment, GA_3 was applied to cotyledons only to examine whether cotyledon-applied GA_3 would have any effect on elongation of roots and shoots. GA_3 applied to the cotyledons restored root elongation at lower concentrations than those that restored shoot elongation (Fig. 5). Recovery of root elongation was saturated at 100 μM GA_3 whereas recovery of shoot elongation was not.

In the following experiments, Anc-treated roots and (Anc+GA)-treated roots were compared with each other since the elongation growth and morphological appearance of GA_3-treated roots and (Anc+GA_3)-treated roots was similar to that of control roots that were not treated with any growth regulator.

Osmotic concentration—Osmotic concentrations (OCs) of consecutive root segments were measured (Table 2). GA_3 did not increase the OC, in any parts of seedlings during 6-h or 24-h treatments. By contrast, GA_3 decreased OC in the apical part of the root during 3- and 5-day treatments.

Acid-enhanced elongation—An interaction between effects of GA_3 and Anc was also observed in the acid-enhanced elongation growth of root segments (Fig. 6). When the roots of seedlings had been treated with Anc with or without GA_3 for 26 h, GA_3-treated roots showed rapid elongation upon acid treatment, whereas Anc-treated roots failed to respond to acid solution. The same effect was ob-

Table 2 Effects of GA_3 on the osmotic concentration of cell sap obtained from four parts of roots and buds

Treatment period		Osmotic concentration (mmol liter^{-1})				
		Parts of roots (distance from tip, mm)				Buds
		1–4	4–8	8–12	12–	
6 h	Anc	329	339	319	384	456
	Anc+GA_3	328 (100%)	336 (99)	332 (104)	387 (101)	437 (96)
1 d	Anc	310	311	317	353	458
	Anc+GA_3	317 (102%)	319 (103)	334 (105)	344 (97)	462 (101)
3 d	Anc	373	317	326	341	401
	Anc+GA_3	309 (82%)	297 (94)	321 (98)	376 (110)	420 (105)
5 d	Anc	366	359	448	311	432
	Anc+GA_3	305 (83%)	361 (101)	368 (82)	295 (95)	439 (102)

The effects of GA_3 over Anc-treatment are shown in parentheses. Means of 3–5 determinations for groups of 30 root segments are shown. Standard errors were less than 2% of means.

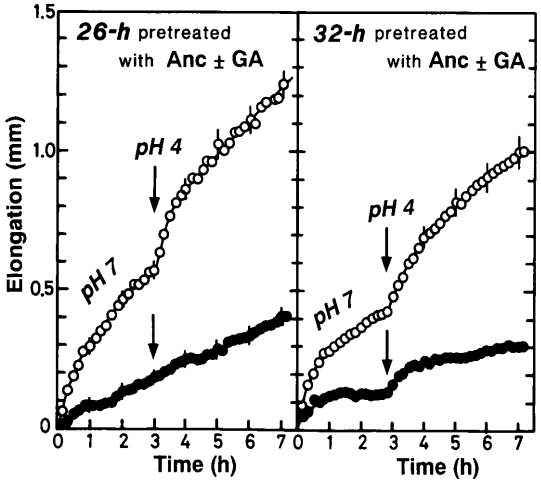

Fig. 6 Acid-induced elongation of segments excised from roots of seedlings that had been pretreated with 10 μM Anc (closed circles) or with 10 μM Anc+0.1 μM GA_3 (open circles) for 26 h (left) and 32 h (right). The elongation of excised segments was determined at 10-min intervals in the rhizometer. Segments were dipped in the standard hydroponic solution at pH 7 for 3 h and then in the solution at pH 4 at the time indicated by arrows.

