Plant Physiol. (1996) 110: 945–955

SGR1, *SGR2*, and *SGR3*: Novel Genetic Loci Involved in Shoot Gravitropism in *Arabidopsis thaliana*[1]

Hidehiro Fukaki, Hisao Fujisawa, and Masao Tasaka*

Department of Botany, Faculty of Science, Kyoto University, Kyoto 606–01, Japan

In higher plants shoots show a negative gravitropic response but little is known about its mechanism. To elucidate this phenomenon, we have isolated a number of mutants with abnormal shoot gravitropic responses in *Arabidopsis thaliana*. Here we describe mainly three mutants: *sgr1–1*, *sgr2–1*, and *sgr3–1* (shoot gravitropism). Genetic analysis confirmed that these mutations were recessive and occurred at three independent loci, named *SGR1*, *SGR2*, and *SGR3*, respectively. In wild type, both inflorescence stems and hypocotyls show negative gravitropic responses. The *sgr1–1* mutants showed no response to gravity either by inflorescence stems or by hypocotyls. The *sgr2–1* mutants also showed no gravitropic response in inflorescence stems but showed a reduced gravitropic response in hypocotyls. In contrast, the *sgr3–1* mutant was found to have reduced gravitropic responses in inflorescence stems but normal gravitropic responses in hypocotyls. These results suggest that some genetic components of the regulatory mechanisms for gravitropic responses are common between inflorescence stems and hypocotyls, but others are not. In addition, these *sgr* mutants were normal with respect to root gravitropism, and their inflorescence stems and hypocotyls could carry out phototropism. We conclude that *SGR1*, *SGR2*, and *SGR3* are novel genetic loci specifically involved in the regulatory mechanisms of shoot gravitropism in *A. thaliana*.

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 pathway can be separated into three sequential steps: gravity perception, signal transduction, and asymmetric growth response by differential cell growth (reviewed by Feldmann, 1985; Pickard, 1985; Sack, 1991; Roberts and Gilbert, 1992; Poff et al., 1994; Kaufman et al., 1995). Nevertheless, the molecular mechanisms of both shoot and root gravitropism still remain unknown.

One approach to elucidate the molecular mechanisms is to identify the genes involved in gravitropism. Many root gravitropic mutants and some shoot gravitropic mutants have been isolated from several plants (reviewed by Roberts and Gilbert, 1992; Okada and Shimura, 1994). In *Arabidopsis thaliana*, at least eight genetic loci have been identified that are involved in root gravitropism (*dwf* [Mirza et al., 1984]; *aux1* [Mirza et al., 1984, Mirza, 1987, Pickett et al., 1990, Okada and Shimura, 1992]; *axr1* [Estelle and Somerville, 1987, Lincoln et al., 1990, Leyser et al., 1993]; *axr2* [Wilson et al., 1990, Timpte et al., 1992]; *agr1* [Bell and Maher, 1990, Maher and Bell, 1990, Okada and Shimura, 1992]; *cop4* [Hou et al., 1993]; *axr4* [Hobbie and Estelle, 1995]; *rgr1* [Simmons et al., 1995]; *eir1* [Roman et al., 1995]). However, few are known at genetic loci involved in shoot gravitropism (unnamed [Bullen et al., 1990]; *axr2* [Wilson et al., 1990, Timpte et al., 1992]; *cop4* [Hou et al., 1993]; *phyB* [Liscum and Hangarter, 1993]).

In shoots of *A. thaliana*, the inflorescence stems and hypocotyls show negative gravitropism. To date, although the hypocotyls have been used for the study of shoot gravitropism (Khurana et al., 1989; Bullen et al., 1990; Hou et al., 1993; Liscum and Hangarter, 1993), with the exception of the observation by Caspar and Pickard (1989) that the horizontally gravistimulated inflorescence stems of wild-type Columbia ecotype achieved 60° curvature in 80 min, the gravitropic properties of inflorescence stems have not been described in detail prior to our recent study (Fukaki et al., 1996). To our knowledge, there has been no report about mutants with abnormal inflorescence stem gravitropism except *axr2* (*auxin resistant 2*) mutant. The inflorescence stems of *axr2* do not orient properly in response to gravity; they twist and frequently grow back toward the soil (Wilson et al., 1990), and the *axr2* also shows defects in both hypocotyl and root gravitropism (Timpte et al., 1992). Thus, it has been generally believed that the mechanism for the gravitropic responses might be the same in inflorescence stems and hypocotyls in *A. thaliana*. Furthermore, the shoot gravitropism mutants of tomato, *diageotropica* (Zobel, 1974) and *lazy-2* (Gaiser and Lomax, 1992, 1993), also show abnormal gravitropism in both stems and hypocotyls. However, whether the mechanisms for the gravitropic responses of stems and hypocotyls are genetically different in higher plants is still an open question.

To investigate the molecular mechanisms of shoot gravitropism, we have isolated a number of Arabidopsis mutants with no or reduced gravitropism in inflorescence stems. Here we describe the identification and initial characterization of several *sgr* (for shoot gravitropism) mutants at three independent loci (*SGR1*, *SGR2*, and *SGR3*) that affect shoot gravitropism. A mutation at

[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.

Abbreviation: EMS, ethyl methansulfonate.

the *SGR1* or *SGR2* locus affects both inflorescence stem and hypocotyl gravitropism, whereas a mutation at the *SGR3* locus affects only inflorescence stem gravitropism. These results suggest that some genetic components of the regulatory mechanisms for gravitropic responses are common to inflorescence stems and hypocotyls, but others are not. In addition, these *sgr* mutants were normal with respect to root gravitropism and their shoots could carry out phototropism. We conclude that the *SGR1*, *SGR2*, and *SGR3* are novel genetic loci specifically involved in the regulatory mechanisms of shoot gravitropism in *A. thaliana*.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana (L.) Heynh. Columbia ecotype was the parental strain of the EMS-mutagenized seed lots, and Wassilewskija ecotype was the parental strain of the T-DNA insertion lots (Feldmann, 1992).

In the experiments on tropic response of inflorescence stems, seeds were sown in vinyl pots and grown under constant white light at 23°C as described in our accompanying paper (Fukaki et al., 1996). In the experiments on tropic response of seedlings, seeds were surface sterilized and plated on Murashige-Skoog plates that contained 4.3 g/L Murashige-Skoog salts (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 10 g/L Suc, 0.1 g/L *myo*-inositol, 0.05% (w/v) Mes adjusted to pH 5.7 with KOH, 20 μg/L thiamine hydrochloride, 1 μg/L nicotinic acid, 1 μg/L pyridoxine hydrochloride, 1 μg/L biotin, and 5 g/L gellan gum (a special agar for plant culture, purchased from Wako Pure Chemical Industries).

Mutagenesis and Screening

About 7,500 seeds of wild-type Columbia ecotype were soaked in 0.3% (v/v) EMS in water for 16 h at room temperature, washed repeatedly with water, and sown in vinyl pots. About 80,000 M_2 seeds harvested from the about 4,500 mutagenized M_1 plants were screened. T-DNA insertion seed stocks (T_4 generation) were obtained from E.I. Dupont de Nemours and Co. (7600 lines). Mutagenized M_2 and T_4 seeds were sown in vinyl pots to screen for mutants with abnormal gravitropism in inflorescence stems. When the primary inflorescence stems of M_2 or T_4 plants were about 4 to 8 cm, they were given the horizontal gravistimulation by setting the pots on their sides for 90 min in darkness at 23°C. The putative mutants that showed no or reduced (less than 30° upward curvature) negative gravitropic response to the 90-min horizontal gravistimulation were then grown in vinyl pots to obtain the M_3 or T_5 seeds. The M_3 or T_5 seeds were sown in vinyl pots and plants were screened to determine whether they exhibited the parental phenotype. Finally, 17 independent mutant lines were isolated. These were further analyzed for the gravitropic responses of seedlings and most of them were genetically analyzed. Among them, three mutant lines, *sgr1–1*, *sgr2–1*, and *sgr3–1*, from EMS-mutagenized lines (Columbia ecotype) were further analyzed in this study. These three *sgr* mutant lines used throughout all experiments were the progeny of F_2 lines that showed mutant phenotypes following backcrossing at least once to wild-type plants.

