Plant Physiol. (1996) 110: 933–943

Gravitropic Response of Inflorescence Stems in *Arabidopsis thaliana*[1]

Hidehiro Fukaki, Hisao Fujisawa, and Masao Tasaka*
Department of Botany, Faculty of Science, Kyoto University, Kyoto 606–01, Japan

We have characterized the gravitropic response of inflorescence stems in *Arabidopsis thaliana.* When the inflorescence stems were placed horizontally, they curved upward about 90° within 90 min in darkness at 23°C, exhibiting strong negative gravitropism. Decapitated stem segments (without all flowers, flower buds, and apical apices) also showed gravitropic responses when they included the elongation zone. This result indicates that the minimum elements needed for the gravitropic response exist in the decapitated inflorescence stem segments. At least the 3-min gravistimulation time was sufficient to induce the initial curvature at 23°C after a lag time of about 30 min. In the gravitropic response of inflorescence stems, (a) the gravity perception site exists through the elongating zone, (b) auxin is involved in this response, (c) the gravitropic curvature was inhibited at 4°C but at least the gravity perception step could occur, and (d) two curvatures could be induced in sequence at 23°C by two opposite directional horizontal gravistimulations at 4°C.

Gravitropism is the growth response whereby a plant orients with respect to the gravity vector. In higher plants shoots show negative gravitropism (upward curvature) and roots show positive gravitropism (downward curvature). The gravitropic response mechanism can be separated into three sequential steps: gravity perception, signal transduction, and asymmetric growth response by differential cell elongation (reviewed by Feldmann, 1985; Pickard, 1985; Roberts and Gilbert, 1992; Poff et al., 1994; Kaufman et al., 1995). Many physiological and cytological studies of these three steps in both shoot and root gravitropism have been performed for more than 180 years, using many different plants. These studies have demonstrated that the amyloplast is involved in the gravity perception step (Iversen, 1969; Heathcote, 1981; Moore and Evans, 1986; reviewed by Sack, 1991) and that auxin (Gillespire and Thimann, 1963; Iwami and Masuda, 1976; Bandurski et al., 1984; Hatfield and LaMotte, 1984; Harrison and Pickard, 1989; Migliaccio and Rayle, 1989; Parker and Briggs, 1990; Young et al., 1990), calcium (Slocum and Roux, 1983; Poovaiah et al., 1987), calmodulin (Stinemetz et al., 1987), and protein phosphorylation (Friedmann and Poovaiah, 1991) are involved in the gravitropic response pathway. Especially the formation of an asymmetric auxin distribution at sites of action is thought to be important for leading the asymmetric growth response, which causes the gravitropic curvature (Gillespire and Thimann, 1963; Iwami and Masuda, 1976; Bandurski et al., 1984; Harrison and Pickard, 1989; Migliaccio and Rayle, 1989; Parker and Briggs, 1990; Young et al., 1990; reviewed by Evans, 1991). In addition, to understand the role of auxin in the gravitropic response, the expression patterns of several auxin-regulated genes within gravistimulated organs have been studied in shoots of tobacco and *A. thaliana* (Li et al., 1991; Wyatt et al., 1993; Hagen, 1995).

Because the molecular mechanisms of the gravitropic responses are still unknown, we attempted to identify genes involved in gravitropism. Many root gravitropic mutants and some shoot gravitropic mutants have been isolated from several plants (for reviews, see Roberts and Gilbert, 1992; Okada and Shimura, 1994). In *Arabidopsis thaliana,* at least eight genetic loci involved in root gravitropism have been identified (*aux1, axr1, axr2, dwf, agr1, cop4,* reviewed by Okada and Shimura, 1994; *axr4,* Hobbie and Estelle, 1995; *eir1,* Roman et al., 1995; *rgr1,* Simmons et al., 1995). However, few genetic loci related to shoot gravitropism are known (unnamed, Bullen et al., 1990; *axr2,* Wilson et al., 1990; *cop4,* Hou et al., 1993; *phyB,* Liscum and Hangarter, 1993). In shoots of *A. thaliana,* both inflorescence stems (flowering stalks) and hypocotyls show negative gravitropism. Although several genetic or physiological studies of hypocotyl gravitropism have been reported in this plant (Khurana et al., 1989; Bullen et al., 1990; Liscum and Hangarter, 1993), to our knowledge no detailed physiological analysis of inflorescence stem gravitropism has been reported, except for the observation that the horizontally gravistimulated inflorescence stems of wild type (Columbia ecotype) achieved 60° curvature in 80 min (Caspar and Pickard, 1989), and no trial to isolate mutants deficient in inflorescence stem gravitropism has been performed.

To understand the molecular mechanisms of shoot gravitropism, especially inflorescence stem gravitropism, we have begun genetic and physiological analyses using *A. thaliana* (Fukaki et al., 1996). Here, as a first step, we describe the basic characteristics of the gravitropic responses of inflorescence stems. Our results indicate that the inflorescence stem of this organism has the common characteristics of the gravitropic responses as observed in other plant shoots and that it is a suitable material for studies of shoot gravitropism in higher plants.

---

[1] This work was supported by Grants-in-Aid Nos. 04804055, 06278209, 07640859, 07270212, U-24, and U-49 from the Ministry of Education, Science and Culture of Japan.

* Corresponding author; e-mail tasaka@fuji.bot.kyoto-u.ac.jp; fax 81–75–753–4122.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana (L.) Heynh, Columbia ecotype, was used. All plants were grown in a 1:1 mixture of perlite and vermiculite in vinyl pots at the density of one individual/4 cm^2 with Arabidopsis mineral nutrient before germination and with one-quarter-strength solution after germination, under constant white light at 23°C in air-conditioned rooms or incubators. The Arabidopsis mineral nutrient solution contained 5 mM KNO_3, 2 mM $MgSO_4$, 2 mM $Ca(NO_3)_2$, 2.5 mM KPO_4, adjusted to pH 5.5 by mixing with 2.5 mM K_2HPO_4 and 2.5 mM KH_2PO_4, 50 μM Fe-EDTA, 70 μM H_3BO_3, 14 μM $MnCl_2$, 0.5 μM $CuSO_4$, 1 μM $ZnSO_4$, 0.2 μM $NaMoO_4$, 10 μM NaCl, and 0.01 μM $CoCl_2$. White light of 40 approximately 120 μmol m^{-2} s^{-1} from a 1:1 mixture of white fluorescent tubes (FL20SS·D; Toshiba, Tokyo, Japan) and fluorescent tubes for plant growth that are enriched in PAR (FL20·SPG; National, Tokyo, Japan) was used for the plant growth.

Measurement of Growth Profiles of Inflorescence Stems

Points were marked in black ink on the distal 4-cm part of primary inflorescence stems (4–8 cm long) at 5 mm and the stem regions were designated 1 to 8 in descending order from the apical part to the basal part of inflorescence stems. The elongation of each part during 24 h at 23°C in light was measured.

Preparation of Stem Segments for Gravitropic Response Assays

Distal 4-cm sections of primary inflorescence stems with total lengths between 4 and 8 cm were used for gravitropic response assays. As shown in Figure 1, the distal 4-cm parts were cut from the primary inflorescence stems and their basal ends were inserted into silicone tubes (1.0 or 1.5 mm i.d., 10 mm long) fixed on acrylic boards that were set in clear plastic boxes so that the stems stood in a vertical position. They were pre-incubated for 12 to 15 h in white light at 23°C with one-quarter-strength Arabidopsis mineral nutrient solution. Three types of inflorescence stem segments were prepared from them (Fig. 1): undecapitated 4-cm parts of primary inflorescence stems (type 1 stem segment); undecapitated 4-cm parts (type 2 stem segment), prepared from the type 1 stem segments by removing all of the lateral shoots; and decapitated 3.5-cm stem segments (type 3 stem segment), prepared from the type 2 stem segments by removing the apical 5-mm region (including all flowers, flower buds, and shoot apices). The basal 5-mm parts of these stem segments were put into gellan gum blocks (8 × 8 × 8 mm, 1.0% [w/v] gellan gum [a special agar for plant culture, Wako Pure Chemical Industries, Ltd., Osaka, Japan] containing one-quarter-strength Arabidopsis mineral nutrient) that were fixed on one face of plastic plates (14.0 × 10.0 × 1.5 cm) so that the stem segments could be in the vertical position (five segments/plate). To keep high humidity in the plates, a wet paper towel was attached in the plate (Fig. 1).

