Restoration of gravitropic sensitivity in starch-deficient mutants of *Arabidopsis* by hypergravity

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Abstract

Despite the extensive study of plant gravitropism, there have been few experiments which have utilized hypergravity as a tool to investigate gravisensitivity in flowering plants. Previous studies have shown that starch-deficient mutants of *Arabidopsis* are less sensitive to gravity compared to the wild-type (WT). In this report, the question addressed was whether hypergravity could restore the sensitivity of starch-deficient mutants of *Arabidopsis*. The strains examined include a WT, a starch mutant and a reduced-starch mutant. Vertical orientation studies with dark-grown seedlings indicate that increased centrifugal acceleration improves orientation relative to the acceleration vector for all strains, even the WT. For starchless roots, growth of seedlings under constant 5 g acceleration was required to restore orientation to the level of the WT at 1 g. In contrast, approximately 10 g was required to restore the orientation of the starchless mutant hypocotyls to a WT level at 1 g. Examination of plastid position in root cap columella cells of the starchless mutant revealed that the restoration of gravitropic sensitivity was correlated with the sedimentation of plastids toward the distal cell wall. Even in WT plants, hypergravity caused greater sedimentation of plastids and improved gravitropic capability. Collectively, these experiments support the hypothesis of a statolith-based system of gravity perception in plants. As far as is known, this is the first report to use hypergravity to study the mechanisms of gravitropism in *Arabidopsis*.

Key words: *Arabidopsis*, chronic acceleration, hypergravity, starch-statolith, graviperception.

Introduction

Plants have intricate mechanisms in order to respond to a variety of environmental stimuli. One of the most essential sensory mechanisms in plants involves gravitropism; the ability to perceive and respond to gravity. Gravitropism can be divided into the following stages: perception, transduction and response (Salisbury, 1993; Kiss, 2000). In roots, gravity perception is hypothesized to occur in the root-cap region in columella cells, and the response (differential growth) occurs in the zone of elongation (Volkman and Sievers, 1979; Sack, 1997). In shoots, the cells of the endodermis appear to be the site of perception (Fukaki *et al*., 1998).

There are two principal models for the perception stage of gravitropism: the starch-statolith hypothesis and the protoplast pressure theory (Kiss, 2000). According to the starch-statolith hypothesis, perception occurs via dense organelles interacting with cytoplasmic structures (Sack, 1997). These dense organelles, or statoliths, are believed to be amyloplasts and are present in almost all gravitropic organs in flowering plants. Unlike the starch-statolith theory, which defines perception as intracellular, an alternate model called the protoplast pressure theory attributes gravitropic sensitivity to the entire weight of the protoplast. The possibility was investigated that an entire cell can sense gravity and it was proposed that the cell senses down where there is an increase in the compression between the plasmalemma and the extracellular matrix (Wayne and Staves, 1996). Likewise, there is tension or relief from compression in the upper portion of the cell. The nature of the gravity perception mechanism in plants is as yet unresolved due in part to convincing evidence for both theories (Barlow, 1995).

Since the starch-statolith theory attributes gravitropic sensitivity to the mass of amyloplasts, mutants that are altered in starch content have been used in several studies to test this model. Compared to wild-type plants,
starch-deficient mutants are not as oriented to the gravity vector, and they respond more slowly to changes in the gravity vector (Kiss et al., 1989, 1996). Orientation and curvature studies have been conducted in Earth’s 1 g acceleration and even in microgravity (Kiss et al., 1998, 1999), but, to date, there has been little published research concerning these factors in a hypergravity environment.

Thus, the major focus of the present research was to use hypergravity as a tool to investigate the theories of graviperception in starch-deficient mutants of Arabidopsis. Studies to measure the gravitropic capability of starchless and reduced-starch mutants following increased gravitational acceleration were performed. Once the sensitivity thresholds for roots and hypocotyls were determined, plastid position studies were conducted in order to determine if this increase in sensitivity correlated with sedimentation of the plastids. The following questions are addressed. (1) Can gravitropic sensitivity be restored in starch-deficient mutants of Arabidopsis using increased gravitational acceleration? (2) If so, is this restoration associated with sedimentation of the plastids? (3) What are the effects of hypergravity on plant development and cell polarity? (4) Are there differences in gravisensitivity between roots and hypocotyls?