Assays for Tropic Responses of Inflorescence Stems

For the gravitropic response assays of inflorescence stems, decapitated 3.5-cm stem segments were prepared from the distal 4-cm parts of the primary inflorescence stems that were 4 to 8 cm long, as described in our accompanying paper (Fukaki et al., 1996). They were put into gellan gum blocks containing 1% (w/v) gellan gum and mineral nutrients (Fukaki et al., 1996), which were fixed on one face of plastic square plates so that the stem segments were vertical. The gravistimulation was given by rotating the plates 90° in darkness at 23°C, except that the photographs were taken under the 10-s illumination of 10 μmol m^{-2} s^{-1} white light from white fluorescence tubes (FL20SS·D; Toshiba, Tokyo, Japan). The gravitropic curvature of stem segments was measured as follows: angles of the growing directions of the tip of stem segments between 0 min (when the gravistimulation was first given) and each time thereafter (usually every 15 min) were measured from the image of negatives enlarged by a projector.

Phototropic responses of inflorescence stems were examined as follows. In the assay for phototropic responses of the intact inflorescence stems attached to the plant, when the inflorescence stems grown in vinyl pots were about 4 to 8 cm long, the vinyl pots were covered by a black opaque box with an opening on one side and illuminated with about 80 μmol m^{-2} s^{-1} white light (FL20SS·D; Toshiba) through the opening for 3 h at 23°C. In the assay for the measurement of the phototropic curvature of stem segments, distal undecapitated 4-cm stem segments (with the apex and the leaves), cut from the primary inflorescence stems that were 4 to 8 cm long, were prepared and put into the gellan gum blocks and set vertically in the plastic plates. Thereafter, the plates were covered by a black opaque box with an opening on one side and illuminated with the 80 μmol m^{-2} s^{-1} white light through the opening at 23°C for 3 h, except that the photographs were taken under the 10-s illumination of 10 μmol m^{-2} s^{-1} red light from red fluorescent tubes (FL20S·R·F; National, Tokyo, Japan). The phototropic curvature of the distal undecapitated 4-cm stem segments was measured as follows: angles of the growing directions of the tip of the undecapitated stem segments between 0 h (when the unilateral illumination was first given) and each time thereafter (every 1 h) were measured from the image of negatives enlarged by a projector.

Assays for Tropic Responses of Seedlings

Seeds were surface sterilized, plated on Murashige-Skoog plates, and incubated at 4°C for 3 d in darkness. The plates were then placed under white light at 23°C for 30 h. For the gravitropic responses of hypocotyls, plates were

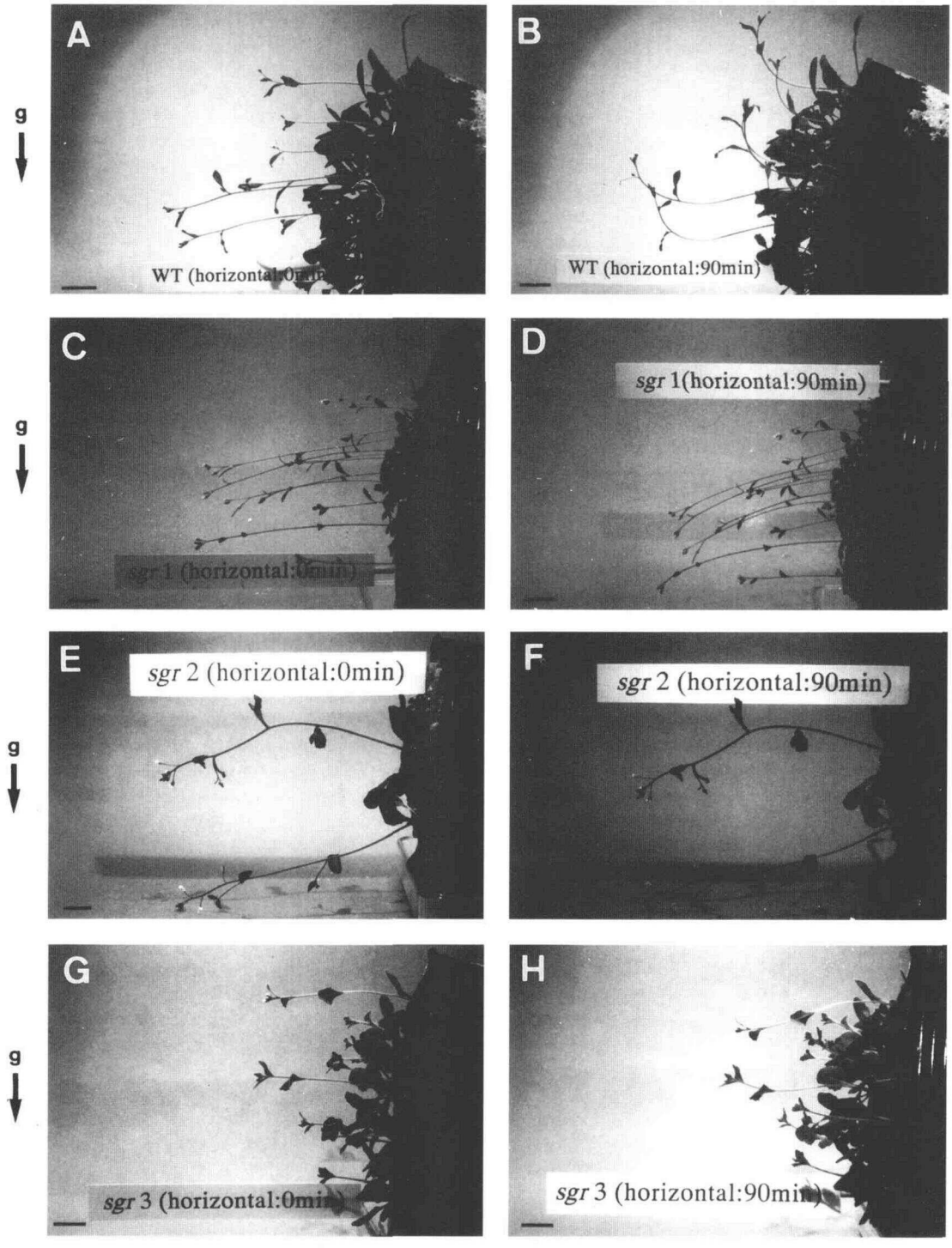

Figure 1. Gravitropic responses of inflorescence stems of wild-type (WT) and *sgr* mutant plants. A and B, Wild type; C and D, *sgr1–1*; E and F, *sgr2–1*; G and H, *sgr3–1*. Inflorescence stems attached to the plant were gravistimulated horizontally by laying the pots down. A, C, E, and G represent the plants at the start of gravistimulation. B, D, F, and H represent the plants 90 min after the gravistimulation. The horizontal gravistimulation was given in darkness at 23°C. Bar = 1 cm. The arrows indicate the direction of gravity (g).

placed on edge in darkness at 23°C for 42 h (vertical position). Next, the plates were rotated through 90° and incubated for 10 h in darkness (horizontal position). Angles between the opposite direction of gravity and the growth directions of hypocotyls were measured in the vertical position and in the horizontal position from the image of negatives enlarged by a projector. Gravitropic responses of roots were observed at the same time.