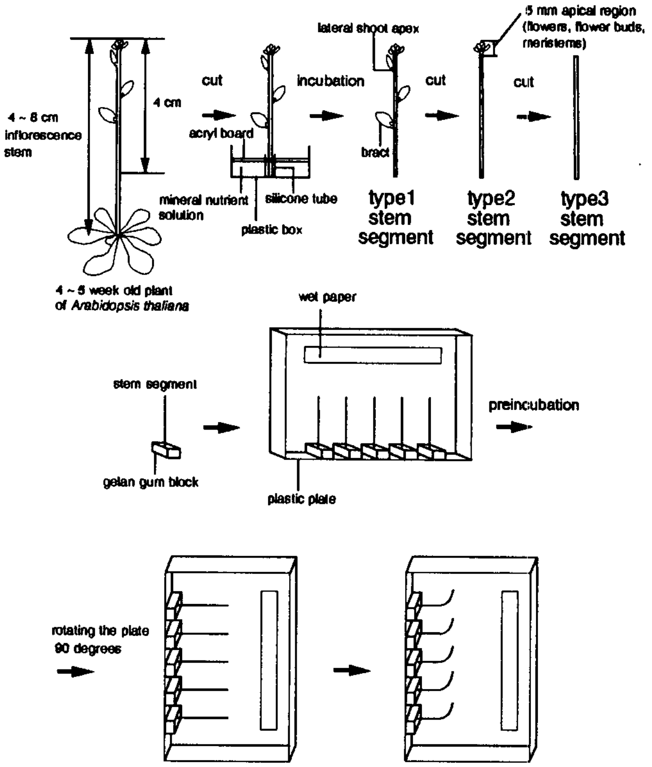


Figure 1. Diagram of preparation and treatment of stem segments for gravitropic response assay. The distal 4-cm sections of the primary inflorescence stems with total lengths between 4 and 8 cm were used for gravitropic response assay. Three types of inflorescence stem segments (types 1, 2, and 3) were prepared from the distal 4-cm sections, and they were set in the plastic plates and gravistimulated horizontally as described in "Materials and Methods."

Gravitropic Response Assays

After the stem segments were incubated in a vertical orientation for 3 h under white light at 23°C in the plates, gravistimulation was begun by rotating the plates through 90° in darkness at 23°C as shown in Figure 1. In assays at low temperature, the stem segments were pre-incubated in the vertical position for 2 h under white light at 23°C and for 1 h in darkness at 4°C before gravistimulation. In the case of auxin addition, auxin was given to the apical ends of the decapitated type 3 stem segments by immersing the stem tip into 10^{-5} M IAA solution for 30 s through a Pasteur pipette just before the gravistimulation. All of the responses were performed in darkness except when the photographs were taken under white light (about 10-s illumination of 10 μmol m^{-2} s^{-1} white light from the white fluorescent tubes). The curvature of stems (stem segments or intact inflorescence stems) was measured as follows. Angles of the growing directions of the free ends of stems between 0 min (when the horizontal gravistimulation was initiated) and subsequent times (usually every 15 min) were measured on the image of negatives enlarged by a projector. In assays at low temperature, angles of the growing directions of stem segments were measured between the time when stems were shifted from 23 to 4°C (1 h before the gravistimulation) and each response time at 23°C.

RESULTS

Gravitropic Responses of Inflorescence Stems of *A. thaliana*

Inflorescence stems of *A. thaliana* Columbia ecotype began to elongate upright after making the rosette leaves (3–4 weeks after germination) (Fig. 2A). When plants were placed horizontally by rotating the pots (Fig. 2B), the inflorescence stems curved about 90° upward in 90 min in darkness at 23°C, exhibiting a strong negative gravitropic response (Fig. 2C). To characterize this response more quantitatively and more easily, we developed an assay system for the gravitropic response of inflorescence stems. To obtain equivalent samples for the assay, the elongation patterns of inflorescence stems of different lengths were examined.

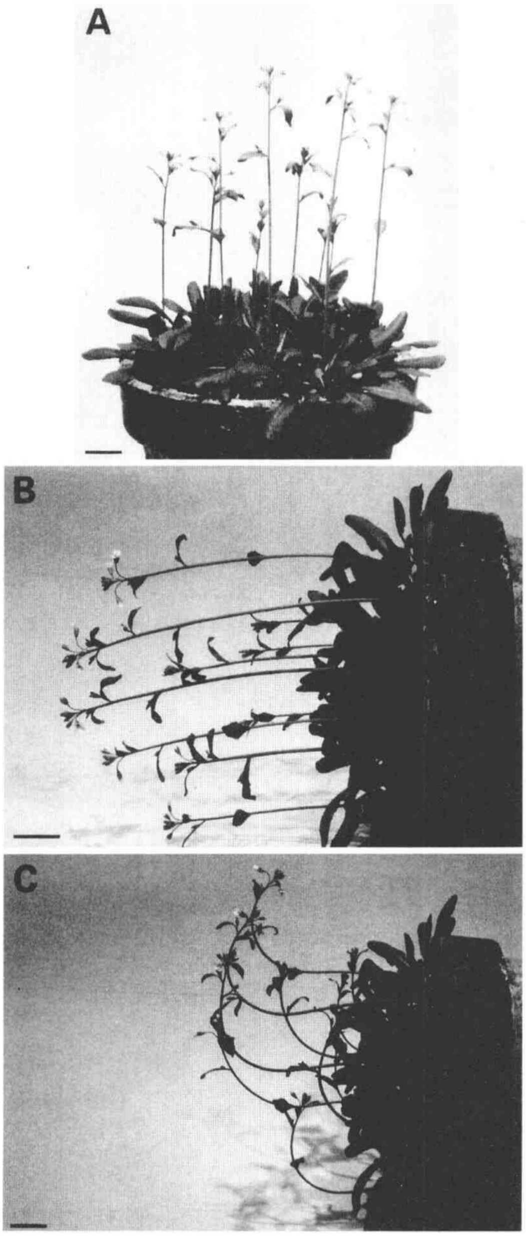


Figure 2. Gravitropic responses of inflorescence stems. A, The inflorescence stems of 4-week-old plants. B, The horizontally gravistimulated inflorescence stems at the start of gravistimulation. C, The horizontally gravistimulated inflorescence stems 90 min after B. The gravistimulation was given in darkness at 23°C. The arrow indicates the direction of gravity (g). Bar = 1 cm.

As shown in Figure 3A, when the total lengths of the primary inflorescence stems were from 4 to 8 cm, the elongation patterns of the distal 4-cm regions were almost the same under our experimental conditions. In this stage, the inflorescence stem consists mainly of three zones: an apical zone, an elongation zone, and a zone that has completed elongation. The apical zone includes the inflorescence meristem, the floral meristems, the flower buds, and the opened flowers. Below the inflorescence meristem, new cells were continuously produced by cell division and differentiated into several tissues (epidermis, cortex, vascular bundle, etc.). This zone was usually within region 1 (the apical 5-mm region; Fig. 3B). The elongation zone includes a part of region 1 and regions 2 to 7, in which region 2 is the most rapidly elongating region (Fig. 3A). The nonelongating zone includes region 8 and the basal part below this region. This zone did not participate in the gravitropic curvature (see below). Furthermore, at this developmental stage, little variation was seen between individuals with a few opened flowers, and there was little elongation of the lateral shoots. Thus, for the assays, we mainly used the distal 4-cm parts of the primary inflorescence stems with total lengths between 4 and 8 cm.

