

Interaction of Root Gravitropism and Phototropism in Arabidopsis Wild-Type and Starchless Mutants¹

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Root gravitropism in wild-type *Arabidopsis* and in two starchless mutants, *pgm1-1* and *adg1-1*, was evaluated as a function of light position to determine the relative strengths of negative phototropism and of gravitropism and how much phototropism affects gravitropic measurements. Gravitropism was stronger than phototropism in some but not all light positions in wild-type roots grown for an extended period, indicating that the relationship between the two tropisms is more complex than previously reported. Root phototropism significantly influenced the time course of gravitropic curvature and the two measures of sensitivity. Light from above during horizontal exposure overestimated all three parameters for all three genotypes except the wild-type perception time. At the irradiance used ($80 \mu\text{mol m}^{-2} \text{s}^{-1}$), the shortest periods of illumination found to exaggerate gravitropism were 45 min of continuous illumination and 2-min doses of intermittent illumination. By growing roots in circumlateral light or by gravistimulating in the dark, corrected values were obtained for each gravitropic parameter. Roots of both starchless mutants were determined to be about three times less sensitive than prior estimates. This study demonstrates the importance of accounting for phototropism in the design of root gravitropism experiments in *Arabidopsis*.

It is well established that shoot growth is oriented by the combined vectors of gravity and light (Hangarter, 1997). Despite reports of root phototropism for over a century (Müller, 1876; Darwin, 1880), the existence of significant negative phototropism in roots was not widely appreciated until the work of Okada and Shimura (1992) with *Arabidopsis*, and interactions between root phototropism and gravitropism have only been shown for a few genera (Mohr, 1961; Schneider, 1964; Okada and Shimura, 1994).

Most such studies examined the effects of prolonged exposure to unilateral light on roots growing in an equilibrium orientation with respect to gravity (Okada and Shimura, 1992; Liscum and Briggs, 1996; Fukaki et al., 1997). For example, *Arabidopsis* roots that had been grown in a vertical orientation were then illuminated unilaterally for 2 d so that the light and gravity vectors acted at right angles. In wild-type roots, the new angle achieved was

intermediate (Okada and Shimura, 1992, 1994). Roots of mutants defective in gravitropism were oriented farther away from the light than the wild type (Okada and Shimura, 1994; Fukaki et al., 1997).

Okada and Shimura (1992, 1994) found that the strengths of phototropism and gravitropism were comparable in *Arabidopsis* roots. But this was determined using lateral light, and the relationship between the tropisms was not analyzed with light in other positions.

In addition, the effects of shorter periods of directional illumination have not been reported for roots of any genotype, especially in conjunction with reorienting roots with respect to gravity. The most frequently determined gravitropic parameter is the time course of curvature after reorientation. Measures of sensitivity include the presentation time (response to a single dose of horizontal stimulation) and the perception time (summed response to repeated doses, i.e. intermittent stimulation; Sack, 1991). The duration of gravitropic stimulation ranges from days (for the time course) to seconds (for intermittent stimulation). In many studies, the light was overhead during the period of horizontal stimulation (e.g. Kiss et al., 1989, 1996; Kiss and Sack, 1989; Blancaflor et al., 1998). If negative phototropism had a significant effect on root growth during these periods, then the extent of gravitropism would have been overestimated. It is important to determine whether directional illumination affects the measurement of all three gravitropic parameters to evaluate the accuracy of many published values.

Accurate values for gravitropic sensitivity are necessary to evaluate hypotheses of sensing. Roots of the starchless *pgm1-1* mutant of *Arabidopsis* have been estimated to be 12 times less sensitive than the wild type (Kiss et al., 1989). While these data support the idea that starch and amyloplast sedimentation are important for sensing, they also reveal the presence of residual sensing in roots that are starchless (Sack, 1997). If directional illumination influenced the above estimates of sensitivity, then it could have misrepresented the degree of residual sensing present in starch-deficient genotypes.

To evaluate the relative strengths of gravitropism and phototropism and the accuracy of previous gravitropic measurements, we analyzed the responses of roots to different positions of exposure to unidirectional light and gravity and to varying periods of illumination. In addition to studying the responses of wild-type *Arabidopsis* and

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pgm1-1 mutant roots, we also analyzed the responses of the *adg1-1* mutant, which has been described as starchless based on light microscopy (Lin et al., 1988). We report that light position significantly influences the measurement of virtually every gravitropic parameter tested, that gravitropism can predominate over phototropism under some conditions in wild-type roots, and that *pgm1-1* roots are less gravitropic than previously estimated.

MATERIALS AND METHODS

Plant Material and Culture

The starch-deficient and recessive mutant alleles *pgm1-1* (also known as TC75) and *adg1-1* (also known as TL255) are defective in plastidic phosphoglucomutase and in the small subunit of ADP Glc-pyrophosphorylase, respectively (Casper et al., 1985; Lin et al., 1988; Wang et al., 1998). Both lines were backcrossed five times and were in the Columbia ecotype of *Arabidopsis*.

