The RHG Gene Is Involved in Root and Hypocotyl Gravitropism in Arabidopsis thaliana

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In higher plants, shoots show negative gravitropism and roots show positive gravitropism. To elucidate the molecular mechanisms of root and hypocotyl gravitropism, we segregated the second mutation from the original phyB-1 mutant line which impaired both root and hypocotyl gravitropism and characterized this novel mutation named rhg (for root and hypocotyl gravitropism). The rhg mutant indicates that root and hypocotyl gravitropism are severely impaired but inflorescence stem gravitropism is not affected by the rhg mutation. In the rhg mutant seedlings, amyloplasts (statoliths for gravity-perception) were present in the presumptive statocytes of roots and hypocotyls. Phototropism by roots and hypocotyls was not impaired in the rhg mutant. These results suggest that the RHG gene product probably acts on the gravity-perception and/or the gravity-signal transduction in root and hypocotyl gravitropism. This is the first report about the genetic locus specifically involved in both root and hypocotyl gravitropism but not inflorescence stem gravitropism, supporting our hypothesis that the mechanisms of gravitropism are genetically different between hypocotyls and inflorescence stems.

Key words: Arabidopsis thaliana — Gravitropism — ROOT AND HYPOCOTYL GRAVITROPISM locus.

In higher plants, shoots show negative gravitropism (upward curvature) and roots show positive gravitropism (downward curvature). Although many studies on gravitropism have been performed, the molecular mechanisms of these phenomena are still unknown. To elucidate the molecular mechanisms of root and shoot gravitropism, several mutational analyses using Arabidopsis thaliana have been reported (Poff et al. 1994, Okada and Shimura 1994, Fukaki et al. 1996b, c, Yamauchi et al. 1997). In Arabidopsis thaliana, there are three gravitropic organs: inflorescence stems and hypocotyls which show negative gravitropism (Fukaki et al. 1996a, b), and roots which show positive gravitropism (Okada and Shimura 1992). Mutational analyses have identified several genes involved in the gravitropic response of one, two or all three organs (reviewed by Fukaki et al. 1996c). The fact that there are several types of mutants with the gravitropic defect in the specific organ(s) has suggested that in Arabidopsis the mechanisms for the gravitropic responses are genetically different among inflorescence stems, hypocotyls, and roots (Fukaki et al. 1996c). To understand such differences in the mechanisms(s), it is necessary to identify more genes specifically involved in the gravitropic response of one or two of three gravitropic organs.

Recently, it was reported that an original phyB-1 mutant of Arabidopsis thaliana, (previously called hy3-Bo64, Koornneef et al. 1980, Reed et al. 1993) exhibited abnormal hypocotyl gravitropism in darkness (Liscum and Hangarter 1993). After that, it was confirmed that this gravitropic defect was seen only in the original phyB-1 line but not in the other phyB alleles, suggesting that this abnormal hypocotyl gravitropism was due to a second mutation in the original phyB-1 line (Robson and Smith 1996, Poppe et al. 1996). However, this second mutation was not analyzed in detail in previous papers (Robson and Smith 1996, Poppe et al. 1996). In this study, we segregated this second mutation from the original phyB-1 line and characterized this novel mutation named rhg (for root and hypocotyl gravitropism). Phenotypic analyses showed that the RHG is a novel genetic locus specifically involved in both root and hypocotyl gravitropism. The rhg mutation supports our hypothesis that the mechanisms of gravitropism are genetically different between inflorescence stems and hypocotyls, although these two organs show negative gravitropism (Fukaki et al. 1996b, 1996c, Yamauchi et al. 1997).