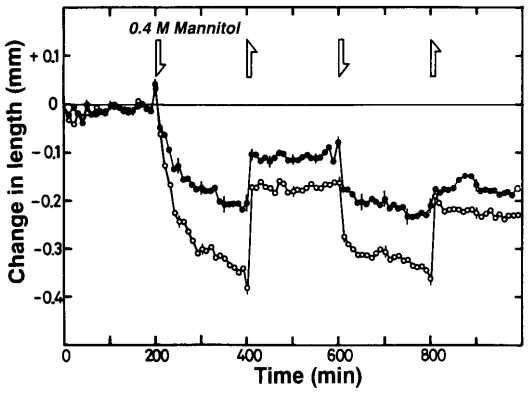

Fig. 7 Decreases and increases in lengths of root segments in hypertonic and hypotonic solution. Roots of seedlings were pretreated with 10 μM Anc (closed circles) or with 10 μM Anc+0.1 μM GA_3 (open circles) for 24 h and root segments were then excised. The segments were set in the rhizometer (Tanimoto and Watanabe 1986) and dipped either in standard hydroponic solution at pH 7 with (downward arrows) or without (upward arrows) 0.4 M mannitol. During each treatment, segments were dipped either in hypotonic or hypertonic solution for 5 min and kept in humid air for 1 min. Four min were required for influx and draining of the solution. Changes in segment lengths were recorded at 10-min intervals for 15 h. Means of 10–12 roots with standard errors (vertical bars) are shown.

served in roots of seedlings that had been treated for 32 h with Anc with or without GA_3.

Cell-wall extensibility in vivo—Cell-wall extensibility of roots was measured by the osmotic method. Root segments shrank quickly in hypertonic solution and elongated in hypotonic solution. Greater elongation and greater shrinkage were observed with GA_3-treated roots than with Anc-treated roots (Fig. 7).

Cell-wall extensibility in vitro—Cell-wall extensibility

Table 3 Effects of GA_3 on the cell-wall extensibility, as measured by stress-relaxation analysis and load-extension analysis with the Tensiron tensile tester in vitro

Stress-relaxation analysis Extension speed (mm min^{-1})		T_0	B
20	Anc	25.0±0.8	4.84±0.09
	GA_3+Anc	25.1±0.9	4.50±0.11
	(Effect of GA_3)	(100%)	(93%)	

Load-extension analysis		Extension (μm)		
Extension speed (mm min^{-1})		A[a]	B[a]	C[a]
0.5	Anc	376±19	23±1.6	−222±11
	GA_3+Anc	519±21	29±1.6	−314±10
	(Effect of GA_3)	(138%)	(126%)	(141%)
10	Anc	608±36	96±5	−466±46
	GA_3+Anc	658±50	87±6	−488±46
	(Effect of GA_3)	(108%)	(90%)	(105%)

Seedling roots were treated for 24 h with 10 μM Anc with or without 0.1 μM GA_3. Root segments were excised, methanol-killed, rehydrated and extended in the tester. Means of 20–30 segments with standard errors are shown.

[a] Extension values shown in Fig. 2.

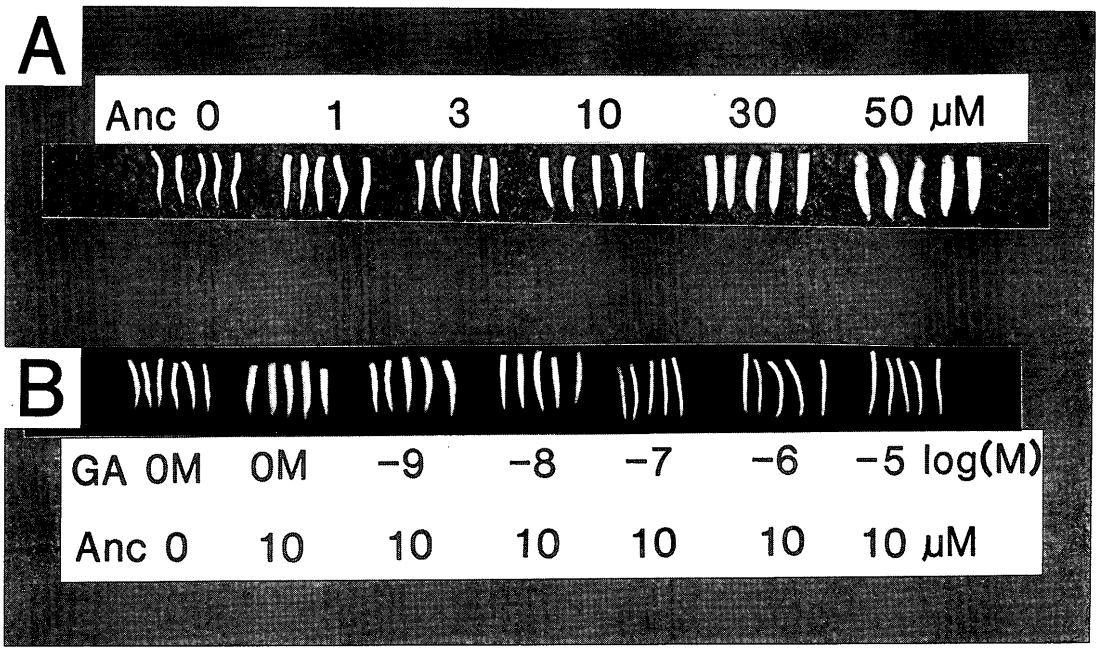

Fig. 8 Photographs of apical 10-mm segments of seedling roots that had been treated with or without Anc and GA_3 for 6 days.