In our system, three kinds of stem segments (types 1, 2, and 3) prepared from inflorescence stems could be incubated by inserting their basal sides into agar blocks in plastic plates, and they could be gravistimulated by rotating the plates through 90° ("Materials and Methods," Fig. 1). The gravitropic responses of three stem segments and intact inflorescence stems attached to the plant were examined. Figure 4 shows the time course for the change in angle of these stem tips in response to the horizontal gravistimulation. When they were placed horizontally, all three kinds of stem segments showed basically similar gravitropic responses as the intact inflorescence stems (Fig. 4). First, all three segments as well as the intact inflorescence stems began to curve upward within 30 min. Second, even after the total curvature reached 90°, it continued to increase and the stems exhibited a "overshooting" shape (Fig. 4). Third, the tip of the overshooting stems reversed its curvature, and finally the growing direction of the stem tip became nearly 90° at 6 h (Fig. 4).

However, there were some quantitative differences among their responses. First, all of the excised stem segments showed a larger curvature than the intact inflorescence stems. These differences may reflect some effects of removal from the intact plants or the incubation in the plate. Second, both type 1 and type 2 stem segments reached the maximum curvature earlier than type 3 stem segments and the reversal of the overshooting by type 1 and type 2 stem segments was also faster than that of type 3 stem segments. These quantitative differences may reflect effects of removal of the apex and/or the lateral shoots. These results indicate that the gravitropic response of inflorescence stems does not require their apical region (including inflorescence meristems and floral meristems, flowers, flower buds) or their lateral shoots, leaves, and roots. Hence, the minimum elements needed for the gravitropic response exist in the decapitated 3.5-cm type 3 stem segments. However, the apical region plays some role when present (Fig. 4), and the other organs may contribute to the gravitropic response. Nevertheless, the gravitropic response of decapitated type 3 stem segments was fundamentally similar to that of the other two stem segment types and intact stems. Thus, to analyze the response of the stem segments as simply as possible, the decapitated type 3 stem segments were mainly used for the subsequent assays.

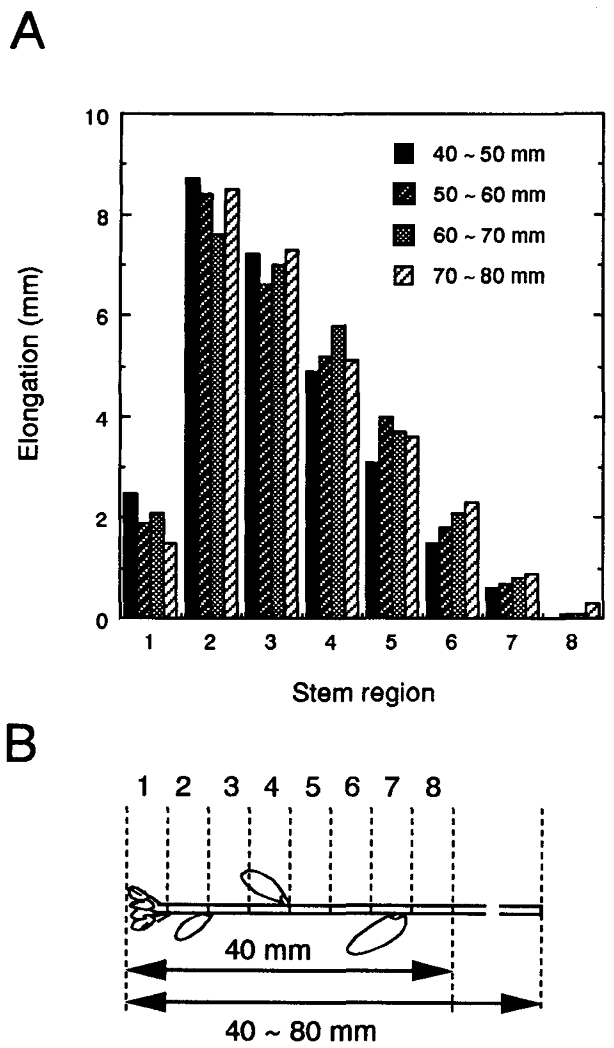


Figure 3. Growth profiles of inflorescence stems. A, The elongation of each stem region. The distal 4-cm part of the primary inflorescence stems of different lengths were each marked with black ink at 5 mm and incubated at 23°C in white light; the lengths between each mark were measured 24 h later. Each box indicates the initial length of inflorescence stems. Means of measurements of more than four individuals in each group are shown. B, The distal 4-cm part of the primary inflorescence stems (4–8 cm long). The stem region numbers (1–8) were given to each 5-mm region from the apical part to the basal part in the distal 4-cm parts of inflorescence stems.

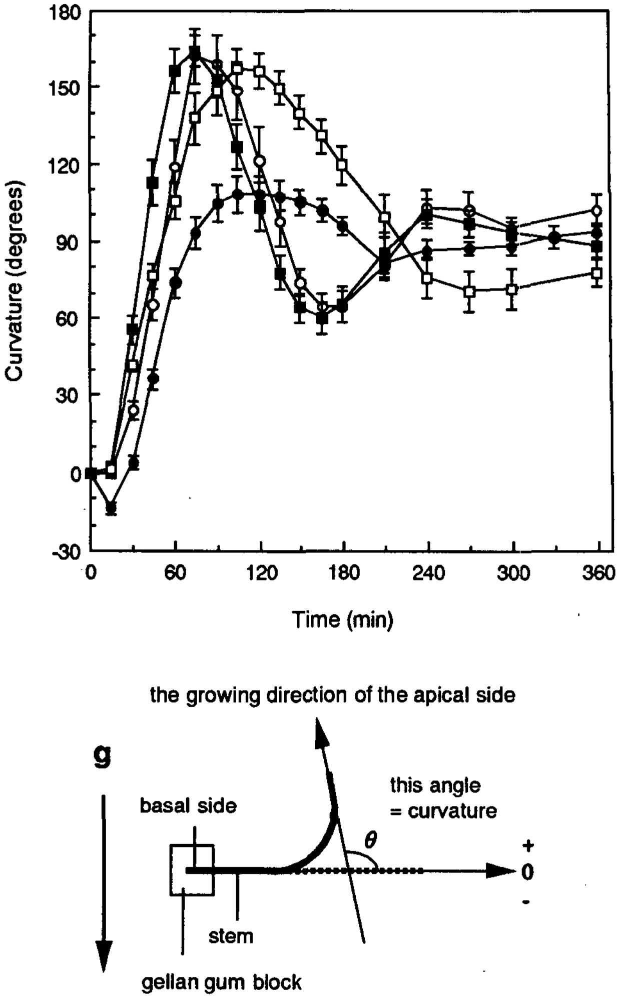


Figure 4. Time course of the curvature of the intact inflorescence stems attached to the plants and the three kinds of stem segments. The intact primary inflorescence stems attached to the plants were horizontally gravistimulated as shown in Figure 2. Three types of stem segments were prepared and horizontally gravistimulated in darkness at 23°C as described in "Materials and Methods." The time courses for the change in angle of the tip of these stem or stem segments are shown. ●, Intact inflorescence stems; ○, undecapitated type 1 stem segments; ■, undecapitated type 2 stem segments; and □, decapitated type 3 stem segments. The vertical error bars represent the SE values. More than eight stems or segments were examined. The drawing below the graph shows the angle measured as the curvature of stems or segments. g, Gravity.