The phototropic responses of hypocotyls were examined as follows. After incubation in the vertical position as described above, the plates were covered by a black opaque sheet with an opening on one side and illuminated with about 80 μmol m^{-2} s^{-1} white light through the opening for 10 h.

RESULTS

Isolation and Genetic Characterization of Arabidopsis Mutants with No or Reduced Gravitropism in Inflorescence Stems

In *A. thaliana* Columbia ecotype, the inflorescence stems begin to elongate vertically after making the rosette leaves (3–4 weeks after germination). When the inflorescence stems were placed horizontally, they curved about 90° upward within 90 min in darkness at 23°C, exhibiting negative gravitropic responses (Fig. 1, A and B). Also, the inflorescence stems of Wassilewskija ecotype curved about 90° upward within 90 min under the same condition (data not shown). On the basis of these responses, we screened from mutagenized seed populations for the mutant lines whose inflorescence stems showed either a reduced gravitropic response or none at all to the 90-min horizontal gravistimulation, and we isolated 17 such mutant lines. Fourteen mutant lines were isolated from EMS-mutagenized M_2 plants (Columbia ecotype), and three mutant lines were isolated from the T_4 generation of two different parental pools arising from T-DNA insertional mutagenesis (Wassilewskija ecotype, Feldmann, 1992). Figure 1 shows the gravitropic responses of inflorescence stems of three such mutants, designated *sgr1–1*, *sgr2–1*, and *sgr3–1* (shoot gravitropism) from EMS-mutagenized lines. The inflorescence stems of the *sgr1–1* and *sgr2–1* mutants failed to curve upward even after 90 min (Fig. 1, C–F), and the inflorescence stems of the *sgr3–1* mutants curved upward very slightly after 90 min (Fig. 1, G and H). The inflorescence stems of the other seven mutant lines, *sgr2–2*, *sgr2–3*, *sgr2–4*, *sgr2–5*, and *sgr2–6* from EMS-mutagenized lines, and *sgr1–2* and *sgr2–7* from T-DNA insertion mutagenized lines also failed to curve upward even after 90 min (data not shown).

The genetic properties of the *sgr1–1*, *sgr2–1*, and *sgr3–1* mutants were examined by crossing the mutant lines with wild-type plants and determining the segregation of the inflorescence stem gravitropism traits in each F_1 and F_2 progeny. In the F_1, all of the progeny as well as wild-type plants showed normal inflorescence stem gravitropism (number of examined F_1 plants: *sgr1–1* = 30, *sgr2–1* = 16, and *sgr3–1* = 11), indicating that these three *sgr* mutations were recessive. In the F_2 progeny, the inflorescence stem gravitropism traits segregated about 3:1 (in F_2 of *sgr1–1*, wild type = 126, mutant = 38, χ^2 [calculation based on 3:1 ratio of wild type to mutant] = 0.29, $P > 0.05$; in F_2 of *sgr2–1*, wild type = 65, mutant = 24, $\chi^2 = 0.18$, $P > 0.05$; in F_2 of *sgr3–1*, wild type = 118, mutant = 33, $\chi^2 = 0.80$, $P > 0.05$), again confirming the recessive character of the inflorescence stem gravitropism traits and demonstrating that the traits in these mutant lines segregated as single Mendelian mutations.

To determine the number of complementation groups, these *sgr* lines were crossed to each other. Complementation tests as shown in Table I demonstrated that *sgr1–1* was allelic to *sgr1–2* and that *sgr2–1* was allelic to *sgr2–2*, *sgr2–3*, *sgr2–4*, *sgr2–5*, *sgr2–6*, and *sgr2–7* but that *sgr1–1*, *sgr2–1*, and *sgr3–1* were not allelic to each other, indicating that these *sgr* lines were single recessive mutations at three different genetic loci designated *SGR1* (*SHOOT GRAVITROPISM1*), *SGR2*, and *SGR3*. From the mapping of these *SGR* loci, it was found that they were located on different chromosomes (data not shown). Hereafter, we focused our efforts on the characterization of the *sgr1–1*, *sgr2–1*, and *sgr3–1* mutants.

Gravitropic Response of Inflorescence Stems of the *sgr* Mutants

Figure 2 shows the time course for the change in angle of the growing apical tip of the decapitated stem segments of wild-type and *sgr* mutant inflorescence stems during horizontal gravistimulation. When the decapitated stem segments of wild type were placed horizontally, they curved upward more than 90° in 90 min (Fig. 2; described in detail

Table I. *Complementation analysis of sgr mutants*

Gravitropic responses of the primary inflorescence stems of about 4-week-old plants were examined in each F_1 progeny from crosses between homozygous *sgr* mutants pairs.

Crosses	No. of Plants		
	Total	Normal response	Abnormal response
sgr1–1 × *sgr1–2*	7	0	7
sgr2–1 × *sgr2–2*	5	0	5
sgr2–1 × *sgr2–3*	2	0	2
sgr2–1 × *sgr2–4*	11	0	11
sgr2–1 × *sgr2–5*	8	0	8
sgr2–1 × *sgr2–6*	10	0	10
sgr2–1 × *sgr2–7*	4	0	4
sgr2–2 × *sgr2–3*	6	0	6
sgr2–2 × *sgr2–4*	6	0	6
sgr2–2 × *sgr2–5*	15	0	15
sgr2–2 × *sgr2–6*	3	0	3
sgr2–3 × *sgr2–4*	11	0	11
sgr2–3 × *sgr2–5*	13	0	13
sgr2–3 × *sgr2–6*	4	0	4
sgr1–1 × *sgr2–1*[a]	13	13	0
sgr1–1 × *sgr3–1*	10	10	0
sgr2–1 × *sgr3–1*[a]	24	24	0

[a] Similar results were obtained with other allele combinations of these loci.

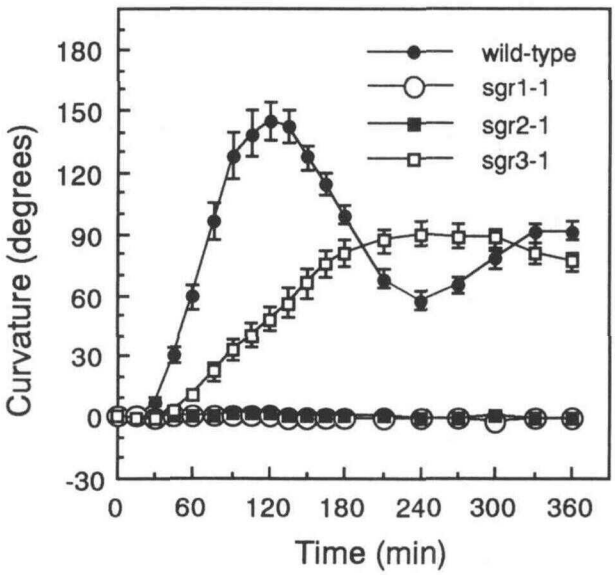

Figure 2. Time courses for gravitropic responses of stem segments of wild type and *sgr* mutants. Decapitated 3.5-cm stem segments from the distal 4-cm parts of the primary inflorescence stems were horizontally gravistimulated in darkness at 23°C (see "Materials and Methods"). The time courses for the change in growing direction of the tip of stem segments are shown. The error bars represent the SE values. At least 11 individuals were examined in each genotype.

by Fukaki et al., 1996). By contrast, the decapitated stem segments of both *sgr1–1* and *sgr2–1* mutants failed to curve upward even after 6 h of horizontal gravistimulation (Fig. 2). In the intact plants, after the inflorescence stems of either *sgr1–1* or *sgr2–1* mutant lay down on the soil, they never curved upward but continued to grow horizontally on the soil (data not shown). These results indicate that the *sgr1–1* and *sgr2–1* mutants appear to be null mutants in the gravitropic response of inflorescence stems, at least in response to gravistimulation of 1*g*.