Seeds were sown in square polystyrene Petri dishes (100 × 100 × 15 mm) on 1% (w/v) agar containing nutrients supplemented with 1% (w/v) Suc (Kiss and Sack, 1990). The dishes were sealed with laboratory film and placed on edge so that the surface of the agar was vertical. Except for some seedlings grown in darkness for 4 d, seedlings were illuminated continuously (irradiance 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) by two 40 W cool white fluorescent lamps (General Electric, Fairfield, CT). For measurements of gravitropism (time course, presentation time, and intermittent stimulation) and of root growth rate, seedlings were grown with light from above until the roots were 5 to 13 mm long. Wild-type and *pgm1-1* roots reached this length after a total of 70 h of illumination. *adg1-1* roots grew more slowly and took 10 h longer to reach a comparable length. In other experiments, freshly plated seeds were placed in racks or in a stationary clinostat and illuminated continuously from above, from below, or from the side.

All experiments were performed at ambient temperatures of 22°C to 24°C. Illuminated dishes were cooled continuously by an electric fan. Thermistor probe measurements indicated that the temperature inside the dishes was $\leq 2^\circ\text{C}$ higher than ambient and failed to detect any temperature gradients within the dishes.

Measurements of Gravitropism

Petri dishes with seedlings were mounted on custom-built, 1-rpm clinostats. Two 20 W fluorescent lamps were positioned at a fixed distance from the clinostat so that the irradiance at the center of the dish was 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

For the time course, dishes were turned 90° so that the seedlings were horizontal, maintained on a stationary clinostat, and illuminated either from above (Fig. 1, A and B) or from below (Fig. 1C). A third set of plants was illuminated "circumlaterally" on a vertical, rotating clinostat (Fig. 1D). Seedlings were photographed intermittently using Technical Pan film (Eastman-Kodak, Rochester, NY). Curvatures of individual roots were measured as an increment over the initial angle from digitally scanned negatives

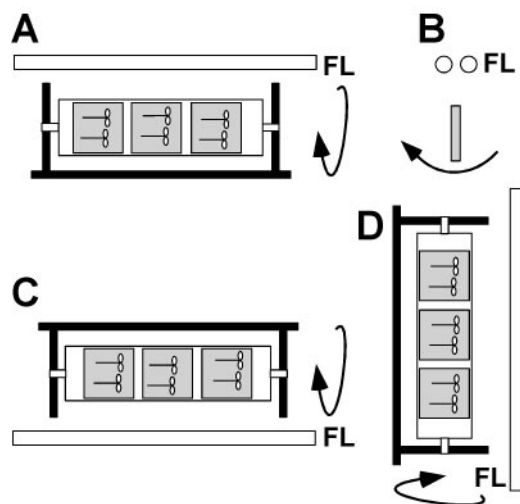


Figure 1. Schematic drawing of the three different illumination configurations used. Petri dishes were mounted on a clinostat and illuminated with two fluorescent lamps (FL). The direction of clinostat rotation is shown by arrows. Both the clinostat and lamps were mounted in a metal frame (not shown) so that the orientation of the whole apparatus could be changed while maintaining the distance between the dishes and lamps. A to C, Clinostat in horizontal orientation with light from above (A, side view; B, end view) or below (C). D, Clinostat in a vertical orientation. For measurement of the time course, seedlings were exposed to "circumlaterally" light upon rotation. The gravity vector is toward the bottom of all figures.

using the public domain NIH Image program (developed at the United States National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image>).

Measurements of presentation times were performed essentially as in Kiss et al. (1989), except that three different illumination modes were used. Seedlings that were grown in a vertical orientation with light from above were turned to the horizontal and immediately placed on the clinostat and left stationary for 2 to 120 min. During this period of horizontal gravistimulation, the roots were illuminated either from above or from below (Fig. 1, A and C). A third set of plants was kept dark during the period of horizontal stimulation. All plants were illuminated during the period of clinostat rotation that followed the stationary gravistimulation. Seedlings were photographed before gravistimulation and then after 2.25 and 3.5 h of clinostat rotation (without stopping the clinostat). The mean root angle after 2.25 and 3.5 h of rotation was then used to determine curvature as an increment over the initial angle.

Two different light treatments were used for intermittent stimulation experiments. In one treatment, roots were illuminated from above during repeated horizontal, stationary gravistimulation (Fig. 1, A and B). In the second treatment, roots were in the dark during each horizontal gravistimulation. In both cases, the roots were illuminated during clinostat rotation (horizontal clinostat, Fig. 1, A and B). As with the presentation time, curvature was measured for each root as an increment over the initial angle. Additional details are described in Kiss et al. (1989). Statistical analyses used Prophet software (BNN Technologies, Cambridge,

MA). The non-parametric Kruskal-Wallis test was used for multiple comparisons.

Characterization of Starch Deficiency

Seedlings were grown on agar for 3 d with continuous light from above, fixed overnight in 3.7% (w/v) formaldehyde, and rinsed, and then whole root tips were stained with I₂KI solution (2% [w/v] KI, 1% [w/v] I) and viewed with a microscope (model IM35, Zeiss, Oberkochen, Germany). Some fixed roots were embedded in Spurr's epoxy resin, and 2- μ m sections were stained with I₂KI or periodic acid-Schiff's reagent (O'Brien and McCully, 1981). For electron microscopy, *adg1-1* roots (grown for 3 d in continuous light) and leaf pieces (20-d-old seedlings grown in continuous light on Promix substrate [Premier Horticultural, Red Hill, PA]) were processed as in Sack and Kiss (1989). Thin sections were viewed using either a Zeiss 10C (Jena, Germany) or a Philips CM12 (Eindhoven, The Netherlands) transmission electron microscope.