We examined the gravitropic response of type 3 stem segments in more detail. Figure 5 shows the representative gravitropic response of type 3 stem segments, and Figure 6 shows the time course of curvature development for each stem region. When the stem segments were gravistimulated horizontally, they began to curve upward within 30 min at the most rapidly elongating regions (regions 2–4) of the inflorescence stem (Figs. 4,

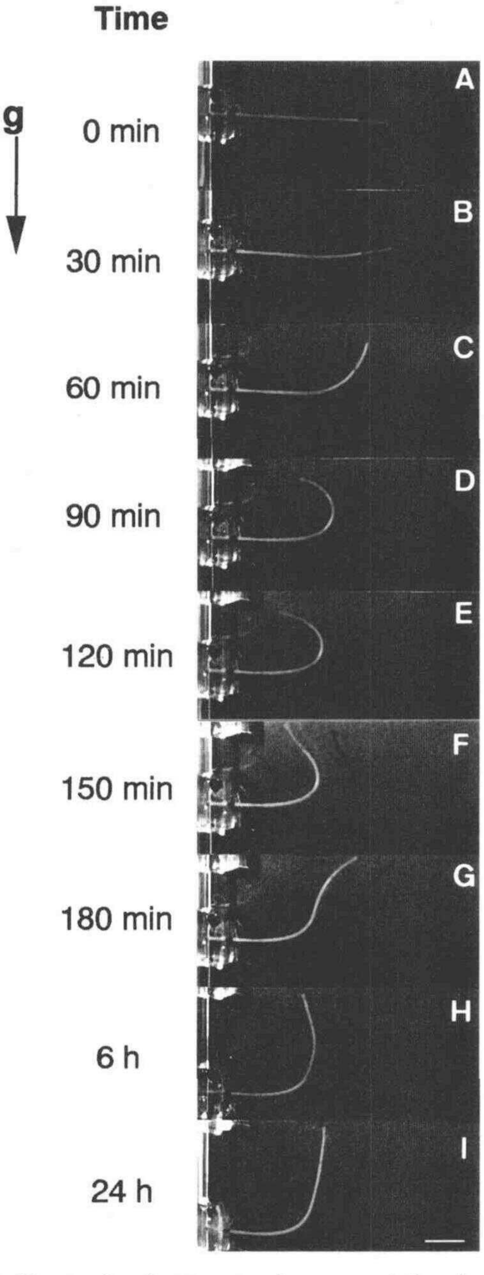


Figure 5. The gravitropic response of a representative decapitated stem segment. The decapitated type 3 stem segment was prepared from the undecapitated 4-cm section of the primary inflorescence stem and it was horizontally gravistimulated in darkness at 23°C as described in "Materials and Methods." Photographs of the same stem segment throughout time are shown. The gravistimulation times are shown beside the photographs. The arrow indicates the direction of gravity. Bar = 1 cm.

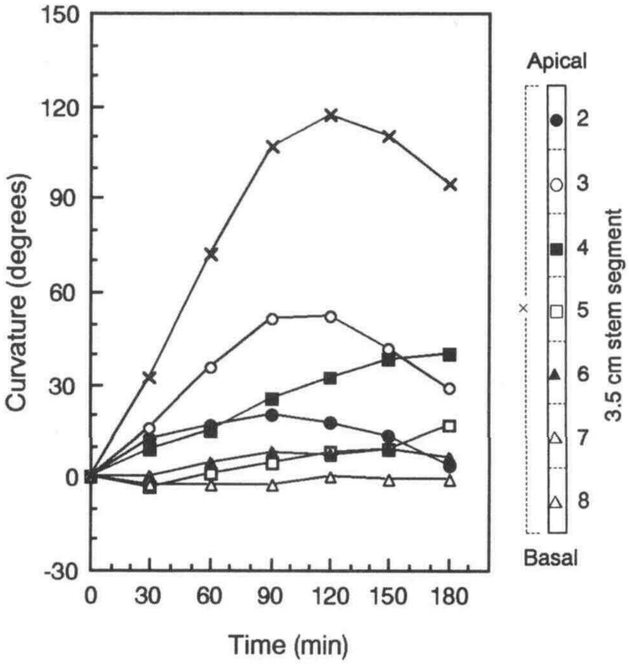


Figure 6. Time course of change of curvatures of each stem region. The decapitated type 3 stem segment was prepared and horizontally gravistimulated in darkness at 23°C. Curvature of each stem region was measured as described in Figure 4. ●, Region 2; ○, region 3; ■, region 4; □, region 5; ▲, region 6; △, regions 7 plus 8; ×, total (regions 2–8). One stem segment was examined.

5B, and 6). Total curvature reached 90° within 90 min (Figs. 4 and 5D) and continued to increase until reaching almost 150° at 2 h (Figs. 4 and 5E). On the other hand, the more basal regions (regions 5 and 6), which were not curving at 30 min, began to curve at 2 h (Figs. 5E and 6). At 2.5 h, the tips of the stem segments began to reverse their curvatures (Fig. 5F). After that, the apical parts passed back through 90° and the stem segments appeared to be S shaped (Fig. 5G). Once again they curved upward at 6 h (Fig. 5H). Finally, when the stem segments stopped these oscillations, the growing direction of the tips was nearly 90° and the curvature was limited to the region that elongates slightly (Fig. 5I).

To determine the location of the gravity perception site, stem segments were divided into two stem segments and the gravitropic responses of both segments were examined. When the decapitated type 3 stem segments were cut between regions 3 and 4 or between regions 4 and 5, the apical segments curved upward strongly and the basal

Table I. *Gravitropic responses of divided stem segments*

Decapitated type 3 stem segments were cut between the stem regions 3 and 4 or regions 4 and 5. The divided stem segments (apical segment [regions 2 and 3, regions 2–4], basal segment [regions 4–8, regions 4–8 treated with 10^{-5} M IAA, regions 5–8]) were horizontally gravistimulated at 23°C in darkness. Curvatures at 90 min for each stem segment and the curvature at 180 min for basal segments (regions 5–8) were measured. Means ± SE are shown. Ten individuals were examined in each treatment. The region numbers are as described in Figure 3.

Region	Curvature (degrees)
Region 2 + 3	62.6 ± 3.4
Region 4 + 5 + 6 + 7 + 8	22.9 ± 7.7
Region 4 + 5 + 6 + 7 + 8 + 10^{-5} M IAA	34.8 ± 7.1
Region 2 + 3 + 4	104.2 ± 8.1
Region 5 + 6 + 7 + 8	−0.4 ± 0.4 (6.6 ± 3.3 at 180 min)

segments curved upward slightly (Table I). These results indicate that the gravity perception site is not localized exclusively to the apical part (regions 1 and 2) and suggest that it exists throughout the entire elongation region of an inflorescence stem.

Moreover, when 10^{-5} M IAA was given to the basal stem segments (regions 4–8) that were cut between regions 3 and 4 of decapitated type 3 stem segments, their curvatures were greater (Table I). This result suggests that auxin is involved in the gravitropic response of inflorescence stems of Arabidopsis, as reported for many other plant shoots (Iwami and Masuda, 1974; Hatfield and LaMotte, 1984; Harrison and Pickard, 1989; Migliaccio and Rayle, 1989; Evans, 1991).

As shown in Figure 7, the decapitated type 3 stem segments that were placed in the horizontal position for 1 min, did not exhibit any curvatures after they were returned to the vertical position, but the stem segments that were placed in the horizontal position for 3 min showed the gravitropic curvature at 30 min. Hence, under this experiment condition, the minimum time required for the perception of gravistimulation was less than 3 min and it took about 30 min for the initial gravitropic curvature in the type 3 stem segments.