Materials and Methods

Plant material and growth condition—Arabidopsis thaliana (L.) Heynh. ecotypes Landsberg erecta (Ler) and Columbia were used as wild-type plants. The original phyB-1 mutant seeds were obtained from the Nottingham Arabidopsis Seed Centre (The Univ. of Nottingham, Nottingham, U.K.). The other phyB alleles (phyB-4, -5, -6, -7, -8, -9, and -10) were obtained from the Arabidopsis Biological Resource Center (The Ohio State Univ. Columbus, U.S.A.). In the experiments on tropic responses of seedlings, seeds were sterilized and sown on the MS plates and then incubated for 3 d at 4°C in darkness as described previously (Fukaki et al. 1996b). To induce the germination of seeds, the plates were then placed for 24 h under about 80 μmol m⁻² s⁻¹ white light at 23°C. For the gravitropic response of inflorescence stems, seeds were sown in vinyl pots and grown under constant white light at 23°C.
phyB-1 mutant line — The second mutation in the original phyB-1 line was reported to result in abnormal hypocotyl gravitropism (Liscum and Hangarter 1993, Robson and Smith 1996). To know whether this second mutation also affects root gravitropism, the gravitropic response of the original phyB-1 mutant seedlings was examined. Figure 1 shows the gravitropic response of the dark-grown wild-type and original phyB-1 mutant seedlings. In wild-type seedlings, hypocotyls grew upright and roots grew downwards; corresponding to negative and positive gravitropism, respectively (Fig. 1A). In contrast, hypocotyls of the original phyB-1 mutant seedlings tended to be highly disoriented, exhibiting abnormal hypocotyl gravitropism as described previously (Liscum and Hangarter 1993, Robson and Smith 1996) (Fig. 1B). At the same time, roots of the original phyB-1 seedlings also tended to be highly disoriented, exhibiting abnormal root gravitropism (Fig. 1B). On the other hand, other phyB alleles exhibited normal root gravitropism (data not shown). These results suggest that the second mutation causes abnormal gravitropism in roots as well as in hypocotyls.

To segregate the second mutation from the original phyB-1 line, this line was backcrossed with wild-type Ler. All the F1 progeny seedlings showed normal hypocotyl and root gravitropism (number of individuals examined = 37). In the F2 progeny, the mutant seedlings exhibiting abnormal gravitropism in both hypocotyls and roots segregated about one quarter (number of seedlings, wild-type = 87; mutant = 88, \( x^2 \) [calculation based on 3:1 ratio of wild-type to mutant] = 0.008, P > 0.05) and there were no seedlings which showed abnormal gravitropism only in either hypocotyls or roots (at least in the population of 355 F2 seedlings). From these results and the mapping data (see below), it was concluded that the phenotype exhibiting abnormal gravitropism in roots and hypocotyls was caused by a single recessive nuclear mutation. We named this novel mutation rhg (for root and hypocotyl gravitropism). From the F2 rhg seedlings, a rhg mutant line was isolated which was confirmed to produce no phyB seedlings (long hypo-
Table 1 Mapping of the rhg mutation using SSLP markers

<table>
<thead>
<tr>
<th>SSLP marker</th>
<th>Linkage group</th>
<th>n</th>
<th>r±SE (%)</th>
<th>(Distance±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nga280</td>
<td>1 (middle, 136.8 cM)</td>
<td>92</td>
<td>28.3±4.7</td>
<td>(32.0±6.9 cM)</td>
</tr>
<tr>
<td>nga111</td>
<td>1 (bottom, 182.1 cM)</td>
<td>94</td>
<td>24.5±4.4</td>
<td>(26.8±5.8 cM)</td>
</tr>
<tr>
<td>nga168</td>
<td>2</td>
<td>54</td>
<td>48.1±6.8</td>
<td></td>
</tr>
<tr>
<td>nga162</td>
<td>3</td>
<td>28</td>
<td>67.9±8.8</td>
<td></td>
</tr>
<tr>
<td>nga8</td>
<td>4</td>
<td>54</td>
<td>51.9±6.8</td>
<td></td>
</tr>
<tr>
<td>nga139</td>
<td>5</td>
<td>52</td>
<td>48.1±6.9</td>
<td></td>
</tr>
</tbody>
</table>