RESULTS AND DISCUSSION

adg1-1 Rootcap Cells Lack Starch

Several low- or intermediate-starch mutants have been used to study root gravitropism in maize, tobacco, and Arabidopsis (Moore, 1987; Kiss and Sack, 1989; Kiss et al., 1996). But the only published data on the gravitropism of starchless mutants are for two different alleles of *pgm1* in Arabidopsis (Caspar and Pickard, 1989; Kiss et al., 1989, 1996). The *adg1-1* mutant of Arabidopsis was reported to be starchless based on light microscopy (Lin et al., 1988), but no ultrastructural data are available. Roots of this mutant were reported to respond to gravity (Caspar and Pickard, 1989), but their sensitivity has not been documented.

To determine whether *adg1-1* is starchless, leaf and rootcap tissues were evaluated using light and electron microscopy. Neither starch nor plastid sedimentation was detected in columella cells of *adg1-1* roots (Fig. 2A; light microscopy not shown), and *adg1-1* resembles *pgm1* in these phenotypes (Kiss et al., 1989, 1996; MacCleery and Kiss, 1999). Thus, *adg1-1* provides an additional mutant at a second locus that can be used to analyze the effect of the elimination of columella starch on gravitropism.

Although not directly relevant to gravitropic studies, small starch grains appear present in some leaf mesophyll chloroplasts in *adg1-1* (Fig. 2, B and C). Murgia et al. (1993) also found trace amounts of starch in the *adg1-1* embryo sac. Presumably, such low levels of starch are below the sensitivity of detection of the biochemical assays originally used to conclude that *adg1-1* leaves contain less than 2% starch of the wild type (Lin et al., 1988). *pgm1-1* appears to completely lack starch in the mesophyll (Lascève et al., 1997).

Relative Strength of Tropisms Depends on Light Position in Wild-Type Roots

According to the vector hypothesis of Okada and Shimura (1992, 1994), root phototropism and gravitropism

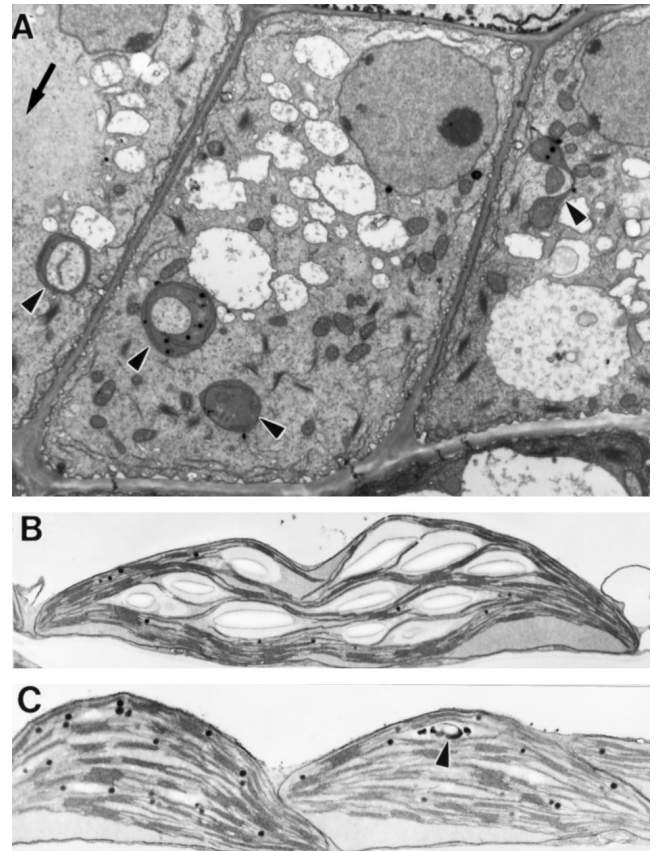


Figure 2. Electron micrographs showing plastids in root columella cells (A) and leaves (B and C) in *adg1-1* (A and C) and in the wild type (B). A, *adg1-1* columella cells contain starchless, mostly un sedimented plastids (arrowheads). Arrow (upper left) indicates the gravity vector. B, Wild-type mesophyll chloroplasts have abundant starch, whereas those of *adg1-1* (C) are severely deficient in starch but occasionally contain what appear to be small starch grains (arrowhead). Magnifications: A, $\times 6,000$; B, $\times 9,500$; and C, $\times 11,000$.

are additive. Thus, the resulting angle of root growth is the sum of responses to the gravity and light vectors. Factors influencing vector magnitude include the force of the physical stimulus and the magnitude of the tropic response. Okada and Shimura (1992, 1994) studied the equilibrium responses of vertically grown Arabidopsis roots to lateral illumination using stimuli of single magnitudes (1g and 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Because wild-type roots grew at an intermediate angle, they concluded that the magnitudes of positive gravitropism and negative phototropism were equal and additive in roots. In contrast, positive phototropism was found to dominate negative gravitropism in hypocotyls.

To test further whether the magnitudes of both tropic responses are comparable in roots, we varied the direction of illumination while holding the physical stimuli constant (1g and 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Wild-type seedlings were grown for 3 to 4 d (starting from germination) in Petri dishes positioned so that the surface of the agar was vertical and with the light coming either from the side (gravity and light vectors acting at 90°) or from below (opposing vectors at 180°).

Table I. Root angles from seedlings grown in darkness or in continuous light from different directions

All seedlings were grown on a vertical agar surface and were not reoriented. Light treatments started at planting. 0°, Toward gravity; 180°, away from gravity. Values are means \pm SD, with sample sizes shown in parentheses.