of methanol-killed root segments was measured by both stress-relaxation analysis and load extension analysis (Table 3). GA_3 did not affect the parameters of stress-relaxation analysis to the extent that they were analyzed under the experimental conditions established by Yamamoto et al. (1970). In this analysis, root segments were quickly extended at a speed of 20 mm min^{-1} to give a 10 g load and then the relaxation process was analyzed to obtain two parameters, T_0 and B. The cell-wall extensibility was also analyzed by the load-extension method, in which root segments were stretched more slowly than in stress-relaxation analysis. When root segments were slowly extended at 0.5 mm

Table 4 Effects of GA_3 on lateral expansion and on the number of cortical cells per cross section of roots in the presence of Anc

	Parts of roots (distance from tip, mm)			
	3–4	5–6	7–8	9–10
Diameter of root (mm)				
Anc+GA_3	0.95±0.03 (100%)	0.94±0.02 (100%)	1.09±0.03 (100%)	1.10±0.03 (100%)
Anc	1.90±0.07 (200%)	1.97±0.05 (210%)	2.20±0.08 (202%)	2.11±0.21 (192%)
Diameter of stele (mm)				
Anc+GA_3	0.36±0.01 (100%)	0.35±0.02 (100%)	0.40±0.02 (100%)	0.39±0.01 (100%)
Anc	0.50±0.02 (139%)	0.51±0.02 (146%)	0.58±0.03 (145%)	0.57±0.01 (146%)
Number of cortical cells				
Anc+GA_3	584±18 (100%)	590±21 (100%)	586±17 (100%)	604±14 (100%)
Anc	1,072±37 (184%)	1,005±44 (170%)	1,072±35 (183%)	1,004±25 (166%)

The diameters of roots and steles and the numbers of cortical cells were measured on every other 1-mm sections excised from 3–10 mm above the root tip of seedling roots that had been treated with 10 μM Anc or with 10 μM Anc+0.1 μM GA_3 for 6 days. Means of 8–12 roots with S.E. are shown.