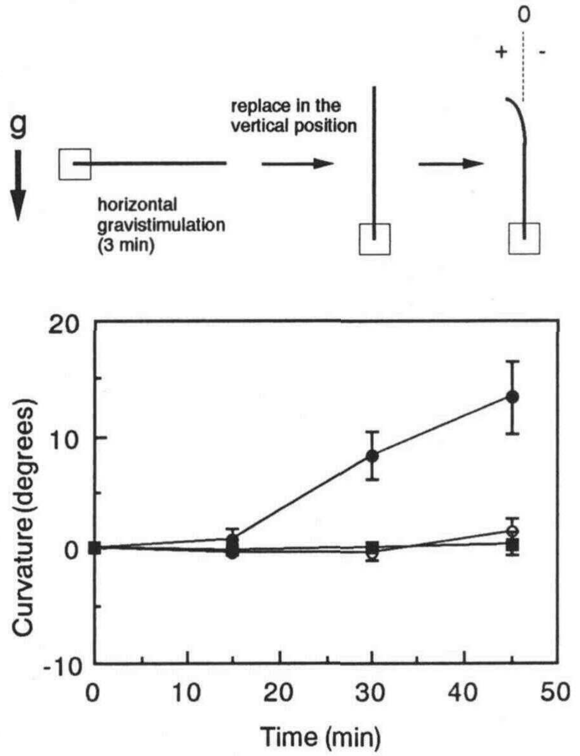


Figure 7. Time course of the gravitropic responses of decapitated stem segments to short gravistimulations. The horizontal gravistimulations were given to the stem segments for 0 min (■), 1 min (○), and 3 min (●) by rotating the plates 90°. After that, the stem segments were replaced in the vertical position. The curvatures shown in the vertical position were measured. The vertical error bars represent the SE values. The horizontal axis represent the time after the start of the gravistimulation. More than 10 stem segments were examined in each treatment. g, Gravity.

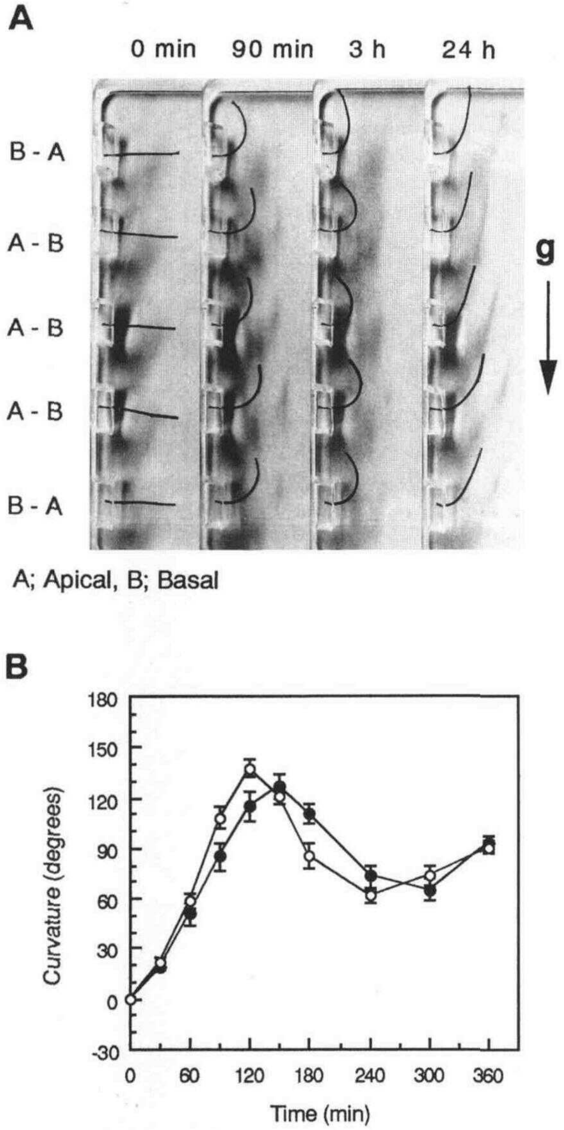


Figure 8. Gravitropic response of decapitated stem segments fixed at the basal or apical ends. After the decapitated 2-cm stem segments (including regions 2–5) were prepared and pre-incubated in the vertical position (all segments were fixed with their basal ends) for 3 h, some of these stem segments were turned upside down and their apical ends were immobilized and these and remaining samples were horizontally gravistimulated in darkness at 23°C. A, Representative gravitropic response of the stem segments fixed at the apical or basal ends (uppermost and lowermost), stem segments fixed at their basal ends (middle three stems), and stem segments fixed at their apical ends. B, Time course for the change in angle of the free ends in the gravitropic response of the stem segments fixed at their apical or basal ends. ●, Stem segments fixed at their apical ends; ○, stem segments fixed at their basal ends. The vertical bars represent the SE values. Ten stem segments were examined in each treatment. g, Gravity.

It is well known that there is a polarity in a stem related to a gradient in age from the younger apical part to the older basal part. As mentioned above, when the basal ends of stem segments were fixed and the stem segments were placed horizontally, the apical part of stem segments curved upward. If shoot gravitropism is the phenomenon that the younger apical part grows upward, the direction of the apical-basal polarity of the inflorescence stems may be strongly related to the direction of gravity in the gravitropic response of inflorescence stems. To examine this, the apical ends of decapitated stem segments were fixed and placed in a horizontal orientation, and the change in angle of free basal ends of the stem segments was observed. The result is shown in Figure 8. The free basal side oriented upward as rapidly and as much as the control, free apical end (Fig. 8A). The tip of the basal end curved more than 90°, reversed its curvature in the same way as the tip of the control segment, and finally stood upright (Fig. 8). This result indicates that the direction of apical-basal polarity of the stem need not be identical with the direction of the gravity vector.

Responses to Gravistimulations at Low Temperature

To examine whether inflorescence stems showed gravitropic responses at low temperature, the decapitated type 3 stem segments were gravistimulated at 4°C. As shown in Figure 9A, the gravistimulation for 3 h at 4°C did not cause any curvature. However, when the stem segments were subsequently placed in the vertical position at 23°C, they curved soon after the temperature shift, depending on the horizontal gravistimulation at 4°C. After that, the tip of the stem segment began to reverse after 240 min (Fig. 9A). Significant curvature at 23°C was induced by 5 min of gravistimulation at 4°C and the curvatures became larger, depending on the gravistimulation time at 4°C (Fig. 9B). These results indicate that the gravity perception step can occur at 4°C but that a part of the gravitropic response is sensitive to low temperature. In other words, the consequence of gravistimulation that was perceived at 4°C remained stored in the inflorescence stems.

To examine how long the memory of gravistimulation remained in inflorescence stems at 4°C, the decapitated type 3 stem segments were gravistimulated horizontally for 30 min at 4°C, additionally incubated in the vertical position at 4°C for different times, and then returned to the vertical position at 23°C. As shown in Figure 10, the stem segments incubated for 30 or 60 min in the vertical position at 4°C, following gravistimulation at 4°C, also curved soon after the temperature shift to 23°C. This result indicates that the gravistimulation perceived at 4°C was stored at that temperature for at least 60 min. However, both the curvature at 23°C and the total time needed to achieve the maximum response became smaller, depending on the incubation time in the vertical position at 4°C. These results suggest that the treatment of the vertical position at 4°C either might be also perceived as a new gravistimulation or might reflect loss of a stored signal. In either case, it might decrease the curvature caused by horizontal gravistimulation.