Genotype	Light Treatment			
	Darkness 4 d	Light from above 3 d	Lateral light 3 d	Light from below 4 d
Wild type	12.2 \pm 9.7 (102)	12.7 \pm 9.5 (333) ^a	44.0 \pm 17.3 (126)	37.0 \pm 42.89 (113)
<i>pgm1-1</i>	25.8 \pm 20.7 (118) ^a	15.3 \pm 11.8 (278) ^a	61.3 \pm 22.7 (138) ^a	139.0 \pm 82.2 (91) ^a
<i>adg1-1</i>	28.5 \pm 35.6 (92) ^a	14.0 \pm 11.1 (193) ^a	65.7 \pm 16.0 (76) ^a	129.4 \pm 70.8 (81) ^a

^a Denotes means within each treatment that are not different statistically ($\alpha = 0.05$; Kruskal-Wallis test).

As in Okada and Shimura (1992, 1994), with the vectors at right angles (light from the side), the mean root angle was intermediate (44°; Table I; Fig. 3), which is consistent with the strength of both tropisms being equal. If a comparable relationship also applied when the light came from below, then the mean root angle should be 90°, which would be intermediate between 0° (full positive gravitropism) and 180° (full negative phototropism). Instead, the absolute angle of 37° for wild-type roots was comparable to or lower than when the vectors acted at right angles (Table I; Fig. 4). This suggests that gravitropism predominates over phototropism in roots when the light comes from below, and there seems to be a limit to how much negative phototropism can overcome positive gravitropism. Perhaps the regulation of the gravitropic set-point angle tolerates only a limited divergence from the vertical.

The conclusions in this and subsequent sections apply to the conditions employed. The irradiance was chosen because it is comparable to that used in many measurements of gravitropism (e.g. Kiss et al., 1989; Okada and Shimura, 1992), and a major purpose of this study was to assess the accuracy of existing values for gravitropism. It remains to be seen whether the same relationship between tropisms holds when different magnitudes of physical stimulation are used. For example, irradiance influences the strength of the phototropic response in many species, and it regulates gravitropism in moss protonemata (Hangarter, 1997; Kern and Sack, 1999). In any case, in *Arabidopsis* roots, the relationship between phototropism and gravitropism is more complex than previously described.

Comparison of Wild-Type and Mutant Equilibrium Responses

As in the wild type, the phototropic positioning of roots of the starchless mutants *pgm1-1* and *adg1-1* is limited by gravitropism, but to a lesser degree. In lateral light, roots of both mutants grew away from the vertical to roughly the same extent, and both diverged from the vertical more than the wild type (Table I, Fig. 3). Unlike wild-type roots, in light from below, *pgm1-1* and *adg1-1* roots showed a net orientation above the horizontal (Table I). Moreover, most mutant roots grew above the horizontal, whereas wild-type roots only rarely did so (Fig. 4).

This greater divergence of starchless mutant roots from the vertical compared with the wild type could be due to reduced gravitropism. Indeed, other *Arabidopsis* mutants thought to be defective in root gravitropism, such as *aux1* and *rhg*, show similar responses to extended unilateral illumination (Okada and Shimura, 1992; Fukaki et al., 1997). Conversely, mutants apparently defective in root phototropism grow closer to the vertical than the wild type in lateral light (Okada and Shimura, 1992, 1994; Liscum and Briggs, 1996). These data support the vector hypothesis in that a reduction in the strength of one tropism increases the net effectiveness of the other tropism (Okada and Shimura, 1994).

But because both tropisms interact, it cannot be ruled out that instead of the mutations depressing one tropism, they actually enhance the other tropism. In principle, mutations in starch synthesis might promote root phototropism rather than reduce gravitropism.

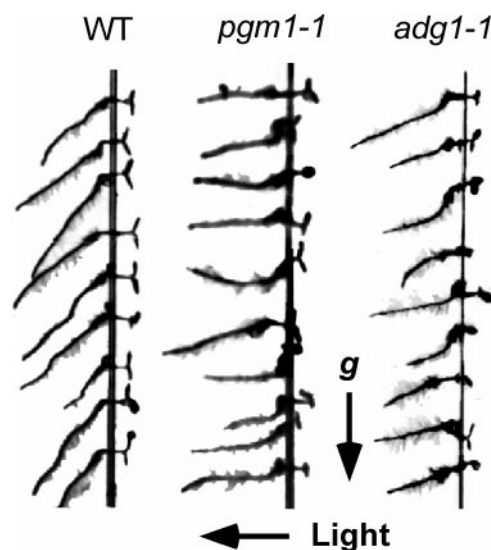


Figure 3. Negative phototropism of *Arabidopsis* roots. Seedlings were germinated and grown for 3 d in unilateral white light. The starchless mutants were strongly negatively phototropic compared with wild-type (WT) roots, which exhibited more gravitropism. Seedlings are shown in outline from digitized photographs with contrast enhancement.