**Materials and methods**

**Plant material**

Wild-type (WT) Arabidopsis thaliana (L.) Heynh. (geographic race Wassilewskija, Ws) and two starch-deficient mutants (lines ACG 21 and ACG 27) were used in these experiments. The mutants were generated by T-DNA insertional mutagenesis and were acquired from the Dupont Company culture collection (Kiss et al., 1996, 1997). Mutant ACG 27 has a reduced-starch content as compared to the wild-type and is non-allelic to previously described pgm1 starch mutants, while ACG 21 is starchless in all tissues and is allelic to the starchless pgm1 mutant (Caspar and Pickard, 1989). All seeds were stored at 4 °C for 6–12 months prior to use in the experiments.

**Culture conditions**

Seeds were surface-sterilized with a 30% (v/v) commercial bleach and 0.01% (v/v) Triton X-100 solution for 20 min. Following three rinses in sterile distilled water, seeds were sown onto Petri dishes (60 x 15 mm) which contained a growth medium (described in Kiss et al., 1996) with 2% (w/v) agar, without sucrose. Approximately 16 seeds were sown in two rows per plate.

For microscopy studies, the seeds were immediately covered with an additional layer of agar growth medium. This was to keep the seedlings from floating during the fixation process (MacCleery and Kiss, 1999), which was performed while the seedlings were on the centrifuge. After the Petri dishes were sealed with Parafilm, the plates were placed under white light of 120 µmol m⁻² s⁻¹ for 24 h to induce germination. To separate gravitropic responses from phototropic responses, dark-grown seedlings were used in all experiments. To accomplish this, the Petri dishes were wrapped in pre-labelled aluminium foil after the illumination period. Seedlings were then grown under various hypergravity conditions, and experiments were performed approximately 3.5 d after the seeds had been sown.

**Centrifuge**

After 24 h of illumination, Arabidopsis seeds were transferred into the hypergravity produced by a variable, low speed centrifuge (range 2–20 g). The centrifuge was constructed with assistance from the Miami University instrumentation laboratory and has a motor with two tiers of twelve swinging buckets. Each bucket held one Petri dish.

**Gravitropism kinetics—vertical orientation**

Three and a half days after the seeds were sown (when seedlings were 10–15 mm long), the orientation of the roots and hypocotyls of young mutant seedlings was observed and compared to WT plants by quantifying the distribution and extent of deviation from the gravity vector. In these experiments, the gravitational acceleration needed to bring starch-deficient mutants to a degree of orientation comparable to that of the WT was determined (see below for details), and approximately 300 seedlings of each strain were measured.

**Gravitropism kinetics—reorientation on the centrifuge**

The time-course of curvature was determined by rotating dishes containing vertically grown (1 g) plants 90° relative to the gravity vector, and then photographing them at 0, 4, 8, 24, and 36 h after reorientation under 1, 5, 10, and 12 g. Angles were measured as an increment over the starting value (as described below). Seedlings which deviated more than 20° from the vertical orientation before gravistimulation by reorientation were excluded from data collection (Kiss et al., 1997). Also excluded from data collection were seedlings that came into contact with other seedlings. Between 80–120 seedlings were used for data collection during these experiments. To avoid light responses in the plants, photographs were taken under a green safe light (from a 15 W incandescent lamp filtered through green plexiglass with a fluence rate of 0.8 µmol m⁻² s⁻¹).

Growth rates were measured from calibrated images during this study. Data were collected from the roots and hypocotyls of randomly selected seedlings from each of the gravity treatments. All gravitropism and growth experiments were replicated at least three times.