The rhg mutant (ecotype Ler) was mapped in a cross to the ecotype Columbia using SSLP markers (Bell and Ecker 1994). n, number of examined chromosomes, r, recombination rate. The map position for nga280 and nga111 are referred to the Latest RI Map using Lister and Dean RI lines (Weeds World 1996 vol. 3 [ii]).* Web address, http://genome-www.stanford.edu/Arabidopsis/ww/Vol3ii/home.html

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The rhg (ecotype Ler) mutant was used for subsequent assays of gravitropism in this report. At the same time, the phyB-1 mutant lines exhibiting normal gravitropism in both roots and hypocotyls (not containing the rhg mutation in the background) were isolated.

For the mapping of the rhg mutation, SSLP markers were used (Bell and Ecker 1994). As shown in Table 1, the rhg mutation was linked to both nga280 and nga111 markers on the lower part of chromosome 1 (Bell and Ecker 1994). Around this region, no genetic loci involved in gravitropism have been known. Thus, the RHG is a newly identified locus in Arabidopsis which is involved in gravitropism.

**Gravitropic responses of the rhg mutant seedlings**—When the rhg seedlings were grown in MS plates in darkness, they exhibited the disoriented growth in hypocotyls and roots as seen in the original phyB-1 seedlings (Fig. 1B).

![Fig. 2 Frequency histograms of growth orientation of vertically-grown populations of wild-type and rhg seedlings with respect to the gravity. A, hypocotyls and B, roots. Seedlings of wild-type Ler and rhg mutant were grown for 3 d in darkness in the plates placed on edge. The angles between the growing direction of hypocotyls and the opposite direction of gravity, and the angles between the growing direction of roots and the direction of gravity were measured and the data were grouped every 20°. Each bar represents a 20° interval (20°=0-20°, etc.). Mean (M) and standard deviation (SD) values are shown. Numbers of examined; Ler=61, rhg=62.](image1)

![Fig. 3 Time courses for gravitropic responses of wild-type and rhg mutant seedlings. Dark-grown seedlings of wild-type Ler and rhg mutant in vertical were horizontally gravistimulated in darkness as described in text. O, Ler (root); rhg (root); D, Ler (hypocotyl); rhg (hypocotyl). The error bar represent the SE values. Numbers of examined seedlings; root (Ler=20, rhg=19); hypocotyl (Ler=31, rhg=32).](image2)
A root and hypocotyl gravitropism mutant in *A. thaliana*

Table 2 Growth rates of the dark-grown wild-type and *rhg* mutant seedlings

<table>
<thead>
<tr>
<th>Genotype (organ)</th>
<th>Growth rate (mm d⁻¹)</th>
<th>n</th>
<th>Growth rate relative to <em>Ler</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ler</em> (hypocotyl)</td>
<td>3.54±0.10</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td><em>rhg</em> (hypocotyl)</td>
<td>3.73±0.09</td>
<td>68</td>
<td>105</td>
</tr>
<tr>
<td><em>Ler</em> (root)</td>
<td>4.96±0.18</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td><em>rhg</em> (root)</td>
<td>3.56±0.11</td>
<td>68</td>
<td>72</td>
</tr>
</tbody>
</table>

Dark-grown seedlings prepared as described in text were used for measurement. Elongation of hypocotyls and roots during 24 h in darkness were measured, respectively. Values of Mean±SE are indicated. n, number of examined seedlings.

The frequency histograms in Figure 2 indicate that the growth orientation of *rhg* seedlings tended to be highly disoriented in both hypocotyls and roots whereas those of wild-type seedlings tended to be parallel to the vertical gravity vector (0°). When seedlings were given the new gravistimulation by rotating the plates 90°, both hypocotyls and roots of wild-type exhibited distinct gravitropic curvatures (Fig. 3). In contrast, both hypocotyls and roots of the *rhg* mutant exhibited reduced gravitropic curvatures (Fig. 3). Table 2 indicates that the growth rates of dark-grown seedlings. While the growth rate of the *rhg* hypocotyls was almost the same as that of wild-type *Ler*, that of the *rhg* roots was slightly reduced (growth rate relative to *Ler*, 72%). Although this reduced growth rate of the *rhg* roots may partially contribute to the reduced root gravitropic curvature, the gravitropism of vertically-grown *rhg* roots was abnormal (Fig. 1B, 2B). These results indicate that the *rhg* mutant is severely impaired in root and hypocotyl gravitropism.