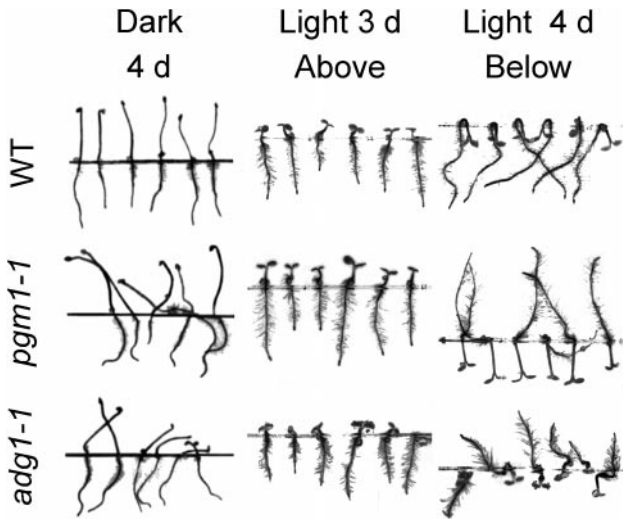


Figure 4. Extended growth of seedlings germinated and grown in continuous darkness or in light either from above or below. Dark-grown mutant roots deviated more from the vertical than those of the wild type. With light from above, roots of all three genotypes seemed to grow uniformly toward gravity. However, light from below caused the mutant roots to grow away from the light and above the horizontal, whereas wild-type roots grew partly away from the light but still below the horizontal. Note the positive phototropism of all light-grown hypocotyls.

It is also important to determine the extent of root phototropism, because Caspar and Pickard (1989) reported that light enhanced gravitropism in *pgm1-1*. They attributed this light promotion to effects independent of phototropism, such as altered carbohydrate reserves or the modulation of gravity sensing by light.

One way to test these hypotheses is to compare the responses of roots grown in the dark with those illuminated from above. When roots were grown so that gravitropism and phototropism acted in the same direction (light from above for 3 d), the roots grew away from the light and toward the gravity vector, and the mean angles and sds of all three genotypes were comparable (Fig. 4;

Table I). Roots grown entirely in the dark were gravitropic in all three genotypes (Fig. 4; Table I). In wild-type roots, the mean angles and the sds were the same in both the light and the dark. But dark-grown starchless mutants were on average about 15° further away from the vertical than light-grown roots, and their sds were much greater in the dark than in the light.

These data allow several conclusions. For the wild type, they suggest that there is no difference between the effectiveness of gravitropism in the dark and in the light. This indicates that when root phototropism and gravitropism act together (light from above) in the wild type, that light has no net effect on the root angle. Presumably, root phototropism is operating simultaneously but the fidelity of the gravitropic response may have been so high that an additional orienting factor cannot reduce the remaining variability in root positioning. This indicates that the use of light from above is not a methodological problem in gravitropism studies with wild-type roots grown for extended periods, because the light in this position does not exaggerate root gravitropism.

In contrast, in the starchless mutants, phototropism has a strong effect on the direction of root growth with light from above. Light from the side or below also has a much stronger effect on the positioning of *pgm1-1* and *adg1-1* roots than on the wild type. Gravitropism appears defective in roots of the starchless mutants, because light from above does not affect the gravitropism of wild-type roots compared with the dark, whereas it does improve the orientation of mutant roots (Table I). In the starchless mutants, negative phototropism probably acts cooperatively with positive gravitropism with light from above, because gravitropism is weaker and not saturated. Because negative phototropism masks defective gravitropism in the starchless mutants, the use of light from above for extended periods is a methodological problem in studying gravitropism in these mutants. Moreover, this masking argues against the idea that light promotes gravitropism in *pgm1-1* independent of phototropism (Caspar and Pickard, 1989).

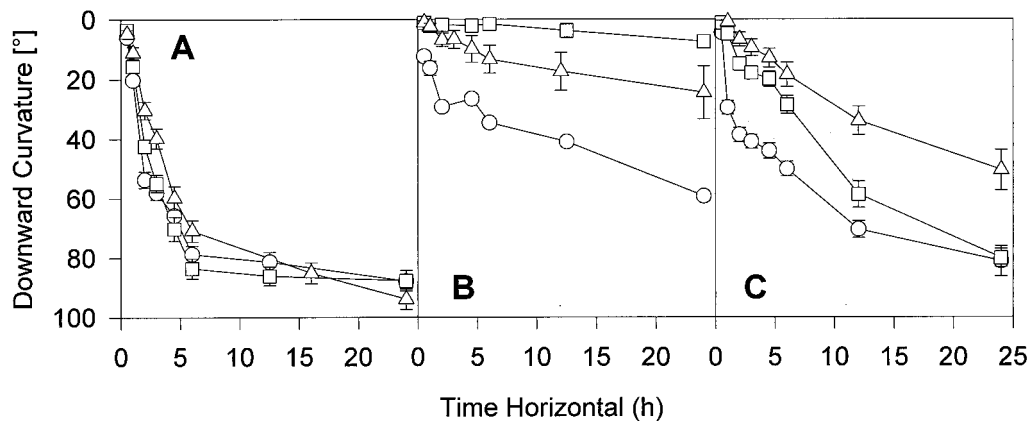


Figure 5. Time course of gravitropic curvature as a function of light position. Roots were turned to the horizontal and illuminated from above (A), below (B), or “circumlaterally” (C; see Fig. 1D). Angles are means ± SE. ○, Wild type; □, *pgm1-1*; △, *adg1-1*.