On the other hand, the decapitated stem segments of the *sgr3–1* mutant curved upward very slightly after 90 min but had curved almost 90° upward by 6 h (Fig. 2). In addition, whereas the decapitated stem segments of wild type, gravistimulated horizontally for 10 min, developed distinct gravitropic curvatures after they were replaced in the vertical position (the curvature at 50 min after the gravistimulation was 32.0 ± 5.0° [mean ± SE]; number examined = 10), the decapitated stem segments of the *sgr3–1* mutant failed to develop distinct gravitropic curvatures (the curvature at 50 min after the gravistimulation was 2.2 ± 1.1° [mean ± SE]; number examined = 10). These results indicate that the *sgr3–1* mutant has reduced gravitropic responses.

Phototropic Responses of Inflorescence Stems of the *sgr* Mutants

The inflorescence stems of wild type showed phototropic curvature in response to the unilateral light (Fig. 3, A and E). It was determined whether the inflorescence stems of the *sgr* mutants showed the phototropic curvature. As shown in Figure 3, B to D and F to H, the inflorescence stems of these three *sgr* mutants showed positive phototropic curvatures to 3 h of unilateral light illumination, and the phototropic curvatures of these *sgr* mutants appeared to be similar to or slightly larger than that of the wild type. In addition, by using the plate assay system, we examined the phototropic responses of the undecapitated 4-cm stem segments of the inflorescence stems in response to long exposure to unilateral light. As shown in Table II, the undecapitated stem segments of these three *sgr* mutants exhibited either the same or significantly larger curvatures than that of wild

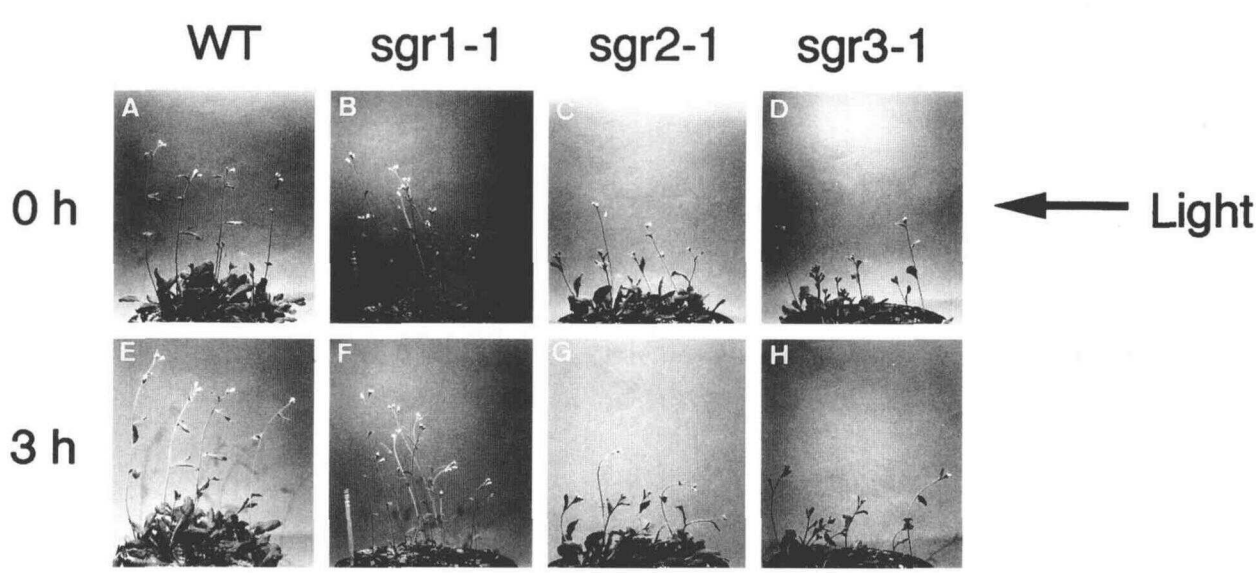

Figure 3. Phototropic responses of inflorescence stems of wild-type and *sgr* mutant plants. A and E, Wild-type (WT); B and F, *sgr1–1*; C and G, *sgr2–1*; D and H, *sgr3–1*. A to D, Before the unilateral white light illumination. E to H, After the unilateral white light stimulation for 3 h from the right side. The arrow indicates the direction of light.

type after 1 to 3 h of exposure to the unilateral light. We did not examine the first positive curvatures of the wild type and these mutants (Khurana and Poff, 1989), but these results indicate that the mechanism of the normal, second positive phototropic response by inflorescence stems in response to long exposure to unilateral light was not directly affected by any *sgr* mutation.

When the wild-type plants were grown from germination with the illumination coming diagonally from above, the basal portion of the inflorescence stem stood upright and the distal portion of the inflorescence stem curved toward the light source (Fig. 4A). By contrast, the inflorescence stems of the three *sgr* mutants became oriented directly toward the light source from the basal portion of the inflorescence stems (Fig. 4, B–D). This light-oriented growth by the inflorescence stems of the three *sgr* mutants would simply result from a normal positive phototropic response and the lack of a normal negative gravitropic response. The same reason could explain the undecapitated stem segments of three *sgr* mutants exhibiting larger phototropic curvatures than that of wild-type in response to the unilateral light (Table II). Therefore, these results support the hypothesis that no *sgr* mutation directly affects the mechanisms of second positive phototropism in inflorescence stems.

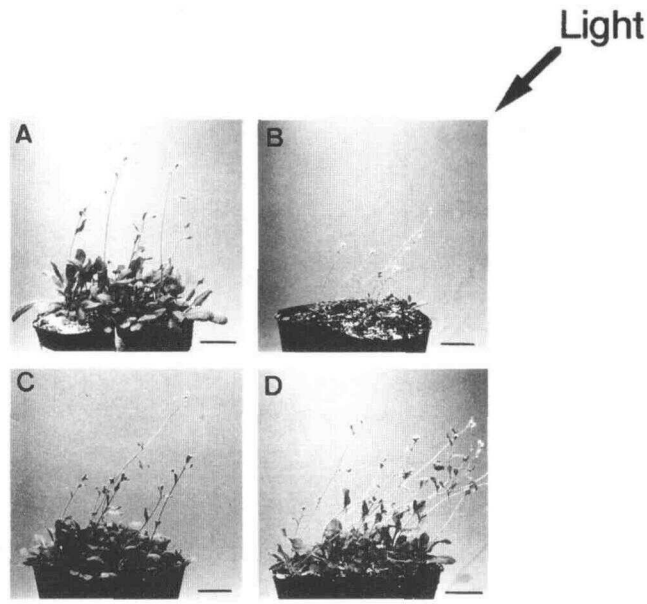

Figure 4. Light-oriented growth by inflorescence stems of *sgr* mutants. Plants were grown under white light coming diagonally from above after the germination. A, Wild type; B, *sgr1–1*; C, *sgr2–1*; D, *sgr3–1*. The arrow indicates the direction of light. Bar = 1 cm.

Growth and Development of the *sgr* Mutants

In the *sgr1–1* plants, the growth rate of inflorescence stems was about 33% of that of wild-type plants (Fig. 5). The *sgr1–1* plants had smaller leaves and thinner and shorter inflorescence stems than wild type (Fig. 6B; Table III), but it had normally produced seeds. In addition, whereas the lateral branches of wild type grew upward by responding to gravity (Fig. 6A), those of *sgr1–1* mutants grew horizontally, indicating that the lateral branches of *sgr1–1* could not respond to gravity as well as the primary inflorescence stems (data not shown). All of these phenotypes co-segregated with *sgr1–1* plants after four backcrosses to wild-type plants. In addition, the *sgr1–2* mutant plants, another independent *sgr1* line, also showed the same phenotypes as *sgr1–1* plants (data not shown). These results suggest that the *SGR1* locus is involved not only in inflorescence stem gravitropism but also in the control of the plant size.