To examine the response of the stem segments to two independent gravistimulations at 4°C, two opposite directional horizontal gravistimulations were given successively for 30 min each at 4°C. The type 3 stem segments showed unexpected but very interesting responses after the tem-

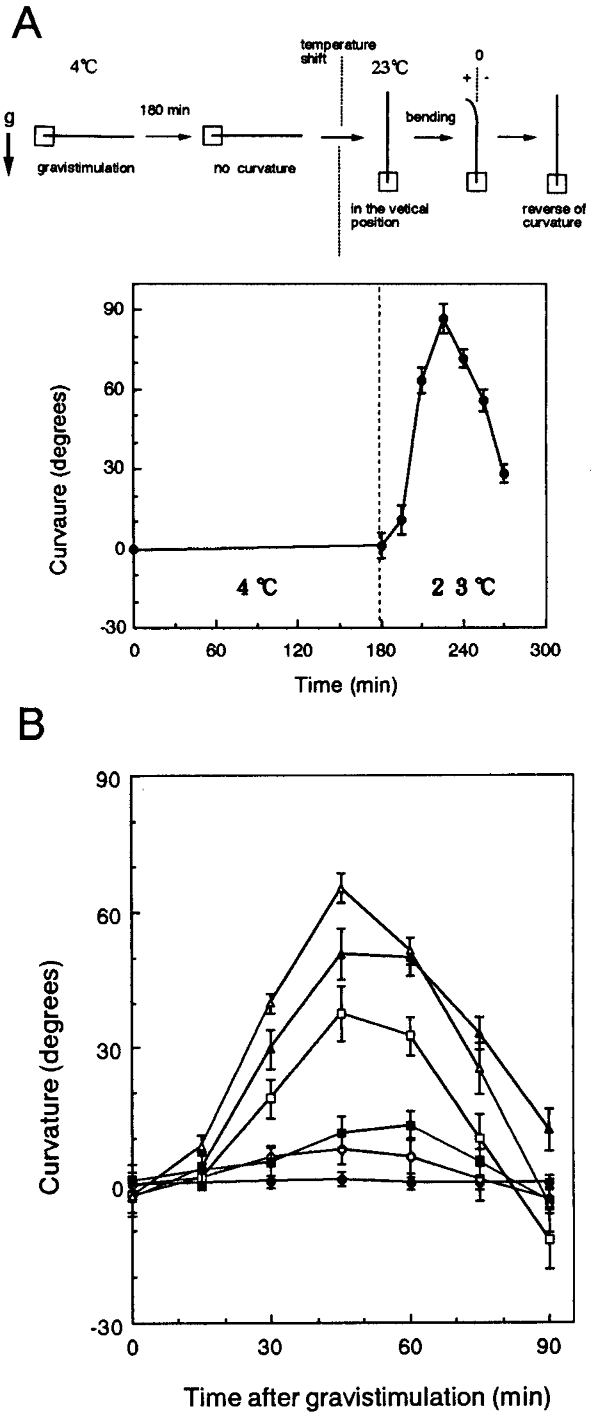


Figure 9. Gravitropic response of decapitated stem segments to gravistimulation at 4°C. Decapitated type 3 stem segments prepared as described in Figure 4 were pre-incubated for 1 h in darkness at 4°C, and then horizontal gravistimulation was given in darkness at 4°C. After each gravistimulation, the stem segments were shifted to 23°C in darkness and were returned to the vertical position. A, Response to 3 h of gravistimulation at 4°C. B, Responses to several gravistimulations at 4°C. The transverse axis represents the time after the stems were reoriented vertically at 23°C. The gravistimulation times were 0 min (●), 5 min (○), 10 min (■), 20 min (□), 60 min (▲), and 90 min (△). The vertical bars represent the SE values. Nine or 10 individuals were examined in each treatment. g, Gravity.

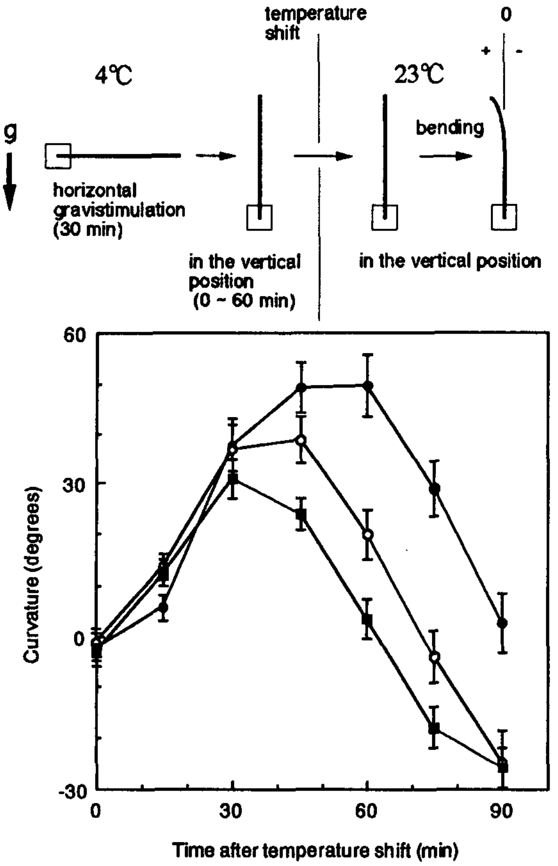


Figure 10. Effect of the vertical gravistimulation at 4°C after the horizontal gravistimulation at 4°C. Decapitated type 3 stem segments were prepared and handled as described in Figure 9. The stem segments were horizontally gravistimulated for 30 min at 4°C and were then incubated in a vertical position at 4°C for 0 min (●), 30 min (○), and 60 min (■) before the temperature shift to 23°C. The transverse axis represents the time after the temperature shift. The vertical bars represent the SE values. Ten individuals were examined in each treatment. g, Gravity.

perature shift to 23°C (Fig. 11A). At first, they curved slightly in response to the first gravistimulation, then they reversed the curvature back toward a vertical orientation, and then they curved in response to the second gravistimulation (toward the opposite direction). This two-step response suggested that the inflorescence stems did not respond once to a sum of two gravistimulations but they could show two independent responses in turn to two gravistimulations. However, because the stem segments were placed in the vertical position at 23°C, the possibility could not be excluded that the second curvature was merely the response to the new gravistimulation that was induced by the first curvature. To test this possibility, the stem segments were placed in the upside down vertical position after the temperature shift to 23°C. In this incubation condition, stem segments that were gravistimulated once at 4°C never induced the reverse bending at 23°C (Fig. 11B). The stem segments that perceived two gravistimulations at 4°C showed the second curvature following the first curvature at 23°C (Fig. 11B). In addition, almost the same stem regions were involved in both curvatures (data not shown). These results indicate that the same regions of inflorescence stems could exhibit two successive curvatures in response to two oppositely oriented horizontal gravistimulations given sequentially, and suggest that the inflorescence stems could perceive and transduce two gravistimulations independently and respond to them in turn.

