Mutations in the SGR4, SGR5 and SGR6 Loci of Arabidopsis thaliana Alter the Shoot Gravitropism

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Shoots of higher plants grow upward in response to gravity. To elucidate the molecular mechanism of this response, we have isolated shoot gravitropism (sgr) mutants in Arabidopsis thaliana. In this report, we describe three novel mutants, sgr4-1, sgr5-1 and sgr6-1 whose inflorescence stems showed abnormal gravitropic responses as previously reported for sgr1, sgr2 and sgr3. These new sgr mutations were recessive and occurred at three independent genetic loci. The sgr4-1 mutant showed severe defect in gravitropism of both inflorescence stem and hypocotyl but were normal in root gravitropism as were sgr1 and sgr2. The sgr5-1 and sgr6-1 mutants showed reduced gravitropism only in inflorescence stems but normal in both hypocotyls and roots as sgr3. These results support the hypothesis that some mechanisms of gravitropism are genetically different in these three organs in A. thaliana. In addition, these mutants showed normal phototropic responses, suggesting that SGR4, SGR5 and SGR6 genes are specifically involved in gravity perception and/or gravity signal transduction for the shoot gravitropic response.

Key words: Arabidopsis thaliana — Gravitropism — Inflorescence stem — Mutant.

Gravitropism is an adaptable mechanism by which plants regulate the orientations of growth in response to gravity. In higher plants, shoots show negative gravitropism (upward growth) and roots show positive gravitropism (downward growth). Many physiological and cytological events are associated with gravitropism (reviewed by Feldman 1985, Poovaiah et al. 1987, Poff et al. 1994, Kaufman et al. 1995); for example, the movement of amyloplasts is involved in gravity perception (Olsen et al. 1984, Song et al. 1988, reviewed by Sack 1991) and the asymmetric distribution of auxin at the elongation zone is thought to be the cause of the asymmetric growth for gravitropic curvature (Harrison and Pickard 1989, MacClure and Guilfoyle 1989, Parker and Briggs 1990, Li et al. 1991). However, the molecular basis for gravitropism is still unknown, including how plants recognize the direction of gravity and transmit this information to the elongation zone, and how the asymmetric distribution of auxin is established.

One approach to investigate the gravitropism at the molecular level is to isolate mutants with altered gravitropism. Many shoot and/or root gravitropism mutants have been isolated from several species of plants (reviewed by Roberts and Gilbert 1992, Okada and Shimura 1994). In Arabidopsis thaliana, at least seven genetic loci (SGR1, SGR2, SGR3, AXR2, PGM, DWF, COP4) have been identified to be involved in shoot gravitropism and nine genetic loci (AXR1, AXR2, AXR4(RGR1), AUX1, AGR, EIR1, PGM, DWF, COP4) involved in root gravitropism (reviewed by Fukaki et al. 1996a). An auxin resistant mutant axr2 and a starchless mutant pgm showed abnormal gravitropic responses in inflorescence stems, hypocotyls and roots (Caspar and Pickard 1989, Kiss et al. 1989, Wilson et al. 1990, Timpte et al. 1992). On the other hand, inflorescence stems and hypocotyls of sgr1-1 and sgr2-1 mutants showed no or reduced gravitropic response but their root showed normal response, whereas sgr3-1 mutant showed reduced gravitropism only in inflorescence stems (Fukaki et al. 1996b). These results suggest that some genetic components of the regulatory mechanisms for gravitropism are common to all three gravity-responsive organs, but others are not.

To elucidate the entire molecular system for shoot gravitropism, it is important to isolate as many agravitropic mutants as possible and to characterize them genetically and at the molecular level. In this study, we isolated and characterized three novel shoot gravitropism mutants at independent genetic loci.

Materials and Methods

Plant materials and growth conditions—Arabidopsis thaliana, Columbia ecotype, was the parental strain of ethyl methanesulfonate (EMS) mutagenized seed lots and the Columbia ecotype homozygous recessive for the glabrous1 (gli) mutation was the parental strain of fast neutron mutagenized seed lots.

For the experiments using inflorescence stems, plants were grown in a 1:1 mixture of perlite and vermiculite in vinyl pots under constant white light at 23°C with one-quarter-strength Arabidopsis mineral nutrient (Fukaki et al. 1996c). The light source was white fluorescent tubes (FL40S EX-N-T, Mitsubishi, Tokyo, Japan) and the light intensity was approximately 40 to 120 μmol m–2 s–1. Surface sterilized seeds were planted on Mura-shige-Skoog medium containing 0.5% (w/v) gellan gum for seedling experiments as described before (Fukaki et al. 1996b).