**Fixation procedures for microscopy**

After 3.5 d of growth on the centrifuge, seedlings were fixed with a 3% (v/v) glutaraldehyde, 1% (v/v) paraformaldehyde solution in 50 mM sodium cacodylate buffer (pH 7.2). The securely wrapped Petri dishes were removed from the centrifuge, and fixative was injected through preformed holes (according to MacCleery and Kiss, 1999). This process took less than 30 s for each plate, and then the plates were returned to the centrifuge for 3 h (to ensure proper preservation of cellular ultrastructure).

After primary fixation, seedlings were excised from the agar with a No. 11 scalpel and placed into vials containing the previously described fixation solution. Seedlings were rinsed in buffer four times with 10 min for each rinse (to ensure that the primary fixative was removed from the both the seedlings and the agar which surrounded the plants). Seedlings remained encased in agar throughout the fixation and embedment process. After the washes in the buffer, specimens were post-fixed with 2% (w/v) OsO₄ for 2 h at 4 °C. After another set of rinses, the seedlings
were dehydrated in a graded acetone series and both the hypocotyls and roots were embedded in either Spurr’s firm resin or Spurtol resin, which is composed of ERL 4206/VCD, Quetol 651, NSA, and DMAE.

Light and electron microscopy
For light microscopy, thick (1 μm) longitudinal sections of the root cap were cut with glass knives on a Reichert Ultracut S microtome. Median longitudinal sections were placed onto glass slides and stained with 0.5% (w/v) toluidine blue in 0.1% (w/v) sodium carbonate (pH 11.1). Images of these sections were taken using an Olympus BH-2 compound microscope with Kodak Technical Pan film at ASA 50 (No. 2415; Eastman-Kodak, Rochester, NY, USA).

For electron microscopy, silver-gold ultrathin longitudinal sections (75–85 nm) of the root cap and hypocotyls were cut using a diamond knife. Median longitudinal sections were collected on formvar-coated, slotted copper grids and stained with uranyl acetate and lead citrate. Specimens were then viewed at 60 kV on a JEOL JEM-100S transmission electron microscope.

Image and data analysis
In all growth and curvature experiments, Kodak Technical Pan black and white film, ASA 50 was used to capture the images of the plants. Negatives were scanned using an Agfa Duoscan Flatbed Scanner (Agfa, Mortsel, Belgium) in conjunction with Corel Photo Paint software (Corel Corp., Ontario, Canada). In studies of growth and curvature, measurements were taken using Image Pro Plus (IPP) software (version 3.0; Media Cybernetics, Silver Spring, MD, USA) on a PC computer. For the plastid position studies, digital images were calibrated, and the cell area and the distance from the plastid centroid to the distal cell wall were quantified using Image Pro Plus (Fig. 1). The cells included in this study were central and flanking storey two and storey three columella cells, as defined earlier (Sack and Kiss, 1989; Kraf t et al., 2000). Statistical significance was determined with Jandel Sigma Stat (version 2.0; SPSS Inc., Chicago, IL, USA) by using ANOVA/Tukey test (P < 0.05) or a Kruskal-Wallis test where ANOVA criteria were not met.

Results
Vertical orientation study
Previous research has shown that seedlings of starch-deficient mutants are disoriented at 1 g (Kiss et al., 1997). In this study, the qualitative observations of starch-deficient mutants indicate that orientation is greatly improved with an increased gravitational acceleration (Fig. 2). At 5 g, the orientation of the starchless mutant is dramatically improved and, at 10 g, the orientation is indistinguishable from WT seedlings.

The distribution and extent of deviation of seedlings from the acceleration vector was quantified and expressed in histograms (Fig. 3). WT seedlings are the most oriented to the gravity vector, the starchless mutant, ACG 21, is severely disoriented relative to the gravity vector, and a reduced-starch mutant (ACG 27) exhibits an intermediate orientation (Kiss et al., 1996, 1997). In all strains, regardless of starch content, orientation was improved with increased gravitational acceleration from 1 g to 12 g. This improvement in orientation caused by hypergravity was most notable in the starchless mutant (Fig. 3A, B). At 1 g, WT roots deviated from the gravity vector with a mean angle of 9° (Fig. 3E), while starchless mutant roots deviated 42° from the vertical (Fig. 3A). However, at 5 g, the orientation of the starchless mutant roots improved to the level of the WT roots (9°). For all strains, at accelerations greater than 5 g, the orientation of the roots is only slightly improved.