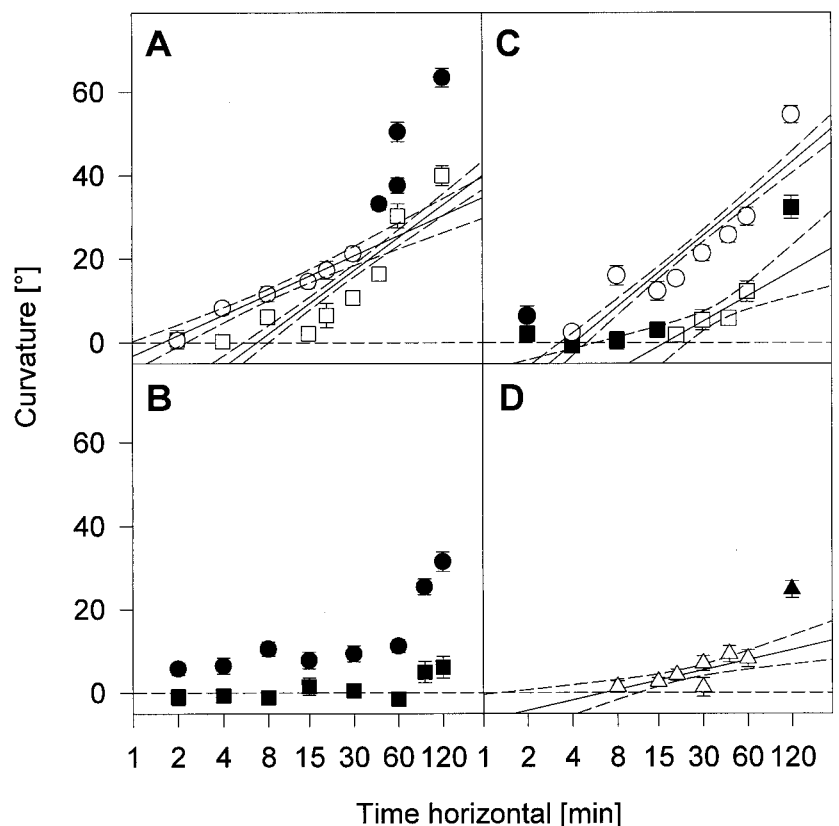
Collectively, these data show that gravitropism but not phototropism is defective in the starchless mutants. They also demonstrate for all three genotypes that gravitropism interacts with phototropism in all light positions tested except for light from above in wild-type roots.

Root Phototropism Influences Measurement of All Gravitropic Parameters

The above conclusions apply to roots that were kept in one position with respect to gravity and that were exposed to prolonged illumination (3–4 d). But most measurements of gravitropism involve reorienting the root. The most commonly used measurement is the time course of downward bending following horizontal placement, which measures the rate of curvature as opposed to the equilibrium angle. Less frequently, sensitivity is measured (see below). To our knowledge, the influence of root phototropism or light position has not been taken into account in any measurement of gravitropism involving root reorientation.

To determine whether negative phototropism operates during the time course of gravitropic curvature, roots from seedlings grown with light from above were then turned to the horizontal and simultaneously illuminated with light from above or below (Fig. 5) All three genotypes showed the same curve when light was from above (Fig. 5A). But with light from below, the starchless mutants, especially *pgm1-1*, barely curved downward, and wild-type roots curved downward much less and more slowly than when illuminated from above (Fig. 5, A and B).

Figure 6. Light position affects determination of presentation time. The light was above (A), below (B), or off (C and D) during the stationary horizontal stimulation. A to C, Wild-type and *pgm1-1* roots. D, *adg1-1* roots. Regression lines are shown as solid lines flanked by the 95% confidence intervals (dotted lines) and the values of the correlation coefficients (r^2) are noted below in parentheses next to the presentation time values for each genotype. Only the data points with white symbols were used for the calculations. Angles are means \pm SE. Black data points were excluded from calculations either because there were repeated zero values (only the longest dose, zero value was included) or because some longer values indicated a significant change in the inflection of the regression. The presentation time is the intercept of the regression line with the abscissa. No regressions are shown for B, because most of the *pgm1-1* data points were not significantly different from zero and because a regression for the wild type yielded a negative value for the presentation time. In A, \circ , Wild type with 1.6 min (0.97) and \square , *pgm1-1* with 6.9-min presentation time ($r^2 = 0.78$); in B, \bullet , wild type and \blacksquare , *pgm1-1*; in C, \circ , wild type with 4.1 min (0.82) and \square , *pgm1-1* with 16.9 min (0.84); in D, \triangle , *adg1-1* with 6.5 min (0.60).



To measure the time course of curvature unaffected by negative phototropism, horizontal roots were exposed to essentially circumlateral light (vertical rotating clinostat, Figs. 1D and 5C). Under these conditions, the initial rate of gravitropic curvature was higher in the wild type compared with both starchless mutants. By 24 h, *pgm1-1* curved downward to the same extent as the wild type, but in *adg1-1* the gravitropic curvature was clearly less than that of the wild type at all time points (Fig. 5C).

These results reinforce the conclusion that gravitropism is defective in both starchless mutants, and that, as a result, root phototropism can play a greater role in masking the gravitropic defect. They also show that the conventional method for determining the time course of gravitropism (light from above) provides an inaccurate assessment, not just for mutants with altered gravitropism, but for the wild type as well. Root phototropism has been reported for several other genera in addition to *Arabidopsis* (Kohlbecker, 1957; Mohr, 1961; Schneider, 1964), but it is not known whether this tropism is ubiquitous. If it is widespread, then many published data on the rate of gravitropic curvature need to be corrected for the influence of root phototropism.