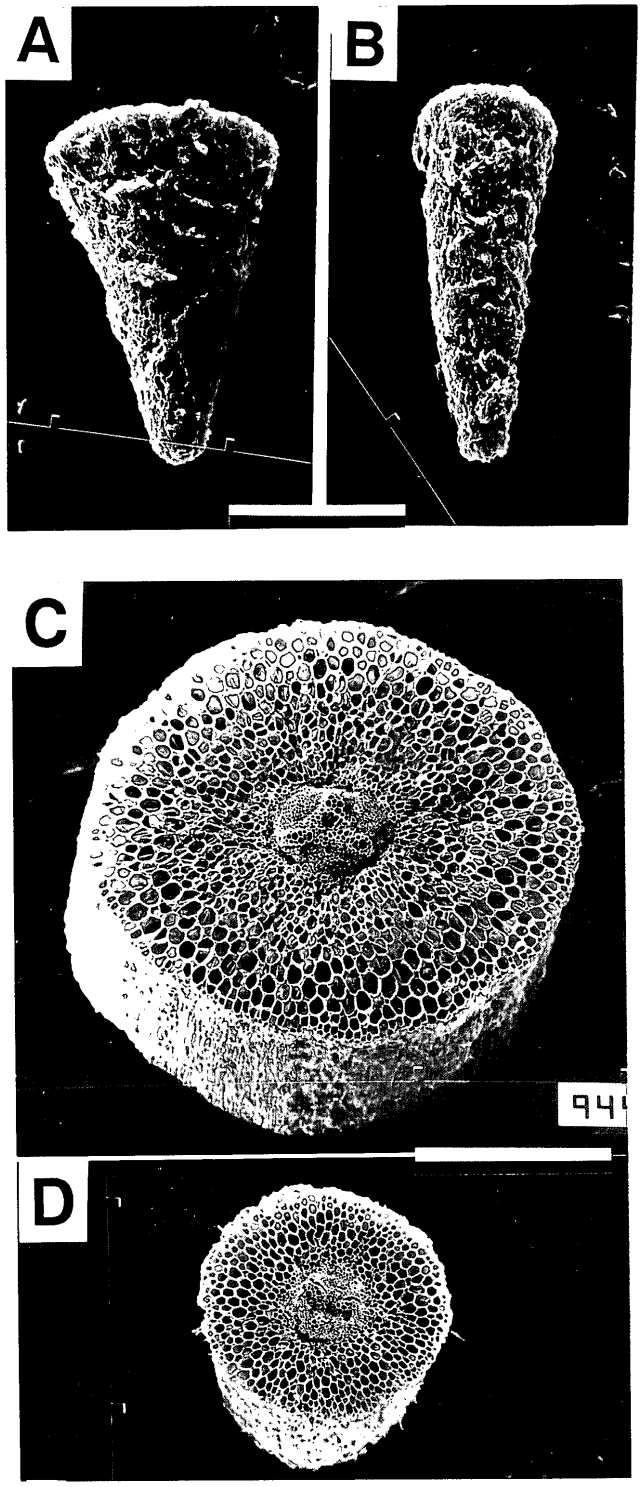

Fig. 9 A and B: Scanning electron micrographs of apical 2-mm segments of seedling roots that had been treated with 10 μM Anc (A) or with 10 μM Anc+0.1 μM GA_3 (B) for 6 days. C and D: Scanning electron micrographs of cross sections 9–10 mm above the root tip of seedling roots that had been treated with 10 μM Anc (C) or with 10 μM Anc+0.1 μM GA_3 (D) for 6 days. Scale bars=1 mm.

min^{-1}, GA_3-treated roots showed greater extensibility (parameter A) than Anc-treated roots. The extension during the application of constant load (parameter B), after an initial slow extension, was also increased by treatment with GA_3. The total shrinkage of GA_3-treated roots after the removal of the load (parameter C) was also greater than that of Anc-treated roots. Conversely, no effect of GA_3 was found on these three parameters when the root segments were stretched more rapidly, namely, at 10 mm min^{-1}.

Morphological changes—Treatment with Anc led to increased thickening of the elongation zone of the roots as the concentration of Anc was increased. The effect of Anc at 10 μM was completely overcome by increasing the concentration of GA_3 (Fig. 8). The thickening was observed in the elongation zone after a two-day treatment with Anc at concentrations above 10 μM. Since GA_3-treated roots and (Anc+GA_3)-treated roots were morphologically identical to control roots, the morphology of cross sections of roots was compared between Anc-treated roots (10 μM) and (Anc+GA_3)-treated roots (0.1 μM GA_3) as shown in Figure 9 and Table 4. After a 6-day treatment, the diameter of Anc-treated roots was twice that of (Anc+GA_3)-treated roots. The numbers of cortical cells per cross section in these segments were nearly constant throughout 3- to 10-mm sections above the root tip after each treatment. There were more cortical cells in Anc-treated roots (166–184%) than in (Anc+GA_3)-treated roots (Table 4). When the cross-sectional area of the cortical region was calculated from the diameters of roots in Table 4, that in Anc-treated roots was found to be four times that in (Anc+GA_3)-treated roots, while the stele area in Anc-treated roots was twice that in (Anc+GA_3)-treated roots. Thus, the Anc-induced expansion of roots was mainly brought about by the expansion of cortical cells, but it was also due in part to an increase in the number of cortical cells.

Discussion

Externally applied GA_3 does not promote the elongation of roots in lettuce and pea (Tanimoto 1987, 1988), whereas it does enhance the elongation of shoots. Low concentrations of GA_3 promote root elongation while root elongation is suppressed by Anc. The elongation of shoots of gibberellin-sensitive dwarf peas is strongly enhanced by application of GA_3, whereas the elongation of roots is unaffected by exogenous GA_3 (Tanimoto 1990). The results of the present study are compatible with those of previous studies and show that GA_3 promotes root elongation in dwarf pea when root growth is suppressed by Anc. The results suggest that a low concentration of gibberellin plays a role in the elongation of roots of both normal and dwarf peas and may explain why the roots of gibberellin-sensitive dwarf pea plants are not stunted.