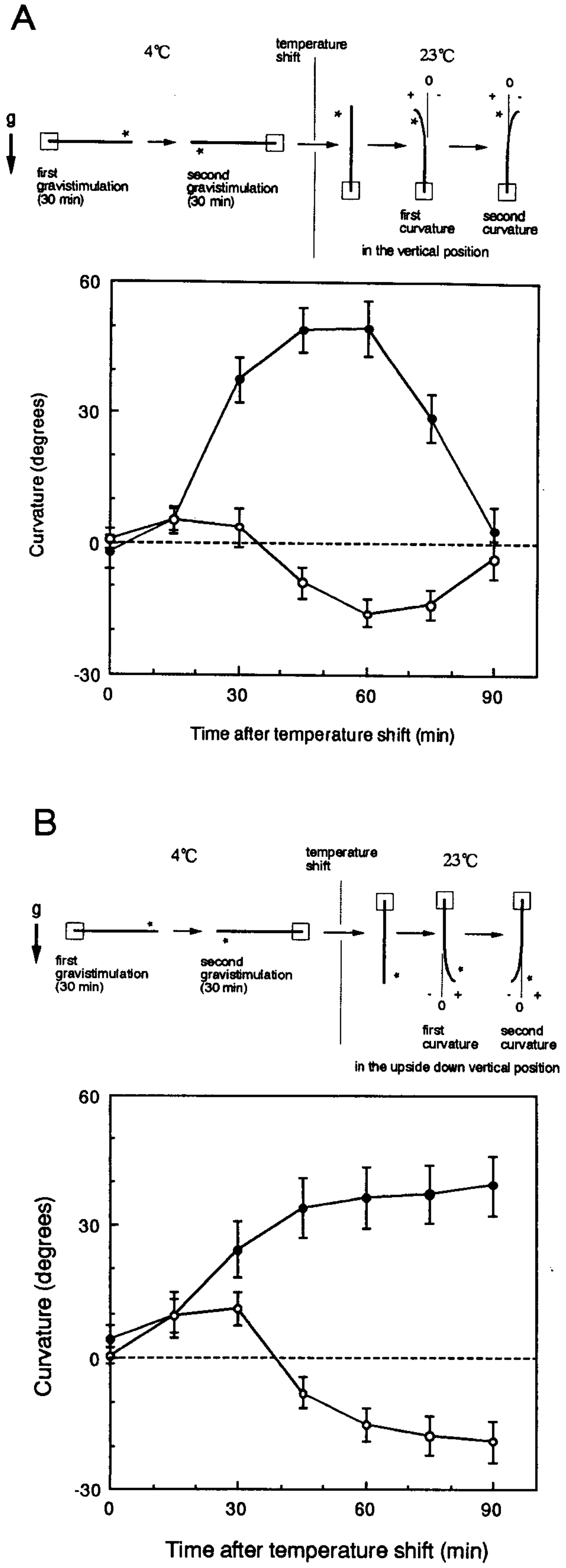
DISCUSSION

Characteristics of the Gravitropic Response of Inflorescence Stems in *A. thaliana*

We have described the basic physiological characteristics of the gravitropic response of inflorescence stems of *A. thaliana*, which exhibited rapid and strong negative gravitropic responses. By using the decapitated stem segments, we have shown that the minimum elements needed for the gravitropic response exist in the decapitated inflorescence stem segments, although the apex is also involved (Figs. 4–6). This characteristic is common in other plant shoots (Iwami and Masuda, 1974; Hart and MacDonald, 1984; Hatfield and LaMotte, 1984). The time course for the gravitropic response of inflorescence stems (Figs. 4–6) and the experimental results using two separated stem segments (Table I) indicate that the gravity perception site is not localized in a specific region of a stem and suggest that it exists throughout the elongation zone of the inflorescence stems in *A. thaliana*. This confirms the hypothesis that the probable sites of gravity sensing in negative gravitropic organs, such as stems, are more spread out along the axis than in roots (Sack, 1991).

The gravistimulated stem segments initiated curvature in the actively elongating regions (regions 2–4), and the basal regions (regions 5 and 6) began curving slightly later (Fig. 6), a consequence of which is that the apical parts overshot the vertical (Figs. 4 and 5). Thereafter, the apical parts reversed the curvature to orient upright but again passed through the vertical. After the tip direction changed several times across 90°, the apical end came to rest (Figs. 4 and 5). Similar movements to reverse of curvature are frequently observed in other plant organs, e.g. hypocotyls of cucumber seedlings (Cosgrove, 1990), coleoptiles of maize seedlings (Bandurski et al., 1984), and roots of maize (Ishikawa et al., 1991).

The stem segments of the basal part (regions 4–8) curved more weakly than the full stem segments (regions 1–8) but its curvature was increased when auxin was applied (Table I). We also found that the gravitropic curvature of stem segments was inhibited by auxin transport inhibitors, *N*-(1-naphthyl)phthalamic acid or 9-hydroxyfluorene-9-carboxylic acid (data not shown). These results indicate that auxin is involved in the gravitropism of inflorescence stems in Arabidopsis as reported in other plant shoots (Iwami and Masuda, 1976; Hatfield and LaMotte, 1984; Harrison and Pickard, 1989; Migliaccio and Rayle, 1989; Evans, 1991).



In the development of stems, new cells are added to pre-existing cells at the shoot apex and therefore there is a polarity of cells from the apical to the basal part, depending on their age. It is well known that auxin is transported from the apical part toward the basal part following this polarity (Goldsmith, 1977). Whether stem segments were attached at their basal or apical ends prior to being placed horizontally, their free ends curved upward (Fig. 8). Similar responses to this were observed in other plant shoots (Hart and MacDonald, 1984; Kaufman and Dayanandan, 1984). When the basal ends were fixed, the direction of the polarity of stems became the same as the direction of gravity. On the other hand, when the apical ends were fixed, the direction of the polarity of stem became opposite to the direction of gravity (Fig. 8). These results indicate that the direction of the polarity of inflorescence stems need not be identical with the direction of the gravity vector in the gravitropic response.

Gravitropic Responses to Gravistimulation at 4°C

The gravitropic response of the stem segments was inhibited at 4°C, but the stem segments gravistimulated at 4°C curved after they were subsequently placed vertically at 23°C (Fig. 9). This result indicates that there is at least one step in the gravitropic response pathway that is inhibited at 4°C, but the gravistimulation is nevertheless perceived at 4°C. The stem segments were not elongated at 4°C (data not shown). Thus, the stem elongation step could be one step of the gravitropic response pathway inhibited at 4°C. Of course, it is also possible that some signal transduction step or steps are sensitive to low temperature. Brauner and Hager (1958) showed that hypocotyls of sunflower (*Helianthus annuus*) failed to develop curvature to a prolonged gravistimulation at 4°C, whereas they developed marked curvature after they were subsequently placed vertically at 25°C.

Our data suggest that the perceived signal is stored in stems at 4°C for some time. When the stem segments that were gravistimulated horizontally at 4°C were incubated in the vertical position at the same temperature, they showed reduced curvature after the temperature shift to 23°C (Fig. 10). It is probable that this reduction is the

Figure 11. Gravitropic responses to two gravistimulations at 4°C. Decapitated type 3 stem segments were gravistimulated for 30 min in the horizontal position at 4°C (first gravistimulation). Thereafter, some of these stem segments were incubated at 23°C in the vertical position or in an upside down vertical position; the others were additionally incubated for 30 min in the horizontal position oriented opposite from the first gravistimulation at 4°C (second gravistimulation) and then incubated at 23°C in the vertical position or in the upside down vertical position. A, Response in the vertical position at 23°C. B, Response in upside down vertical position at 23°C. Curvatures in response to the first gravistimulation are indicated as positive values. ●, Response to one gravistimulation; ○, response to two gravistimulations. The transverse axis represents the time after the temperature shift. The vertical error bars represent the SE values. Ten individuals were examined in each treatment. g, Gravity.

response to a sum of both the horizontal gravistimulation and the vertical gravistimulation at 4°C. However, the stem segments exhibited two successive curvatures in turn in response to two opposite directional horizontal gravistimulations given at 4°C (Fig. 11). This result indicates that stems do not respond to a simple sum of two independent signals received at 4°C and that these two signals are stored independently at 4°C. When the temperature was shifted to 23°C, the first signal presumably induced the first curvature and soon thereafter the second signal presumably induced the second curvature. This result suggests that the inflorescence stems can perceive more than one gravistimulation in succession and can transduce each signal sequentially. The hypocotyls of sunflower could exhibit two successive curvatures in response to two horizontal gravistimulations given at 4°C (Brauner and Hager, 1958). Although the mechanism of the response to the double gravistimulations is not known, this response could be a useful phenotype to study the adaptation of inflorescence stems to the changes of strength and direction of the gravistimulation.