We then checked whether measurements using even shorter periods of horizontal exposure were affected by phototropism as well. Gravitropic sensitivity has been estimated by two different parameters, the presentation time and the perception time (Sack, 1991; Kiss et al., 1996). In the former, a single, short horizontal dose is followed by an extended period of rotation on the clinostat. In the latter,

Table II. Comparison of estimates of presentation times

Data are from Figure 6 (top three lines) or from previous studies (bottom three lines).

Light Conditions	Genotype			Ratio (Mutant: wild type)
	<i>pgm1-1</i>	<i>adg1-1</i>	Wild type	
	<i>min</i>			
Above	6.9	–	1.6	4.3
Dark	16.9	–	4.1	4.1
Dark	–	6.5	4.1	1.6
Above ^a	1.3	–	0.4	3.3
Above ^b	–	–	1.2	–
Above ^c	9.5 ^{d,e}	–	5.3 ^e	1.8

^a Kiss et al. (1989). ^b Blancaflor et al. (1998). ^c Kiss et al. (1996). ^d *pgm1* allele ACG 21; all others are *pgm1-1*. ^e Ws (Wassilewskija) background; all others Columbia.

intermittent stimulation is used; for example, a short horizontal dose (e.g. 1 min) is followed by a set period of clinostat rotation (e.g. 9 min) and then this cycle is repeated (here 12 times).

To determine the effects of light position on the presentation time, the light was either positioned above (Fig. 6A) or below the roots (Fig. 6B) or it was turned off (Fig. 6, C and D) during the period of horizontal stimulation. To maintain overall root growth in all treatments, the light was turned on during clinostat rotation.

The results show that phototropism significantly affects the estimation of the presentation time in wild-type and *pgm1-1* roots (Fig. 6; Table II). Light from below essentially blocks gravitropic curvature in *pgm1-1*, and it precludes the measurement of the presentation time in the wild type, since the extrapolation of the regression produces a negative time value. Light from above exaggerates the presen-

tation time by a factor of about 2.5, the value derived with the light off during stimulation divided by that with the light overhead (Table II). Both wild-type and *pgm1-1* roots show the same degree of overestimation with the light overhead. The minimum period of overhead illumination that influenced the measurement of gravitropism was 45 min for both the wild-type and for *pgm1-1*. This was the shortest period in which the root angles for light from above compared with light off were statistically different (ANOVA confidence level $\alpha = 0.05$).

To determine whether shorter periods of light exposure affect the measurement of gravitropism when those periods are summed (intermittent stimulation), the perception time was estimated for all three genotypes (Table III). The perception time is estimated as the lowest horizontal dose that when repeated produces gravitropic curvature (see description in Table III). The perception time for the wild type, ≤ 10 s, was the same regardless of whether the roots were illuminated from above or whether the lights were off during horizontal stimulation. Six but not 2 min of light from above exaggerated the gravitropic curvature of wild-type roots, whereas 2 min affected *pgm1-1* and perhaps *adg1-1* roots. Thus, the shortest period of illumination that seems to induce phototropism (at the irradiance used) is a total of 24 min in 2-min doses of intermittent irradiation (summed over 2 h) for the starchless mutants. These data suggest that phototropism influences the measurement of the perception times of both starchless mutants but not wild-type roots.

Thus, Arabidopsis roots respond phototropically to periods of stimulation that are much shorter than previously thought or tested. Because virtually all measurements of root gravitropism can be influenced by phototropism, future studies should incorporate methodological precau-

Table III. Determination of perception times by intermittent stimulation of roots

During periods of stationary, horizontal gravistimulation, the roots were either illuminated from above or kept in the dark. Each 10-min cycle of horizontal exposure + clinostat rotation was repeated 12 times. Experiments were repeated two to three times and the data for each treatment were pooled for calculation of the mean. The angles shown are means \pm SE. The 0 + 10 column indicates divergence from 0° in control roots that have been rotated on the clinostat but not given any stationary gravity stimulation. The roots in the remaining columns were horizontally stimulated for 0.17 to 8 min. Negative angles are above the horizontal. * and **, Curvatures greater than 0° at confidence levels $\alpha = 0.05$ and 0.01, respectively. Note that even wild-type roots that were not horizontally stimulated (0 + 10; upper left) can show values that are statistically different from zero. Thus, only values substantively and consistently (over several time points) greater than vertical values were taken as actual threshold gravitropic curvature. The first time points tested that appeared to indicate gravitropic curvature are shown in boldface for each row. Data from Kiss et al. (1989) are included for comparison.