Although, in the present study, both shoots and roots

elongated more rapidly in darkness than in the light, the dose-response relationships for treatment of roots with GA_3 and Anc were the same in darkness and in the light. Thus, the promotion of root growth by GA_3 found in the present experiments was light-independent. Comparison of the effectiveness of GA_3 application at three sites (roots, cotyledons and whole plants) suggests that the requirement of shoot growth for GA_3 is greater than that of root growth. Root-applied GA_3 had a several-hundred-fold greater effect on root growth than on shoot growth (Fig. 4). Cotyledon-applied GA_3 had a ten-fold greater effect on roots than on shoots (Fig. 5). In a previous study (Tanimoto 1990), GA_3 applied only to shoots promoted root elongation over the same range of concentrations as those that promoted shoot elongation. All these dose-response relationships are compatible with the hypothesis that roots require less gibberellin for normal elongation growth than do shoots. The difference between saturation concentrations of GA_3 for roots and shoots also supports this hypothesis (Fig. 4, 5). However, the translocation and concentration of the applied GA_3 and the concentration of GA_3 at its site of action at the cellular level must be determined before we can draw any firm conclusions.

Promotion of shoot growth by GA_3 appears to involve two mechanisms: regulation of the orientation of cellulose microfibrils and regulation of the osmotic concentration in elongating cells (Katsumi et al. 1980, Miyamoto and Kamisaka 1988a, b). GA_3 had little effect on the osmotic concentration of root cells. Thus, regulation of the osmotic concentration may not be the major action of GA_3 in the promotion of root growth, at least under the present experimental conditions.

Anc led to thickening of the root in the elongation zone while GA_3 applied together with Anc prevented the effect of Anc and roots remained slender. Anc-induced thickening of the roots was mainly brought about by lateral expansion of cortical cells and was partially due to an increase in the number of cells in the cortex.

The interactions of the effects of gibberellin and a growth retardant have also been examined cytologically in roots of *gib-1* dwarf tomato and *d-5* dwarf maize by Barlow et al. (1991) and Baluska et al. (1993). They found that cortical cells in the roots of GA-deficient dwarf mutants or of paclobutrazol-treated wild-type plants elongated more slowly and reached a shorter final length than those of the untreated wild type or gibberellin-treated counterparts. Their findings are compatible with the present results for dose-response relationships in root elongation and the morphological effects of gibberellin on dwarf pea roots.

An increase in cell-wall extensibility in response to GA_3 was demonstrated by the osmotic method. The results shown in Figure 7 indicate that GA_3-treated roots incur greater shrinkage and extension than Anc-treated roots. Furthermore, they indicate that GA_3 makes cell walls in roots more extensible than those in Anc-treated roots since the osmotic concentration of root cells in the elongation zone was not increased by GA_3. Cell-wall extensibility, as measured with a tensile tester, indicated that GA_3 makes cell walls of roots more extensible. The effect of GA_3 on the extensibility was detected when the root segments were extended at 0.5 mm min^{-1} but it was not found when the speed of extension was 10 mm min^{-1}. A higher speed of extension (20 mm min^{-1}) has been used to measure effects of auxin on the mechanical properties of cell walls in stems and coleoptiles by stress-relaxation analysis (Yamamoto and Masuda1971, Yamamoto et al. 1970). In stress-relaxation analysis, the relaxation of the cell wall is analyzed after the cell wall has been rapidly extended to bear a certain load. Auxin has been shown to decrease the initial relaxation time (T_0) of oat coleoptiles and pea stems (Yamamoto and Masuda 1971, Tanimoto and Masuda 1971). In the present study, GA_3 had little effect on the extensibility of root cell walls, as analyzed by the same stress-relaxation method (Table 3). The effect of GA_3 on the extensibility was only found when the cell walls were stretched slowly in load-extension analysis. Thus, it seems that the biochemical mechanism of GA_3-enhanced extensibility of root cell walls is different from that of the auxin-enhanced extensibility of stem cell walls. In stress-relaxation analysis, the thickness of specimens does not affect T_0 but it may affect the extension in load-extension analysis. However, GA_3-induced changes in cell-wall extensibility (parameter A in Table 3) may possibly reflect the properties of the cell wall since the thickness of Anc-treated roots was not significantly different from that of (Anc+GA_3)-treated roots after 24 h. Possible biochemical changes in root cell walls induced by GA_3 are under investigation.

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