Mutagenesis and screening—Mutant strains sgr5-1 and sgr6-1 were derived from the EMS mutagenized M2 seeds harvested from about 4,500 M1 plants as described previously (Fukaki et al. 1996b).
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1996b). Finally, 14 independent mutant lines were isolated. Among them, eight mutant lines were described before as sgr1-1, sgr2-1, 2-2, 2-3, 2-4, 2-5, 2-6 and sgr3-1. The other four mutant lines showed weak gravitropic curvature in inflorescence stems but their elongation rates were seriously reduced. After some additional phenotypic analyses, we concluded that these are not shoot gravitropism mutants. The remaining two mutant lines, sgr5-1 and sgr6-1, were further analyzed in this study.

The sgr4-1 mutant was initially isolated by Drs. H. Tsukaya and T. Tuge from fast neutron mutagenized seed lots (Lehle Seeds). This mutant was screened for its characteristic phenotype of inflorescence stems and rosette leaves. We were kindly given its seeds by them and tested its tropic responses.

All data are based on the use of sgr mutants obtained after backcrossing with wild-type more than two times.

**Assay for tropic responses of inflorescence stems and seedlings**—To examine the tropic responses of inflorescence stems, plants with a primary inflorescence stem of 4 to 8 cm were selected. Stem segments were prepared and their tropic responses were examined as described before (Fukaki et al. 1996b). For the assay for phototropic responses, stem segments (with shoot apices and all lateral organs) were prepared from the distal 4 cm of primary inflorescence stems and illuminated with about 80 μmol m⁻² s⁻¹ white light from a horizontal direction. Gravitropic responses were examined with decapitated inflorescence stem segments, which were the distal 4 cm of primary inflorescence stems from which all lateral organs and approximately 5 mm of the apices including shoot meristems were removed.

Tropic responses of seedlings were examined in the plastic plates containing Murashige-Skoog medium. Surface sterilized seeds were planted on the medium, stored at 4°C for 2 to 3 days in darkness. Then, plates were stood vertically under white light at 23°C for 30 h to induce germination and incubated for 42 h in darkness. For the assay for gravitropic response, the plates were rotated through 90° to change the direction of gravity and angles between growing directions of hypocotyls at 0 min and at indicated times were measured. Phototropic responses were examined by illuminating seedlings with about 80 μmol m⁻² s⁻¹ white light from horizontal direction.

**Results**

*Isolation and genetic characterization of shoot gravitropic mutants*—When the inflorescence stems of Columbia wild-type were placed horizontally in darkness at 23°C, they were curved about 90° upward within 90 min. By screening for altered gravitropism mutants whose inflorescence stems showed either reduced or no gravitropic response to the 90 min horizontal gravistimulation, we have identified three genetic loci as SGR1, SGR2 and SGR3 which were involved in inflorescence stem gravitropism (Fukaki et al. 1996b). By additional screenings, two mutant lines (sgr5-1 and sgr6-1) were isolated from EMS mutagenized M₂ seeds and one line (sgr4-1) isolated from fast neutron mutagenized M₂ seeds.

Each new mutant line was crossed to wild-type plants and all plants in the F₂ generation showed normal gravitropic responses in the inflorescence stems. Abnormal gravitropic response phenotype segregated about one quarter in the F₂ generation, indicating that each mutant has a single recessive nuclear mutation (Table 1). The results of a complementation test made by crossing these mutants to each other and to the previously isolated sgr mutants are shown in Table 2. All F₂ plants showed normal gravitropic responses, indicating that three new mutations occurred at different genetic loci, named SGR4, SGR5 and SGR6.

**Tropic responses of inflorescence stems**—Decapitated inflorescence stem segments were prepared in order to examine the gravitropic responses. Fig. 1 shows the time courses of gravitropic curvatures of horizontally gravistimulated stem segments in wild-type and mutants. The angles of the direction of shoot apex between 0 min and the indicated times were measured. Stem segments of wild-type plants curved upward more than 90° in 90 min. In contrast, those of sgr4-1 showed only a slight gravitropic curvature (21.9 ± 4.3° [mean ± SE] upward) in 360 min. Moreover, inflorescence stems of intact plants showed almost no gravitropic curvature even 24 h after being placed horizontally (data not shown). These results indicate that the inflorescence stem gravitropism is severely affected in sgr4-1.