In contrast, WT hypocotyls at 1 g deviated from the gravity vector 8° (Fig. 3F), while the starchless hypocotyls deviated 54° (Fig. 3B). In this case, 7.5–10 g was required to bring the level of response of the starchless mutant to the level of response of the WT at 1 g. For all strains, there is little difference between the orientation of the hypocotyls at 12 g and the orientation of hypocotyls grown at 7.5 and 10 g. Thus, the orientation studies suggest a difference in gravitropic sensitivity between roots and shoots.

Reorientation time-course of curvature
Under a 1 g acceleration, starch-deficient roots and hypocotyls exhibit a slower response to gravity in comparison to the WT as indicated by time-course studies (Kiss et al., 1996, 1997). When subjected to chronic 5–10 g, roots and hypocotyls of starch-deficient mutants exhibit increased curvature relative to the 1 g control (Fig. 4). For
example, 36 h after reorientation at 1 g, roots of the starchless mutant had only curved 40°, and under 5 g, the response was increased to 70° (Fig. 4A). This increase in response was observed in all strains. However, in roots of all strains, 10 g caused a decreased response relative to 5 g, and 12 g inhibited curvature in all strains completely (Fig. 4A, C, E). These results indicate that beyond 5 g, there is an inhibiting effect of hypergravity in roots, which is probably due to cell damage.

In contrast, at 1 g, hypocotyls of the starchless mutant only curved 30° after 36 h (Fig. 4B). This response was enhanced at accelerations up to 10 g. At 12 g, the curvature of the hypocotyls was inhibited in comparison to the curvature at 10 g. Thus, in hypocotyls, there is an inhibitory effect of hypergravity beyond 10 g, also probably due to cell damage (Fig. 4B, D, F). Once again, these data suggest a difference in relative gravitropic sensitivity between roots and hypocotyls.

**Fig. 2.** Etiolated seedlings of an *Arabidopsis* starchless mutant (ACG 21) grown under 1, 5 and 10 g accelerations. Seedlings grown at 1 g are not well-oriented to the gravity vector, but the orientation of seedlings grown under constant 5 g is greatly improved. At 10 g, the starchless mutant seedlings are indistinguishable from WT plants grown at 1 g (Kiss et al., 1997). The direction of the acceleration vector is towards the bottom of the figure. Bar=0.5 cm.
Growth rates under hypergravity conditions

Growth rates were measured over a 36 h period for WT and the two starch-deficient mutants. The mean growth rates for roots and hypocotyls were similar (data not shown), and, thus, the effects of hypergravity observed were not due to differences in growth rates.

Plastid position using light microscopy

Plastids in the root cap columella cells of WT and starchless mutants respond to alterations in the gravity vector by settling to the new lower cell wall after reorientation of the plants (Sack, 1997; MacCleery and Kiss, 1999). In this set of experiments, the effects of hypergravity on cellular ultrastructure and plastid position from the distal cell wall were determined for each strain under 1, 5, and 10 g accelerations (Fig. 1). Under 1 g, starchless mutant plastids appear to be scattered throughout the cell (Fig. 5). After 5 g stimulation, these starchless plastids are found in the distal portions of the cell, and this sedimentation of the plastids is slightly amplified at 10 g (Fig. 5). In WT cells, the amyloplasts are located in the lower portion of the cell at 1 g, but can be further sedimented with increasing accelerations of 5 g and 10 g (Fig. 5).