The size of the *sgr2–1* mutant plants and the growth rate of their inflorescence stems were almost the same as those of wild-type plants (Figs. 5 and 6C). However, the inflorescence stems of *sgr2–1* mutants twisted as they elongated and the lateral branches twisted downward (Fig. 6C). These phenotypes were seen in the other six *sgr2* alleles but not in the *sgr1* alleles and *sgr3–1*.

Table II. *Phototropic response of wild-type and sgr inflorescence stems*

The decapitated 4-cm stem segments of the primary inflorescence stems were illuminated by a unilateral light and the phototropic curvature was measured after 1, 2, and 3 h of exposure to the light as described in "Materials and Methods." Data represent the means ± SE. *n*, Number examined.

Genotype	Curvature (degrees)		
	1 h	2 h	3 h
Wild type (*n* = 19)	14.3 ± 2.4	17.8 ± 4.1	36.5 ± 4.1
sgr1–1 (*n* = 28)	19.2 ± 2.6	29.6 ± 3.4	39.2 ± 3.3
sgr2–1 (*n* = 19)	20.6 ± 3.9	47.9 ± 7.7	68.4 ± 6.8
sgr3–1 (*n* = 24)	13.9 ± 2.8	30.0 ± 3.6	39.1 ± 4.2

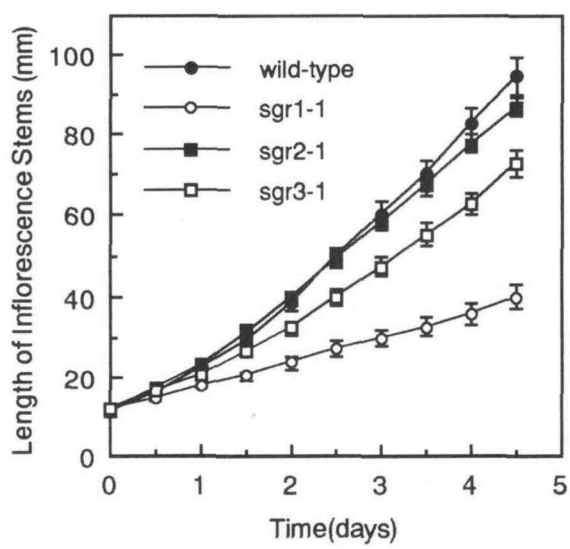

Figure 5. Growth of the primary inflorescence stems of wild type and *sgr* mutants. Elongation of the primary inflorescence stems during 4.5 d (lengths at the 0 h were 10–15 mm) were measured. Growth rates (mutant/wild type) during 108 h: *sgr1–1*, 33%; *sgr2–1*, 90%; *sgr3–1*, 73%. The error bars represent the SE values. At least six individuals were examined in each genotype.

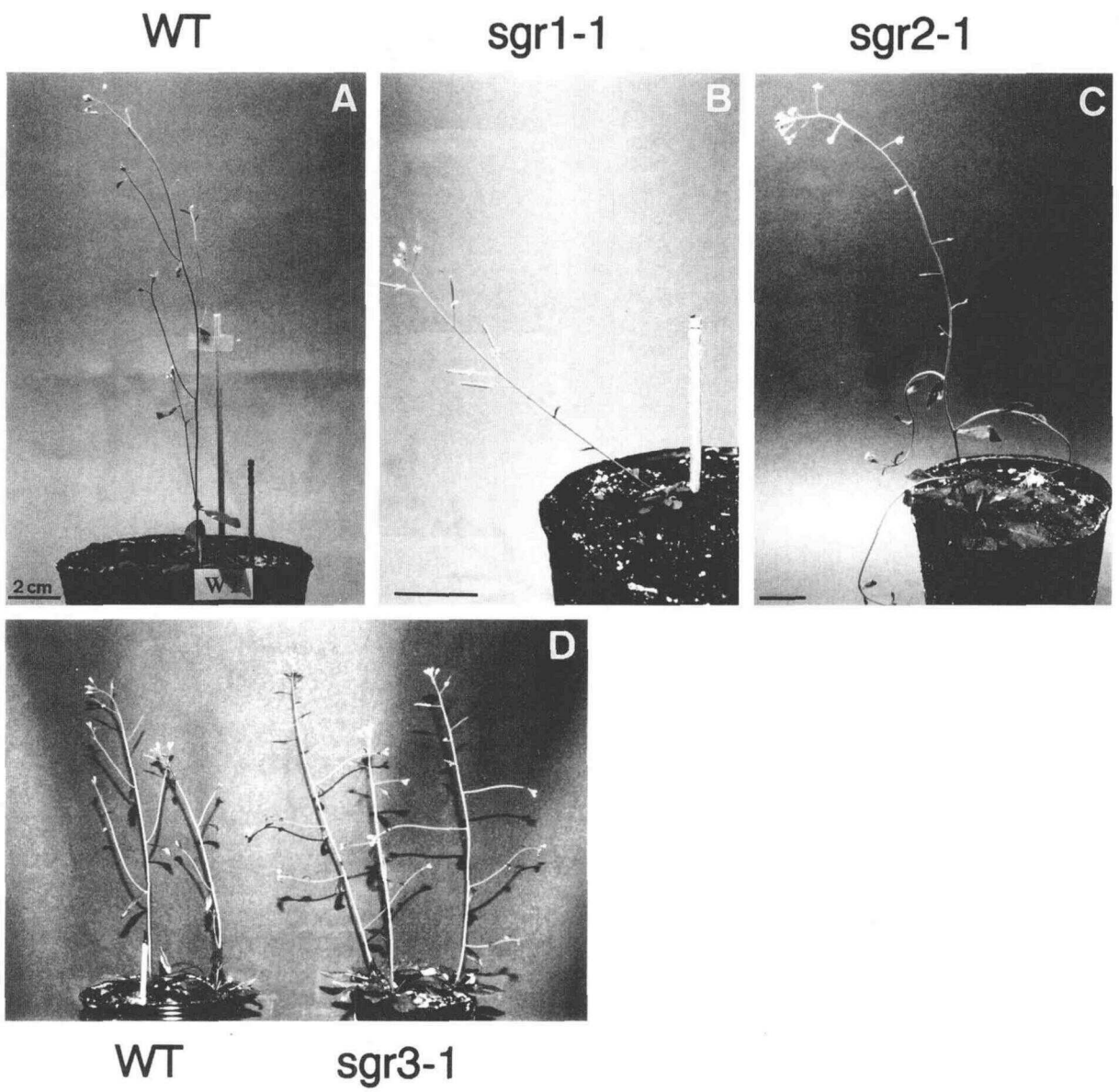

Figure 6. Photographs of 4-week-old wild-type and *sgr* mutant plants. A, Wild type (WT); B, *sgr1–1*; C, *sgr2–1*; D, wild type (WT; left) and *sgr3–1* (right). Note that the lateral branches of *sgr2–1* and *sgr3–1* grow downward or horizontally, whereas those of wild type grow upward. Bar = 2 cm.

In the *sgr3–1* plants, the growth rate of inflorescence stems was slightly reduced (about 70% of that of wild type; Fig. 5) but the final size of the *sgr3–1* plants was almost the same as that of wild type. The possibility is not excluded that its reduced growth rate caused the reduced gravitropic response. However, the lateral branches of the *sgr3–1* plants grew nearly horizontally (Fig. 6D). Additionally, despite their reduced growth rate, the inflorescence stems of the *sgr3–1* exhibited either the same or larger phototropic curvatures than that of wild type in response to the unilateral light (Table II) and showed the light-oriented growth seen in *sgr1–1* and *sgr2–1* plants (Fig. 4). The stem segments of *sgr3–1* mutants failed to show distinct curvatures after the 10-min horizontal gravistimulation, although those of wild type showed distinct curvatures in

Table III. *Phenotypes of rosette leaves and inflorescence stems of wild type and sgr1–1 mutant plants*

Data represent means ± SE.