Lighting during Horizontal Stimulation	Min Stationary (Horizontal Stimulation) + Min Rotating						
	0 + 10	0.17 + 9	0.5 + 9	1 + 9	2 + 8	6 + 4	8 + 2
Above ^a							
Wild type ^a	*2.3 \pm 1.0 (86)	**5.3 \pm 1.0 (44)	**6.2 \pm 1.0 (63)	**18.7 \pm 1.1 (98)	**27.1 \pm 1.6 (55)	**27.7 \pm 1.0 (87)	ND ^b
<i>pgm1-1</i> ^a	–2.0 \pm 1.2 (80)	1.1 \pm 0.9 (31)	*2.2 \pm 1.1 (31)	*1.7 \pm 0.7 (90)	**5.3 \pm 1.4 (52)	**8.0 \pm 1.4 (56)	ND
(TC7)							
Wild type ^c	1.0 \pm 0.9 (163)	**7.0 \pm 0.9 (249)	**12.0 \pm 1.3 (150)	**17.6 \pm 1.1 (184)	**23.8 \pm 1.3 (167)	**41.4 \pm 1.3 (97)	ND
<i>pgm1-1</i> ^c	–0.2 \pm 1.1 (80)	1.7 \pm 1.1 (141)	*2.8 \pm 0.9 (141)	*1.6 \pm 0.9 (154)	**8.1 \pm 1.3 (121)	**18.3 \pm 2.9 (75)	ND
<i>adg1-1</i> ^c	1.6 \pm 1.9 (71)	1.3 \pm 1.0 (137)	1.5 \pm 0.8 (188)	*2.9 \pm 1.4 (81)	**3.5 \pm 1.1 (156)	**17.1 \pm 1.7 (60)	ND
No light ^b							
Wild type ^c	ND	**5.4 \pm 1.8 (68)	*10.9 \pm 2.5 (25)	**12.7 \pm 1.1 (173)	**21.5 \pm 1.3 (163)	**32.0 \pm 3.4 (40)	**33.6 \pm 2.0 (61)
<i>pgm1-1</i> ^c	ND	0.0 \pm 0.7 (66)	–0.3 \pm 1.0 (44)	1.7 \pm 1.1 (133)	0.9 \pm 1.1 (144)	**10.2 \pm 1.4 (94)	**14.0 \pm 1.9 (50)
<i>adg1-1</i> ^c	ND	–0.4 \pm 1.1 (66)	–1.4 \pm 1.4 (53)	*3.0 \pm 1.1 (148)	**2.5 \pm 0.7 (208)	**6.9 \pm 1.8 (62)	**6.2 \pm 1.2 (64)

^a Data from Kiss et al. (1989).

^b ND, Not determined.

^c Data from current study.

tions to remove the influence of directional light. Phototropic effects can be removed by providing circumlateral illumination (time course) or by keeping plants in the dark during short periods of horizontal exposure (presentation and perception times). This is necessary to provide accurate values for gravitropic parameters.

Reduced Estimates of Mutant Gravitropic Sensitivity

The ratio of presentation times (*pgm1-1* to wild type) derived with light from above (Table II) was comparable to that with the light off during horizontal stimulation. Thus, even though light from above exaggerates the presentation time, it did so to the same extent in *pgm1-1* and the wild type. This ratio is close to that found by Kiss et al. (1989; Table II), suggesting that by the measure of the presentation time, the relative sensitivity of *pgm1-1* does not require revision. The presentation time of the second starchless mutant, *adg1-1*, was much closer to the wild type than *pgm1-1* (Fig. 6, C and D; Table II).

Although the presentation time has been used in many studies of sensitivity, the perception time is probably a more discriminating method, because, given once, horizontal doses too small to induce curvature may result in curvature if given repeatedly (Sack, 1991). With light from above during horizontal stimulation, the first dose tested that yielded gravitropic curvature was ≤ 2 min for *pgm1-1* and ≤ 10 s for the wild type, suggesting that wild-type roots are 12 times more sensitive. A 12-fold difference with light from above was also found in Kiss et al. (1989) for *pgm1-1* (Table III). However, when the light was off during the horizontal doses, *pgm1-1* did not show a significant response at 2 min, but did at 6 min (intermediate doses were not tested), a 36-fold difference in sensitivity. *adg1-1* roots appeared to show perception times similar to *pgm1-1*. These differences are not likely to be due to indirect effects, because growth rates were not affected by genotype or by the presence or absence of light during horizontal stimulation (data not shown). Thus, once the perception times are corrected for the influence of phototropism, both starchless mutants can be considered to be 36 times less sensitive than wild-type roots, not 12 times, as previously estimated for *pgm1-1*.

The low sensitivity of starchless mutants is presumably due to the decreased mass of the plastids in columella cells of the rootcap. This supports the hypothesis that sensing relies directly on the mass of amyloplasts that sediment (Sack, 1997; Blancaflor et al., 1998; Chen et al., 1999). If so, then the residual sensing present in starchless roots might also be plastid based if the mass of the starchless plastid produces a high enough signal-to-noise ratio to trigger receptors specialized to interact with plastid mass (Sack, 1997). The revised perception times suggest that the level of residual sensing in the starchless mutants is as low as 3% of that of the wild type. The finding that sensing is noisier than previously thought is consistent with the increased variability found for starchless roots growing in an equilibrium vertical orientation once the masking effect of negative phototropism is separated from actual gravitropism (Table I). While these data do not resolve the controversy

about whether gravitropic sensing relies on plastid mass or that of the whole cell (Sack, 1997; Staves, 1997; Chen et al., 1999; MacCleery and Kiss, 1999), they do provide a more accurate assessment of the consequences that the loss of starch has on sensitivity.

CONCLUSIONS

Root phototropism can overestimate various measures of gravitropism in mutants defective in gravitropism as well as in wild-type *Arabidopsis* roots. For this reason, studies of *Arabidopsis* gravitropism need to employ methods that account for the influence of phototropism. Corrected values for the gravitropic perception time indicate that the elimination of starch reduces threshold sensitivity more than previously estimated. Finally, over extended periods of stimulation, the relative strength of root gravitropism and phototropism was found to depend upon the direction of illumination. The nature of the relationship between these two tropisms clearly requires further study, such as the effects of different wavelengths, of varying irradiance and the magnitude of the gravity vector (via centrifugation and spaceflight), and of altering the expression of genes known to function in root phototropism (Christie et al., 1998; Sakai et al., 1999).

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