These observations of plastid position were quantified by image analysis (Fig. 6). In storey two central columella cells (Fig. 6A), the mean position of the starchless mutant plastids was approximately 4 μm from the distal cell wall, whereas the mean distance of WT plastids from the distal cell wall was 2.5 μm at 1 g. At 5 g, the starchless plastids were brought to 2.4 μm away from the distal cell wall, which is approximately the same position of WT plastids at 1 g. At 10 g, the starchless plastids are 2 μm away from the distal cell wall. In addition, WT plastids in storey two central columella cells (Fig. 6A) also became increasingly sedimented with greater gravitational accelerations, between 1.5 μm (5 g) and 1.2 μm (10 g). In storey three central columella cells (Fig. 6B), a similar distribution of plastids was observed. The distance of starchless plastids from the distal cell wall was decreased from 5.3 μm at 1 g to 3.5 μm at 5 g and 2.7 μm at 10 g. In the WT, the plastids were positioned from 3.2 μm at 1 g to 2.2 μm at 5 g and 2.1 μm at 10 g (Fig. 6B). As in the other cells, 5 g brought the position of starchless plastids in storey three flanking cells (Fig. 6C), to the same position as the plastids.
in WT cells at 1 g (i.e. mutant=3.8 μm at 5 g, and WT=3.9 μm at 1 g).

In columella cells from all treatments, the position of the nucleus remained unchanged, and it was consistently found in the proximal area of the cell (Figs 5, 7). This indicates that the polarity of the cell, other than the position of the plastids, was not altered during centrifugation up to 10 g. Qualitative observations indicate that the positions of other organelles (i.e. lipid bodies, mitochondria, Golgi bodies) were not affected by hypergravity, as can be seen in Fig. 7.

The cell area was also quantified to determine whether hypergravity had an effect on this parameter of the seedlings (Table 1). There was no significant difference ($P<0.05$) between the cell areas at higher g-forces, as determined by either an ANOVA or, in some cases, a Kruskal-Wallis test.

A similar type of structural analysis was attempted in endodermal cells, the presumptive gravity-perceiving cells in stems. However, the quality of fixation was very poor in endodermal cells, and previous studies have shown that these cells are difficult to fix due to their highly vacuolate nature (Sack, 1987; Kiss and Sack, 1990).

**Discussion**

**Comparison with other hypergravity studies**

While the effects of hypergravity have been studied in cress roots (Sievers and Heyder-Caspers, 1983), sunflower hypocotyls (Zachariassen et al., 1987), Chara (Braun, 1996; Hodick and Sievers, 1998), and various unicellular organisms (reviewed in Hemmersbach et al., 1999), there have been few studies with Arabidopsis to date (Brown et al., 1976). In contrast to the present study, many

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**Table 1. Cell area (μm²) of columella cells from seedlings grown under various gravity treatments**

The mean ± SE is provided for the WT and starless mutant (MT).

<table>
<thead>
<tr>
<th></th>
<th>WT St 3-Central</th>
<th>MT St 3-Central</th>
<th>WT St 3-Flanking</th>
<th>MT St 3-Flanking</th>
<th>WT St 2-Central</th>
<th>MT St 2-Central</th>
<th>WT St 2-Flanking</th>
<th>MT St 2-Flanking</th>
</tr>
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<tbody>
<tr>
<td>1 g</td>
<td>132 ± 4.86 (35)</td>
<td>126 ± 4.70 (29)</td>
<td>126 ± 6.67 (19)</td>
<td>112 ± 5.43 (32)</td>
<td>62 ± 2.43 (20)</td>
<td>67 ± 3.19 (23)</td>
<td>56 ± 2.69 (13)</td>
<td>53 ± 6.78 (17)</td>
</tr>
<tr>
<td>5 g</td>
<td>143 ± 5.74 (30)</td>
<td>135 ± 4.99 (23)</td>
<td>128 ± 8.03 (18)</td>
<td>110 ± 5.91 (21)</td>
<td>71 ± 5.49 (17)</td>
<td>68 ± 3.94 (17)</td>
<td>59 ± 6.67 (8)</td>
<td>54 ± 5.18 (9)</td>
</tr>
<tr>
<td>10 g</td>
<td>145 ± 6.43 (25)</td>
<td>124 ± 2.31 (44)</td>
<td>116 ± 5.70 (24)</td>
<td>116 ± 3.35 (51)</td>
<td>74 ± 3.85 (19)</td>
<td>59 ± 4.38 (23)</td>
<td>64 ± 4.97 (11)</td>
<td>48 ± 2.99 (16)</td>
</tr>
</tbody>
</table>