Phenotype	Wild Type	*sgr1–1*
Length of inflorescence stems (cm)[a]	45.1 ± 1.2	25.5 ± 1.1
Width of inflorescence stems (mm)[b, c]	0.87 ± 0.04	0.45 ± 0.03
No. of rosette leaves[c]	11.4 ± 0.4	8.2 ± 0.2
Length of rosette leaves (mm)[c, d]	35.7 ± 1.32	8.33 ± 0.76

[a] Lengths of the primary inflorescence stems of about 8-week-old plants (more than seven individuals) were measured. [b] Widths of the point at 1 cm above the base of the primary inflorescence stems were measured. [c] About 4-week-old plants (more than five individuals) were used. [d] Lengths of the longest rosette leaves were measured.

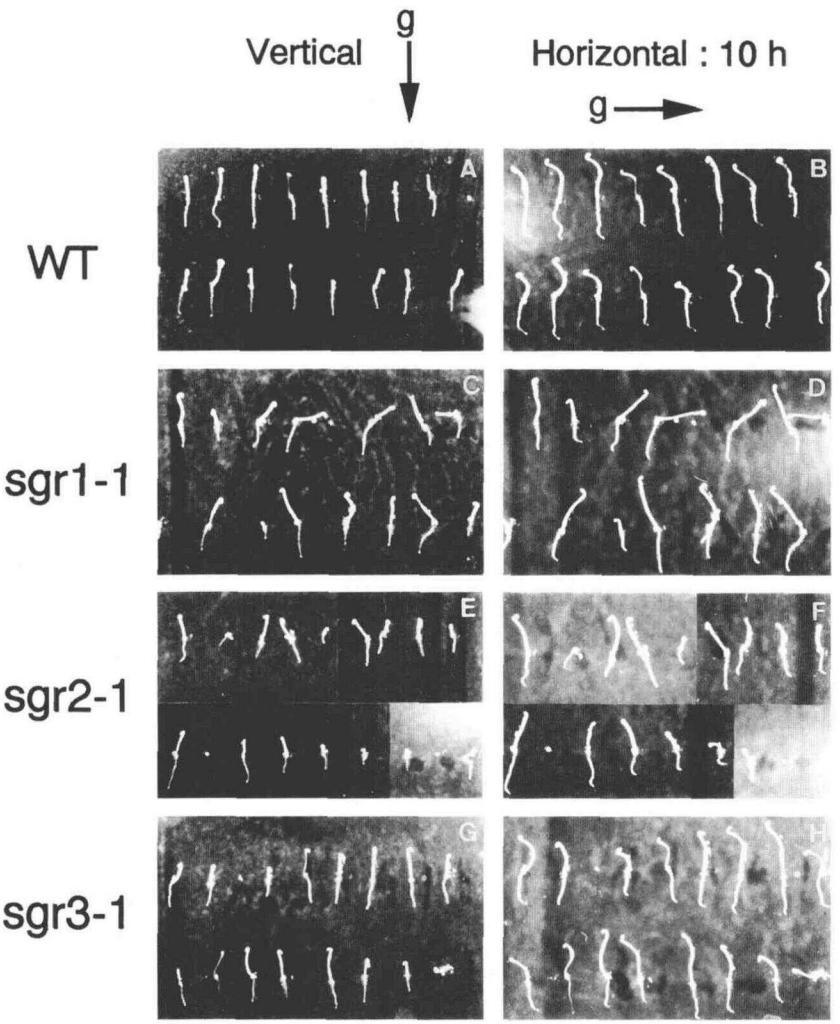

Figure 7. Representative gravitropic responses of dark-grown *sgr* mutant seedlings. A and B, Wild type (WT); C and D, *sgr1–1*; E and F, *sgr2–1*; G and H, *sgr3–1* mutant seedlings. A, C, E, and G represent the dark-grown seedlings kept in the vertical position. B, D, F, and H represent the seedlings 10 h after the horizontal gravistimulation was given in darkness to the seedlings shown in A, C, E, and G, respectively. The arrows indicate the directions of gravity (g).

response to the treatment (see above). These results indicate that the *sgr3–1* is a reduced-response-type mutant of inflorescence stem gravitropism.

Tropic Responses of Seedlings of the *sgr* Mutants

Whether a mutation at the *SGR1*, *SGR2*, or *SGR3* locus causes some abnormal gravitropic or phototropic responses in hypocotyls or roots of the seedlings was examined. In etiolated seedlings of wild-type Arabidopsis, the hypocotyls show negative gravitropism (Khurana et al., 1989; Bullen et al., 1990; Liscum and Hangarter, 1993). Figure 7 shows representatives of these responses in wild-type and three *sgr* mutant seedlings, and Figure 8 shows the frequency-distribution histograms for the gravitropic response of hypocotyls. In the vertical position, the hypocotyls of wild type grew upward uniformly (Figs. 7A and 8A). However, the growth directions of both *sgr1–1* and *sgr2–1* hypocotyls ranged from +90 to −90° in comparison to wild-type (Figs. 7, C and E, and 8, C and E). In contrast, the hypocotyls of *sgr3–1* mutants grew upward uniformly as well as the wild type (Figs. 7G and 8G). When the direction of gravistimulation was changed by 90°, the hypocotyls of wild type curved upward (Figs. 7B and 8B). In contrast, the hypocotyls of *sgr1–1* mutant seedlings failed to curve upward (Figs. 7D and 8D), and the hypocotyls of *sgr2–1* mutant seedlings exhibited reduced curvature (Figs. 7F and 8F), indicating that *sgr1–1* and *sgr2–1* mutants are abnormal in hypocotyl gravitropism. Once again, the hypocotyls of *sgr3–1* showed normal negative gravitropic responses, similar to those of wild type (Figs. 7H and 8H). In addition, from the kinetics of the hypocotyl gravitropic response in wild-type and *sgr3–1* mutant seedlings, it was confirmed that the hypocotyls of *sgr3–1* mutants did not show reduced gravitropic responses in comparison to wild type (Fig. 9). These results indicate that the *sgr3–1* mutant is normal in hypocotyl gravitropism.

Furthermore, the roots of all three classes of *sgr* mutants showed normal positive gravitropic responses (Fig. 7). Thus, a mutation at either the *SGR1* or *SGR2* locus affects the gravitropic responses of both inflorescence stems and hypocotyls but not that of roots; a mutation at the *SGR3* locus affects only the gravitropic response of inflorescence stems and not those of both hypocotyls and roots.