Our data indicate that inflorescence stems of *A. thaliana* exhibit rapid and strong negative gravitropic responses and they have some characteristics of the gravitropic response in common with other plant shoots. The inflorescence stem will be one of the suitable materials for physiological and genetic studies of shoot gravitropism in higher plants, especially in combination with the use of the auxin-regulated genes (Li et al., 1991; Wyatt et al., 1993; Hagen, 1995) and the mutants with defects in inflorescence stem gravitropism (Fukaki et al., 1996).

ACKNOWLEDGMENT

We wish to thank Dr. W.R. Briggs for critical reading of the manuscript and valuable suggestions.

Received July 21, 1995; accepted November 27, 1995.
Copyright Clearance Center: 0032-0889/96/110/0933/11.

LITERATURE CITED

Bandurski RS, Schulze A, Dayanandan P, Kaufman PB (1984) Response to gravity by *Zea mays* seedlings. Time course of the response. Plant Physiol **74:** 284–288

Brauner L, Hager A (1958) Versuche zur analyse der geotropischen perzeption. I. Planta **51:** 115–147

Bullen BL, Best TR, Greg MM, Barsel SE, Poff KL (1990) A direct screening procedure for gravitropism mutants in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol **93:** 525–531

Caspar T, Pickard BG (1989) Gravitropism in a starchless mutant of *Arabidopsis*. Planta **177:** 185–197

Cosgrove DJ (1990) Rapid, bilateral changes in growth rate and curvature during gravitropism of cucumber hypocotyls: implications for mechanism of growth control. Plant Cell Environ **13:** 227–234

Evans ML (1991) Gravitropism: interaction of sensitivity modulation and effector redistribution. Plant Physiol **95:** 1–5

Feldmann LJ (1985) Root gravitropism. Physiol Plant **65:** 341–344

Friedmann M, Poovaiah BW (1991) Calcium and protein phosphorylation on the transduction of gravity signal in corn roots. Plant Cell Physiol **32:** 299–302

Fukaki H, Fujisawa H, Tasaka M (1996) *SGR1*, *SGR2* and *SGR3*: novel genetic loci involved in shoot gravitropism in *Arabidopsis thaliana*. Plant Physiol **110:** 945–955

Gillespire B, Thimann KV (1963) Transport and distribution of auxin during tropistic response. I. The lateral migration of auxin in geotropism. Plant Physiol **38:** 214–225

Goldsmith MHM (1977) The polar transport of auxin. Annu Rev Plant Physiol **281:** 439–478

Hagen G (1995) The control of gene expression by auxin. *In* PJ Davies, ed, Plant Hormones. Kluwer Academic, Dordrecht, The Netherlands, pp 228–245

Harrison MA, Pickard BG (1989) Auxin asymmetry during gravitropism by tomato hypocotyls. Plant Physiol **89:** 652–657

Hart JW, MacDonald IR (1984) Is there a role for the apex in shoot gravitropism? Plant Physiol **74:** 272–277

Hatfield RD, LaMotte CE (1984) IAA-induced growth responses of decapitated corn seedlings. Plant Physiol **74:** 302–306

Heathcote DG (1981) The geotropic reaction and statolith movements following geostimulation of mung bean hypocotyls. Plant Cell Environ **4:** 131–140

Hobbie L, Estelle M (1995) The *axr4* auxin-resistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. Plant J **7:** 211–220

Hou Y, Arnin AG, Deng X-W (1993) A new class of *Arabidopsis* constitutive photomorphogenic genes involved in regulating cotyledon development. Plant Cell **5:** 329–339

Ishikawa H, Hasenstein KH, Evans ML (1991) Computer-based video digitized analysis of surface extension in maize roots. Planta **183:** 381–390

Iversen TH (1969) Elimination of geotropic responsiveness in roots of cress (*Lepidium sativum*) by removal statolith starch. Physiol Plant **22:** 1251–1262

Iwami S, Masuda Y (1974) Geotropic response of cucumber hypocotyls. Plant Cell Physiol **5:** 121–129

Iwami S, Masuda Y (1976) Distribution of labeled auxin in geotropically stimulated stems of cucumber and pea. Plant Cell Physiol **17:** 227–237

Kaufman PB, Dayanandan P (1984) Hormonal regulation of the gravitropic response in grass shoots. *In* SS Purohit, ed, Hormonal Regulation of Plant Growth and Development, Vol 1. Agro Botanical, Bikaner, India, pp 369–386

Kaufman PB, Wu LL, Brock TG, Kim D (1995) Hormones and the orientation of growth. *In* PJ Davies, ed, Plant Hormones. Kluwer Academic, Dordrecht, The Netherlands, pp 547–571

Khurana JP, Best TR, Poff KL (1989) Influence of hook position on phototropic and gravitropic curvature by etiolated hypocotyls of *Arabidopsis thaliana*. Plant Physiol **90:** 376–379

Li Y, Hagen G, Guilfoyle TJ (1991) An auxin-promoter is differentially induced by auxin gradients during tropism. Plant Cell **2:** 1071–1080

Liscum E, Hangarter RP (1993) Genetic evidence that the red-absorbing form of phytochrome B modulates gravitropism in *Arabidopsis thaliana*. Plant Physiol **103:** 15–19

Migliaccio F, Rayle DL (1989) Effect of asymmetric auxin application on *Helianthus* hypocotyls curvature. Plant Physiol **91:** 466–468

Moore R, Evans ML (1986) How roots perceive and respond to gravity. Am J Bot **73:** 574–587

Okâda K, Shimura Y (1994) Modulation of root growth by physical stimuli. *In* EM Meyerowitz, CR Somerville, eds, Arabidopsis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 665–684

Parker KE, Briggs WR (1990) Transport of indole-3-acetic acid during gravitropism in intact maize coleoptiles. Plant Physiol **94:** 1763–1769

Pickard BG (1985) Early events in geotropism of seedling shoots. Annu Rev Plant Physiol **36:** 55–75

Poff KL, Janoudi AK, Rosen ES, Orbović V, Konjević R, Fortin MC, Scott TK (1994) The physiology of tropisms. *In* EM Meyerowitz, CR Somerville, eds, Arabidopsis. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 639–664

Poovaiah BW, McFadden JJ, Reddy ASN (1987) The role of calcium ions in gravity signal perception and transduction. Physiol Plant **71:** 401–407

Roberts JA, Gilbert I (1992) Gravitropism research—will mutants prevent us from going around the bend? *In* CM Karssen, LC van Loon, DV Vreugdenhil, eds, Progress in Plant Growth Regulation. Kluwer Academic, Dordrecht, The Netherlands, pp 913–920

Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR (1995) Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics **139:** 1393–1409

Sack FD (1991) Plant gravity sensing. Int Rev Cytol **127:** 193–252

Simmons C, Migliaccio F, Masson P, Caspar T, Söll D (1995) A novel root gravitropism mutant of *Arabidopsis thaliana* exhibiting altered auxin physiology. Physiol Plant **93:** 790–798

Slocum RD, Roux SJ (1983) Cellular and subcellular localization of calcium in gravistimulated oat coleoptiles and its possible significance in the establishment of tropic curvature. Planta **157:** 481–492

Stinemetz CL, Kuzmanoff KM, Evans ML, Jarret HW (1987) Correlation between calmodulin activity and gravitropic sensitivity in primary roots of maize. Plant Physiol **84:** 1337–1342

Wilson AK, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet **222:** 377–383

Wyatt RE, Ainley WM, Nagao RT, Conner TW, Key JL (1993) Expression of the *Arabidopsis AtAux2-11* auxin-responsive gene in transgenic plants. Plant Mol Biol **22:** 731–749

Young LM, Evans ML, Hertel R (1990) Correlations between gravitropic curvature and auxin movement across gravistimulated roots of *Zea mays*. Plant Physiol **92:** 792–796