Gravitropic responses of the *rhg* mutant shoots

—in *Arabidopsis thaliana*, inflorescence stems also showed negative gravitropic curvatures when placed horizontally in darkness (Fukaki et al. 1996a). In the *rhg* mutant, the primary inflorescence stems grew upright and their lateral branches grew upward, exhibiting normal negative gravitropism as well as those of wild-type *Ler*. When inflorescence stems of the *rhg* mutant were placed in the horizontal position in darkness (Fig. 4B), the negative gravitropic curvature occurred rapidly as well as those of wild-type (Fig. 4C, D). Figure 5 shows the time courses for gravitropic responses of wild-type and *rhg* mutant inflorescence stems during horizontal gravistimulation. The kinetics of *rhg* mutant was almost the same as that of wild-type (Fig. 5). These results indicate that inflorescence stem gravitropism is not affected by the *rhg* mutation. Moreover, because the *rhg* mutant shoots could not be phenotypically

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**Fig. 4** Gravitropic response of inflorescence stems of wild-type and *rhg* mutant. A and C, wild-type *Ler*; B and D, *rhg*. Inflorescence stems attached to the plant were gravistimulated horizontally in darkness by laying the pots down. A and B represent the plants at the start of the gravistimulation. C and D represent the plants 3 h after the gravistimulation. The arrow indicates the direction of gravity (g).

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**Fig. 5** Time courses for gravitropic responses of inflorescence stems of wild-type and *rhg* mutant. The intact primary inflorescence stems attached to the plants were horizontally gravistimulated in darkness. The time courses for the change in angle of the tip of the stems are shown. The angles between the growing directions both at the start of the gravistimulation and at each time were measured as the curvature. The error bars represent the SE values. At least nine stems were examined in each genotype.
distinguished from wild-type shoots, shoot development also appears to be unaffected by the rhg mutation.

Phototropic responses of the rhg mutant seedlings—It was examined whether the rhg mutation also impairs the phototropic responses of seedlings. Figure 6 represents the phototropic responses of wild-type and the rhg seedlings. When seedlings of wild-type were illuminated with white light for 4 d from one side after germination, hypocotyls grew toward the direction of the light, exhibiting positive phototropism and roots grew diagonally downward, exhibiting negative phototropism (Okada and Shimura 1992, 1994). In contrast, in the rhg mutant seedlings, hypocotyls grew toward the light as well as those wild-type and roots grew toward the opposite direction of the light (Fig. 6). Similarly, when dark-grown seedlings of wild-type in the vertical position were illuminated with white light for 24 h from one side, hypocotyls exhibited a positive phototropic curvature and roots exhibit a negative phototropic curvature (Table 3). Hypocotyls of the rhg mutant seedlings also exhibited the same positive phototropic curvature as

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**Fig. 6** Phototropism of wild-type and rhg mutant seedlings. Seedlings of wild-type Ler (upper two lines) and rhg mutant (lower two lines) were illuminated with white light for 4 d from one side (the arrows indicate the direction of light [L] and gravity [g]) after germination.