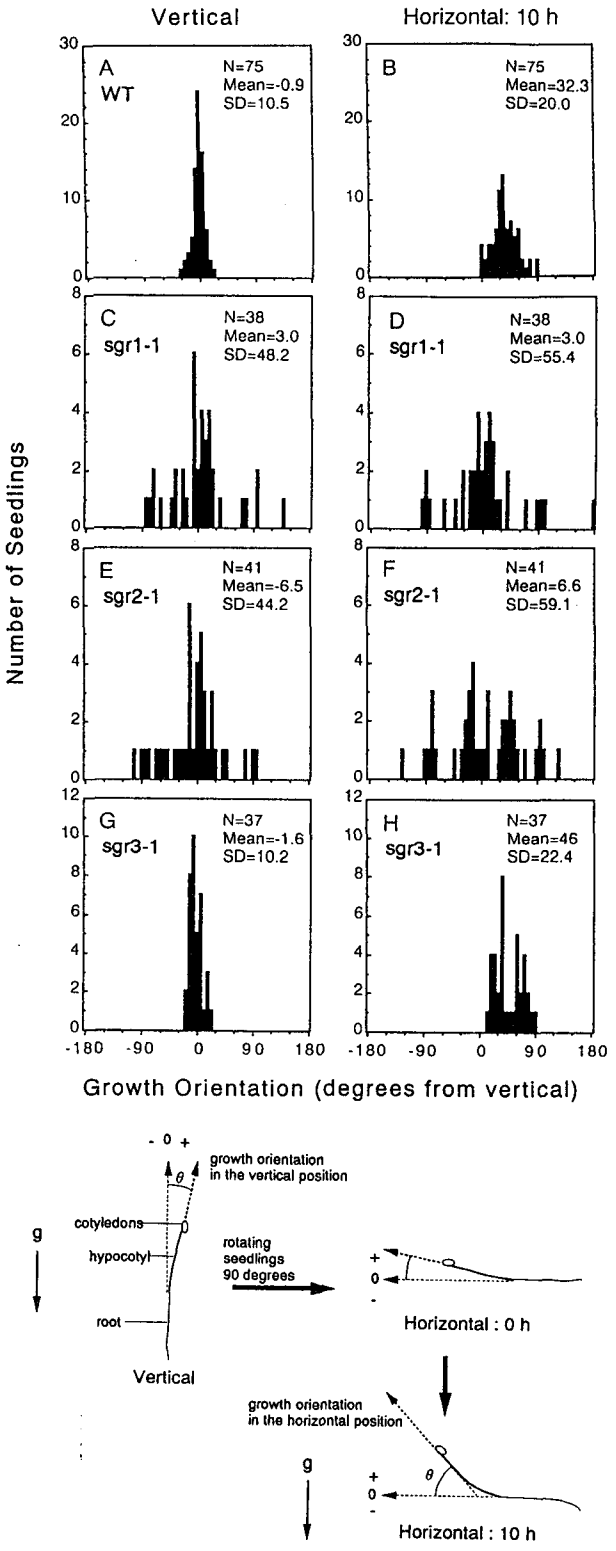

Figure 8. Frequency-distribution histograms for gravitropic curvatures of dark-grown hypocotyls of wild-type (WT) and *sgr* mutant seedlings. A, C, E, and G show the growth orientations of hypocotyls in the vertical position. B, D, F, and H show the growth orientations of hypocotyls after the 10-h horizontal gravistimulation. A and B, Wild type (WT); C and D, *sgr1–1*; E and F, *sgr2–1*; G and H, *sgr3–1*. N, Number of seedlings examined. Means and SDs of negative gravitropic curvatures are given on each histogram. As indicated in the drawings below the histograms, positive or negative values were assigned when the hypocotyl was to the right or left of the direction opposite that of gravity (g), respectively, and the angles between the growing direction of hypocotyls and the direction opposite that of gravity were measured.

Finally, the phototropic curvatures of hypocotyls of wild-type and *sgr* mutant seedlings to the unilateral light source were examined. The hypocotyls of three *sgr* mutant seedlings could carry out phototropic curvatures as well as those of wild type (Fig. 10). We did not examine the first positive curvatures of wild type and these mutants (Khurana and Poff, 1989). But this result indicates that normal second positive curvature by hypocotyls in response to long exposure to unilateral light is unaffected by each *sgr* mutation.

DISCUSSION

SGR Genes Are Essential for Shoot Gravitropism in *A. thaliana*

Our screening procedure for shoot gravitropism mutants is simple, involving screening directly for alterations in the gravitropic response of inflorescence stems. However, with this procedure, it is possible to obtain mutants with the alterations in steps that are not directly involved in the gravitropic response. An example might be the dwarf mutants or those that have reduced growth in the inflorescence stems. Phenotypes of three mutants isolated and characterized in this study, *sgr1–1*, *sgr2–1*, and *sgr3–1*, are summarized in Table IV. The inflorescence stems of these *sgr* mutants either failed to respond or exhibited reduced responses to gravity (Figs. 1 and 2) and their lateral branches grew horizontally or downward (Fig. 6). In addition, the inflorescence stems of *sgr* mutants exhibited light-oriented growth when they were grown under illumination coming from a diagonal upper direction (Fig. 4) and their stem segments exhibited larger phototropic curvatures to the unilateral light than those of wild-type (Table II), despite the reduced growth rates of inflorescence stems (Fig. 5). The stronger phototropic responses of these *sgr* mutants under conditions in which gravitropism and phototropism are forced to work against each other demonstrate that these *sgr* mutants are specifically affected in the regulatory mechanisms of the gravitropic response of inflorescence stems. Moreover, a mutation at either *SGR1* or *SGR2* locus causes a gravitropic defect in hypocotyls (Figs. 7 and 8). Therefore, *SGR1*, *SGR2*, and *SGR3* loci appear to be novel genetic loci that are essential for shoot gravitropism in *A. thaliana*.

SGR Gene Products Probably Act on the Gravity Perception or the Signal Transduction Mechanisms

Current concepts of gravitropism advance three steps: gravity perception; signal transduction, which includes an asymmetric auxin distribution; and asymmetric growth response by differential cell elongation, resulting

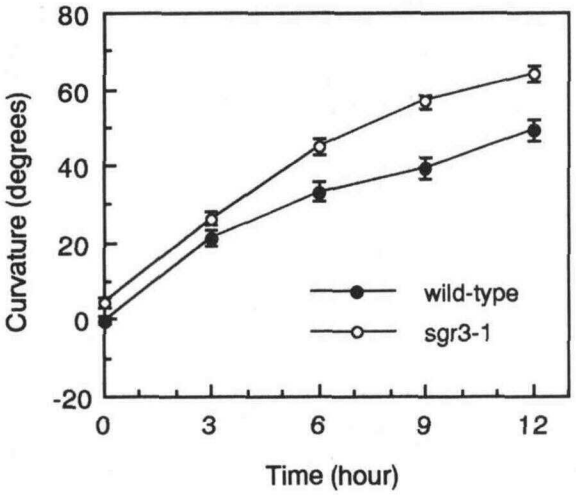

Figure 9. Time course of gravitropic responses of hypocotyls of wild-type and *sgr3–1* mutant seedlings. Dark-grown hypocotyls of wild-type (●) and *sgr3–1* (○) mutant seedlings were horizontally gravistimulated in darkness. The angles between the growing direction of hypocotyls and the horizontal direction were measured as the curvature. The error bars represent the SE values. At least 42 seedlings were examined in each genotype.

in a tropic curvature (reviewed by Feldmann, 1985; Pickard, 1985; Roberts and Gilbert, 1992; Poff et al., 1994; Kaufman et al., 1995). It has been thought that gravitropism and phototropism are related to the transport and distribution of auxin. In gravitropic responses of both tomato hypocotyls and maize coleoptiles, the lateral transport of auxin occurs within the horizontally gravistimulated organs, the consequence of which results in the gravitropic curvature (Harrison and Pickard, 1989; Parker and Briggs, 1990). In phototropic responses of maize coleoptiles, unilateral light induces the lateral transport of auxin in the apical parts of the coleoptiles.

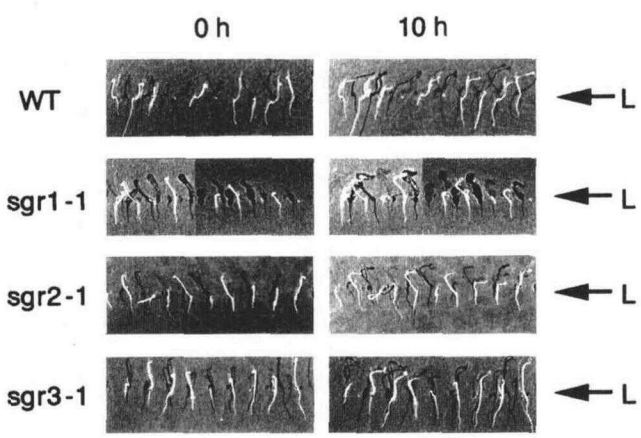

Figure 10. Representative phototropic responses of hypocotyls of wild-type and *sgr* mutant seedlings. Dark-grown wild-type (WT), *sgr1–1*, *sgr2–1*, and *sgr3–1* mutant seedlings were illuminated with white light for 10 h from one side (the arrows indicate the direction of light [L]). The left four photographs show the seedlings before the illumination and the right four photographs show those after the illumination.