**Table 1** Gravitropic response phenotype in the F₂ generation

<table>
<thead>
<tr>
<th></th>
<th>Normal response</th>
<th>Abnormal response</th>
<th>( \chi^2 _a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>sgr4-1</td>
<td>129</td>
<td>30</td>
<td>2.04 ( b )</td>
</tr>
<tr>
<td>sgr5-1</td>
<td>104</td>
<td>28</td>
<td>1.01 ( b )</td>
</tr>
<tr>
<td>sgr6-1</td>
<td>137</td>
<td>45</td>
<td>0.01 ( b )</td>
</tr>
</tbody>
</table>

Gravitropic responses of primary inflorescence stems were examined in each F₂ progeny from crosses to wild-type plant.

* a expected ratio, 3 normal : 1 abnormal.  

 b \( P > 0.05 \).

**Table 2** Complementation analysis of sgr mutants

<table>
<thead>
<tr>
<th>Crosses</th>
<th>Normal response</th>
<th>Abnormal response</th>
</tr>
</thead>
<tbody>
<tr>
<td>sgr4-1 × sgr5-1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>sgr4-1 × sgr6-1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>sgr5-1 × sgr6-1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>sgr4-1 × sgr1-1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>× sgr2-1</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>× sgr3-1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>sgr5-1 × sgr1-1</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>× sgr2-1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>× sgr3-1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>sgr6-1 × sgr1-1</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>× sgr2-1</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>× sgr3-1</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>

Gravitropic responses of primary inflorescence stems were examined in each F₁ progeny from crosses between sgr mutants.
Decapitated inflorescence stem segments of sgr5-1 and sgr6-1 showed reduced gravitropic responses (Fig. 1). They began to bend within 60 min as did those of wild-type but their response was considerably slower. In 180 min, sgr6-1 curved 81.7 ± 5.8° and almost stopped bending thereafter (86.5 ± 3.5° in 24 h), whereas sgr5-1 curved slower than sgr6-1 (52.6 ± 4.2° in 180 min) and reached 64.2 ± 2.4° of curvature in 24 h. Inflorescence stems of intact sgr5-1 and sgr6-1 plants also showed very reduced gravitropic curvatures. Especially, those of sgr5-1 stopped bending before they reached 90° upward curvature (data not shown).

To test the phototropic responses of these mutants, their stem segments were stuck vertically and illuminated unilaterally with white light. Phototropic curvatures of wild-type, sgr5-1 and sgr6-1 were almost the same and the curvature of sgr4-1 was enhanced (Table 3). These data indicate that the mechanism of second positive phototropic responses of inflorescence stems is not directly affected by these sgr mutations.

When the wild-type plants of A. thaliana were grown under unilateral white light from the time prior to bolting, their inflorescence stems stood vertically upward in the basal region and curved toward the light in the apical region (Fig. 2A). In contrast, the inflorescence stems of sgr4-1, sgr5-1 and sgr6-1 tended to elongate straight toward the light from basal region (Fig. 2B, C, D). Such light-oriented growth of inflorescence stems was also observed in previously reported mutants, sgr1, sgr2 and sgr3.

**Morphological characteristics and growth rates**—Fig. 3 shows the aerial parts of wild-type and mutant plants. In these mutants, lateral branches elongated in a horizontal direction compared with those of wild-type, although those of sgr5-1 grew upward to some extent. This phenotype was also observed in sgr1-1, sgr2-1 and sgr3-1. Inflorescence stems of sgr4-1 elongated zigzag, which bent at the nodes and curved in the internodes (Fig. 3B). Its rosette leaves were small and wrinkled. However, we did not observe any morphological changes in sgr5-1 and sgr6-1 except the growing direction of lateral branches.

There is a possibility that the reduced gravitropic responses in the inflorescence stems of these mutants were caused by the reduced growth rates of their inflorescence stems as observed in dwarf mutants. To examine this possibility, growth rates of primary inflorescence stems of each

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**Table 3** Phototropic responses of inflorescence stem segments of wild-type and sgr mutants

<table>
<thead>
<tr>
<th></th>
<th>Curvature (degree)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Wild-type</td>
<td>12.9 ± 4.0</td>
</tr>
<tr>
<td>sgr4-1</td>
<td>59.5 ± 6.6</td>
</tr>
<tr>
<td>sgr5-1</td>
<td>5.0 ± 1.8</td>
</tr>
<tr>
<td>sgr6-1</td>
<td>13.4 ± 4.0</td>
</tr>
</tbody>
</table>

Inflorescence stem segments with shoot apex were stuck vertically and illuminated with white light from horizontal direction at 23°C. Phototropic curvatures were measured at the indicated times after the illumination of white light. At least 15 individual segments were examined in each. Data represents the mean ± standard error.
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Fig. 3 Aerial parts of wild-type and sgr mutant plants A, wild-type (5 weeks old); B, sgr4-1 (6 weeks old); C, sgr5-1 (5 weeks old); D, sgr6-1 (5 weeks old).