**Fig. 7** Whole mounts of dark-grown wild-type and rhg mutant hypocotyls and roots stained for starch with IKI. Hypocotyl of wild-type Ler (A) and rhg mutant (B); Root tip of wild-type Ler (C) and rhg mutant (D). Arrows indicate the stained amyloplasts. Rc, root cap; En, endodermis. Bar = 50 μm.
Table 3 Phototropic responses of the dark-grown wild-type and rhg seedlings

<table>
<thead>
<tr>
<th>Curvature (degrees)</th>
<th>Hypocotyl</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>77.6±3.4</td>
<td>30.6±4.4</td>
</tr>
<tr>
<td>rhg</td>
<td>74.3±3.2</td>
<td>64.7±5.4</td>
</tr>
</tbody>
</table>

Dark-grown seedlings of wild-type Ler and rhg mutant in vertical position were illuminated with white light from one side as described in text. After the illumination for 24 h, positive phototropic curvatures of hypocotyls and negative phototropic curvatures of roots were measured. Values of Mean ±SE of the curvature are shown. Numbers of examined seedlings; (hypocotyl) n=19, (root) n=25.

Discussion

In this study, we characterized the novel rhg mutation segregated from the original phyB-1 mutant line of Arabidopsis which caused the abnormal gravitropism in roots and hypocotyls. Our analyses indicated that root and hypocotyl gravitropism were severely impaired but inflorescence stem gravitropism was not affected by the rhg mutation.

In general, the mechanism of the gravitropic response can be separated into three sequential steps; gravity perception, signal transduction, and an asymmetric growth response (reviewed by Feldmann 1985, Roberts and Gilbert 1992, Kaufman et al. 1995, Fukaki et al. 1996c). Among these steps, the asymmetric growth is the common final response in the gravitropic responses, which follows their upstream pathway(s) including the signal (gravity or light) perception and the signal transduction. The rhg mutation impaired both root and hypocotyl gravitropism (Fig. 2, 3) but did not affect both root and hypocotyl phototropism (Fig. 6 and Table 3). These results indicate that the RHG gene is not directly involved in the mechanism of the asymmetric growth responses of roots and hypocotyls. Furthermore, the rhg mutant had amyloplasts which function as statoliths for the gravity perception in the presumptive statocytes of roots and hypocotyls (Fig. 7). This observation indicates that the RHG gene is not involved in the statolith mechanism for the gravity perception. From these, we think that the RHG gene product probably acts on the gravity perception and/or the gravity-signal transduction in root and hypocotyl gravitropism.

The characteristics of gravitropism are different between positive gravitropic (roots) and negative gravitropic organs (hypocotyls and inflorescence stems). Statocytes in roots are columnella cells of the root cap, which is not located in the elongation zone exhibiting a gravitropic curvature (reviewed by Sack 1991, Kiss et al. 1996). On the other hand, presumptive statocytes in both hypocotyls and inflorescence stems are starch sheath cells consisting of the endodermis, which is located through the elongation zone exhibiting a gravitropic curvature (Kiss and Sack 1990, reviewed by Sack 1991, Fukaki, Fujisawa and Tasaka unpublished data). These results suggest that the mechanisms of gravitropism are different between roots and hypocotyls but similar between hypocotyls and inflorescence stems. However, the RHG gene would play an essential role(s) as a common component for root and hypocotyl gravitropism but not inflorescence stem gravitropism.

In Arabidopsis, the RHG gene is the first characterized genetic locus specifically involved in root and hypocotyl gravitropism but not inflorescence stem gravitropism (reviewed by Fukaki et al. 1996c). The existence of this rhg class of mutants supports our hypothesis that the mechanism of inflorescence stem gravitropism is genetically different from that of hypocotyl gravitropism in Arabidopsis (Fukaki et al. 1996b, c). This hypothesis is also supported by the other class of mutants such as the sgr3, sgr5, and sgr6 which show abnormal gravitropism in inflorescence stems but show normal gravitropism in hypocotyls (Fukaki et al. 1996b, Yamauchi et al. 1997). It is unknown what
differences there are in the molecular mechanisms between two negative gravitropic organs (hypocotyls and inflorescence stems). Isolation and characterization of the RHG gene will be helpful to understand the role of this gene in root and hypocotyl gravitropism and to clarify the differences in the mechanisms of gravitropism between hypocotyls and inflorescence stems.

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References