Table IV. *Summary of phenotypes of sgr mutants*

−, No response; ±, reduced response; +, normal response.

Phenotype	*sgr1–1*	*sgr2–1*	*sgr3–1*
Gravitropism			
Inflorescence stem[a]	−	−	±
Hypocotyl[b]	−	±	+
Root	+	+	+
Phototropism			
Inflorescence stem[c]	+	+	+
Hypocotyl[c]	+	+	+
Growth rate of inflorescence stem	33%[d]	90%[d]	73%[d]
Other phenotypes	Small plant	Twisting stem	

[a] Response to 6 h of horizontal gravistimulation. [b] Response to 10 h of horizontal gravistimulation. [c] Phenotype of second positive curvature. First positive curvature was not examined (Khurana and Poff, 1989). [d] Percentage of mutant to wild-type.

Thereafter, auxin is transported asymmetrically to their basal part, which results in the phototropic curvature (Baskin et al., 1986). Our observation that shoots of all three *sgr* mutants can carry out phototropic curvatures (Figs. 3 and 10) suggests that the mechanisms for the transport and distribution of auxin for phototropic response are normal in these *sgr* mutants. In addition, the elongation of the stem segments of these *sgr* mutants were stimulated by exogenous auxin, suggesting that these *SGR* genes are not directly involved in the cell elongation induced by auxin (H. Fukaki, H. Fujisawa, and M. Tasaka, unpublished data). Therefore, it is probable that *SGR1*, *SGR2*, and *SGR3* gene products are acting on the gravity perception mechanism or the signal transduction mechanism such as that which regulates the auxin distribution within the gravistimulated shoots.

Gravitropic Responses of Inflorescence Stems and Hypocotyls Are Genetically Separable in Arabidopsis

A mutation at either the *SGR1* or the *SGR2* locus affected the gravitropic response of both inflorescence stems and hypocotyls, and a mutation at the *SGR3* locus affected only the gravitropic response of inflorescence stem (Figs. 1, 2, and 7–9). Unfortunately, other *sgr3* allelic mutants in addition to the *sgr3–1* mutant could not be obtained. It is possible that the *sgr3–1* mutant is a weak allele mutant, since the inflorescence stems showed reduced gravitropic responses (Figs. 1 and 2). Nevertheless, normal negative gravitropism of hypocotyls in the *sgr3–1* mutant is unaffected by this mutation (Figs. 7–9). These results suggest that some genetic components of the regulatory mechanisms for gravitropic responses are not the same in inflorescence stems and hypocotyls of *A. thaliana*. Recently, it was reported that the *PHYTOCHROME B* gene is required for normal hypocotyl gravitropism in *A. thaliana* (Liscum and Hangarter, 1993). However, we found that the *phyB* mutants showed normal negative gravitropism in inflorescence stems (H. Fukaki, H. Fujisawa, and M. Tasaka, unpublished data). This observation also supports our suggestion that inflorescence stem and hypocotyl gravitropism are geneti-

cally separable. We think that the different mechanisms for gravitropic responses between inflorescence stems and hypocotyls may be related to the fact that the developmental origin of inflorescence stems and hypocotyls are different; the former is differentiated from the shoot apical meristem originated from the apical domain of the early embryo, whereas the latter is the embryonic organ that differentiated from the central domain of the early embryo (West and Harada, 1993).

Additionally, our results that three classes of *sgr* mutants showed normal root gravitropism (Fig. 7) are also consistent with the idea that shoot and root gravitropism are genetically separable (Mirza et al., 1984; Bell and Maher, 1990; Bullen et al., 1990; Lincoln et al., 1990; Okada and Shimura, 1994). Thus, we propose that inflorescence stem, hypocotyl, and root gravitropism are genetically separable in *A. thaliana*.

ACKNOWLEDGMENTS

We wish to thank Y. Yamauchi for his help with the screening of *sgr1–2* and *sgr2–7* mutants. We are also grateful to Dr. T. Caspar and E.I. Du Pont de Nemours and Co. for the seed of T-DNA insertion lots. We also wish to thank Drs. W.R. Briggs and M. Filoso for critical reading of the manuscript and valuable suggestions.

Received July 21, 1995; accepted November 27, 1995.

LITERATURE CITED

Baskin TI, Briggs WR, Iino M (1986) Can lateral auxin redistribution account for phototropism of maize coleoptiles? Plant Physiol **81:** 306–309

Bell CJ, Maher EP (1990) Mutants of *Arabidopsis thaliana* with abnormal gravitropic responses. Mol Gen Genet **220:** 289–293

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

Estelle MA, Somerville CR (1987) Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology. Mol Gen Genet **206:** 200–206

Feldmann KA (1992) T-DNA insertion mutagenesis in Arabidopsis: seed infection/transformation. *In* C Koncz, N-H Chua, J Schell, eds, Methods in Arabidopsis Research. World Scientific, Singapore, pp 274–289

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

Fukaki H, Fujisawa H, Tasaka M (1996) Gravitropic response of inflorescence stems in *Arabidopsis thaliana*. Plant Physiol **110:** 933–943

Gaiser JC, Lomax TL (1992) The gravitropic tomato mutant *lazy-2* altered in signal transduction. *In* CM Karssen, LC van Loon, DV Vreugdenhil, eds, Progress in Plant Growth Regulation. Kluwer Academic, Dordrecht, The Netherlands, pp 928–937

Gaiser JC, Lomax TL (1993) The altered gravitropic response of the *lazy-2* mutant of tomato is phytochrome regulated. Plant Physiol **102:** 339–344

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

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

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

Khurana JP, Poff KL (1989) Mutants of *Arabidopsis thaliana* with altered phototropism. Planta **178:** 400–406

Leyser HMO, Lincoln CA, Timpte C, Lammer D, Turner J, Estelle M (1993) *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature **364:** 161–164

Lincoln C, Britton JH, Estelle M (1990) Growth and development of the *axr1* mutants of *Arabidopsis*. 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

Maher EP, Bell CJ (1990) Abnormal responses to gravity and auxin in mutants of *Arabidopsis thaliana*. Plant Sci **66:** 131–138

Mirza JI (1987) The effects of light and gravity on the horizontal curvature of roots of gravitropic and agravitropic *Arabidopsis thaliana* L. Plant Physiol **83:** 118–120

Mirza JI, Olsen GM, Iversen T-H, Maher EP (1984) The growth and gravitropic responses of wild-type and auxin-resistant mutants of *Arabidopsis thaliana*. Physiol Plant **60:** 516–522

Okada K, Shimura Y (1992) Mutational analysis of root gravitropism and phototropism of *Arabidopsis thaliana* seedlings. Aust J Plant Physiol **19:** 439–448

Okada 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

Pickett FB, Wilson AK, Estelle M (1990) The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. Plant Physiol **94:** 1462–1466

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

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, Keiber 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

Timpte CS, Wilson AK, Estelle M (1992) Effects of the *axr2* mutation of *Arabidopsis* on cell shape in hypocotyl and inflorescence. Planta **188:** 271–278

West MAL, Harada JJ (1993) Embryogenesis in higher plants: an overview. Plant Cell **5:** 1361–1369

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

Zobel RW (1974) Control of morphogenesis in the ethylene-requiring tomato mutant, diageotropica. Can J Bot **52:** 735–741