Table 4 Growth rate of the primary inflorescence stems of wild-type and sgr mutants

<table>
<thead>
<tr>
<th></th>
<th>Growth (mm)*</th>
<th>% of wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>172.8 ± 9.0</td>
<td>100</td>
</tr>
<tr>
<td>sgr4-1</td>
<td>103.5 ± 6.7</td>
<td>59.6</td>
</tr>
<tr>
<td>sgr5-1</td>
<td>144.8 ± 8.8</td>
<td>83.8</td>
</tr>
<tr>
<td>sgr6-1</td>
<td>154.2 ± 6.5</td>
<td>89.2</td>
</tr>
</tbody>
</table>

* Growth in seven days after the length of primary inflorescence stems were approximately 5 mm. At least 5 individual plants were examined in each. Data represent the mean ± standard error.

Fig. 4 Time courses for gravitropic responses of etiolated hypocotyls of wild-type and mutants. Seedlings were grown about 2 days after germination at 23°C and given gravitropic stimulation by rotating plastic plates through 90°. Gravitropic curvatures were determined at the indicated times thereafter. The vertical error bars represent the values of standard error. At least 5 individuals were examined in each genotype. C, wild-type; ●, sgr4-1; □, sgr5-1; ■, sgr6-1.

Mutant were determined (Table 4). Stems of sgr4-1 elongated more slowly than wild-type but both sgr5-1 and sgr6-1 showed no severe reduction in their growth rate.

Tropic responses of hypocotyls and roots—To examine whether the mutations in SGR4, SGR5 or SGR6 loci affect the gravitropism in hypocotyls and roots, surface sterilized seeds were germinated and grown on Murashige-Skoog medium in the plastic plates which stood vertically. In wild-type, angles between the growing directions of hypocotyls and the vertical were 8.0 ± 11.2° (mean ± SD). Three sgr mutants also grew almost upward (sgr4-1: 22.5 ± 20.9°, sgr5-1: 6.9 ± 6.4°, sgr6-1: 11.9 ± 15.1°). Growing directions of sgr4-1 hypocotyls were relatively random, but their range was within 90° from the vertical. Thereafter, etiolated seedlings were given different directional gravitropic stimulation by rotating plates through 90° and curvatures of hypocotyls were measured (Fig. 4). Hypocotyls of sgr4-1 showed reduced gravitropic curvature whereas sgr5-1 and sgr6-1 showed nearly the same curvature as wild-type seedlings in the 10 h horizontal gravistimulation. The gravitropic responses of roots were also examined. Roots of wild-type grew downward initially and curved in the direction of gravity when the plates were rotated through 90°. All sgr mutants showed normal positive gravitropism as wild-type (data not shown).

When etiolated seedlings were illuminated with white light from a horizontal direction, hypocotyls of wild-type seedlings curved and elongated straight toward the light whereas roots grew obliquely downward in response to both gravity and light (Okada and Shimura 1992). In all sgr mutants, hypocotyls and roots showed the same bending pattern as that of wild-type in response to unilateral light, indicating that the mutations in SGR4, SGR5 and SGR6 loci do not affect the phototropic responses in both hypocotyls and roots.

Discussion

In this study, we isolated and characterized three novel shoot gravitropism mutants. In sgr4-1, as in sgr1-1 and sgr2-1, inflorescence stems and hypocotyls of etiolated seedlings failed to achieve gravitropic curvature, whereas roots
showed normal gravitropic response. Moreover, in sgr5-1 and sgr6-1, as in sgr3-1, inflorescence stems showed reduced gravitropic responses but both hypocotyls and roots showed almost normal responses. The following characteristics were common to all sgr mutants (sgr1-1 to sgr6-1): (1) Altered gravitropic curvature of inflorescence stems. (2) Normal phototropic of inflorescence stems. Strong phototropic curvature of inflorescence stems of sgr4-1 (Table 3) seems to reflect their severe defect in gravitropism. Such phototropic responses were also observed in other gravitropism mutants such as sgr2-1 and tomato Lazy-1 mutant (Roberts 1984). Light oriented growth of sgr mutants is thought to be caused by a normal phototropic response in the reduced gravitropism background (Fig. 2). (3) Horizontal growth of lateral branches (Fig. 3). This characteristic suggests that the mechanism of gravitropism of lateral shoots is similar to that of primary inflorescence stems. These common characteristics in all sgr mutants are related to abnormal shoot gravitropisms.

The gravitropic response pathway can be separated into three steps: gravity perception, signal transduction and asymmetric growth in the elongation zone (reviewed by Feldman 1985, Björkman 1988, Kaufman et al. 1995). The pgm mutant cannot accumulate starch in amyloplasts, showing reduced gravitropic responses in inflorescence stems, hypocotyls and roots (Caspar and Pickard 1989, Kiss et al. 1989). This result suggests that the amyloplasts work as statoliths in all three gravitropic organs during gravity perception. All sgr mutants accumulated starch in amyloplasts of root caps (unpublished data), indicating that these are not starchless mutants. Furthermore, sgr1-1, sgr2-1 and sgr4-1 mutants were normal in root gravitropism, and sgr3-1, sgr5-1 and sgr6-1 mutants were normal in both hypocotyl and root gravitropism. These results suggest that all SGR genes are not directly involved in starch synthesis for amyloplast development related to gravity perception.

Asymmetric growth in both gravitropism and phototropism is considered to be caused by asymmetric auxin distribution (Baskin et al. 1986, Harrison and Pickard 1989, Parker and Briggs 1990). When auxin (2,4-D) was exogenously applied to seedlings, all sgr mutants showed almost the same auxin sensitivity as wild-type (unpublished data). Additionally, sgr mutants were normal in root gravitropism and in phototropism of all tropic organs. These results suggest that all the mutants can normally regulate elongation of inflorescence stems and hypocotyls in response to endogenous auxin. Therefore, it is probable that all SGR genes are acting on the gravity perception step or the signal transduction pathway.

In the roots, gravity is perceived at columella cells in the root cap and this signal is transduced to the epidermal or cortex cells in the elongation zone (Feldman 1985, Moore and Evans 1986). In the shoots, it is considered that gravity perception occurs in the endodermal cells around the vascular bundles, then the signal is transduced to the epidermal cells in the elongation zone (Sack 1991). Because the cell types and their organization in these organs are different, it is not unexpected that these organs would use different molecular components to transduce the gravitropic signal. The mutations at SGR1, SGR2 and SGR4 loci affected the gravitropic responses in both inflorescence stems and hypocotyls but not in roots, and the mutations at SGR3, SGR5 and SGR6 loci affected only the gravitropic response of inflorescence stems. Furthermore, the mutation at COP4 and the second mutation of the phyB-1 mutant affected the gravitropic responses in both hypocotyls and roots but not in inflorescence stems (Hou et al. 1993, Fukaki et al. 1996a). These facts support the hypothesis that some genetic components involved in gravitropism are different among these three organs in A. thaliana.

As previously reported, sgr1-1 had small leaves and thin, short inflorescence stems, and sgr2-1 had slightly twisted inflorescence stems and showed unusual embryo-genesis (Fukaki et al. 1996b, unpublished data). Inflorescence stems of sgr4-1 elongated in a zigzag fashion and rosette leaves were small and wrinkled (Fig. 3B). These features suggest that some SGR loci are concerned not only with shoot gravitropism but also with growth regulation of the plant. However, it is possible that sgr4-1 has a large deletion disrupting two or more genes since the mutagen used to produce sgr4-1 was fast neutrons (Sun et al. 1992, Lukowitz et al. 1996). In contrast, sgr5-1, sgr6-1 and sgr3-1 plants did not show any distinct morphological changes. It is probable that the SGR3, SGR5 and SGR6 loci are specifically involved in inflorescence stem gravitropism.

Up to now, our attempts to isolate shoot gravitropism mutants have resulted in mutations at six separate loci (SGR1 to SGR6). But alleles of some loci have not yet been found. This result suggests that many genes may be involved in the responses to gravity. To fully elucidate the molecular mechanism of shoot gravitropism, more sgr mutants should be studied. Recently, we have isolated some additional shoot gravitropism mutants and analysis of them is now in progress.

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