The number of cells analyzed is given in parentheses, and a separate statistical analysis was performed for values within each column. There were no significant differences ($P>0.05$) as determined by an ANOVA. Where one-way ANOVA criteria were not met, a Kruskal–Wallis (K–W) test was used. St 3, storey three cells; St 2, storey two cells.

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**Fig. 4.** The time-course of curvature (following gravistimulation by reorientation) in the dark of roots and hypocotyls of etiolated Arabidopsis seedlings. (A) Starchless mutant roots. (B) Starchless mutant hypocotyls. (C) Reduced-starch roots. (D) Reduced-starch hypocotyls. (E) Wild-type roots. (F) Wild-type hypocotyls. In roots, 5 g increased curvature relative to the 1 g control for all strains, but 10 g was less effective compared to 1 g, while 12 g inhibited curvature. In contrast, in hypocotyls, both 5 g and 10 g increased curvature relative to the control, and 12 g was less effective in stimulating curvature.
previous hypergravity experiments have used accelerations greater than 50 g (i.e. with cress roots, Sievers and Heyder-Caspers, 1983; and with maize shoots, Soga et al., 1999). In those experiments involving lower gravitational accelerations (comparable to the 2.5–12 g reported in this study), the primary objective was to study sunflower circumnutation (Zachariasen et al., 1987) or Arabidopsis morphology (Brown et al., 1976). Experiments addressing the graviresponsiveness of protists in hypergravity have shown that an increase in gravi-reaction can be accomplished with increased gravitational acceleration (reviewed in Hemmersbach et al., 1999). While these studies have provided data on gravisensing in protists and algae, as far as is known by the authors, this is the first study to use hypergravity to investigate gravisensing in a flowering plant.

**Restoration of gravitropic sensitivity in starch-deficient mutants**

The major objective of this study was to determine if increased gravitational acceleration could increase the gravitropic response in starch-deficient mutants of Arabidopsis. In both stems and roots, increased gravi-sensitivity correlates with the total mass of the plastids and, thus, to the amount of starch in plastids (reviewed in Sack, 1997; see also Kiss et al., 1997; Weise and Kiss, 1999). In contrast to WT seedlings, starchless mutants are not as responsive to gravity due to a decreased mass of plastids (Kiss et al., 1996).

One criterion to measure graviresponsiveness is to measure the orientation of seedlings relative to the gravity vector during vertical growth. These results demonstrate that the graviresponsiveness of starch-deficient mutants can be increased to a WT level of response with increased gravitational acceleration. Thus, under 1 g, starchless mutants are not well-oriented to the gravity vector. However, when the seedlings are grown under a continuous 10 g acceleration, they are indistinguishable from WT seedlings (Fig. 2). It is important to note that the orientation even of WT seedlings is improved with greater accelerations. This increase in orientation of all strains by hypergravity suggests that the gravisensing mechanism is capable of perceiving and actively responding to greater accelerations than the normal 1 g.

**Correlation between plastid sedimentation and increased sensitivity**

There is a great deal of evidence linking plastid sedimentation to gravity perception. For instance, laser

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**Fig. 5.** Light micrographs of median longitudinal thick sections of wild-type (WT) and starchless mutant (MT) root caps stained with toluidine blue. Arrows indicate the plastids in storey three columella cells. For both MT and WT, there was an increase in sedimentation with increased gravitational acceleration. M, meristem; P, peripheral cells; 2, storey two cells; 3, storey three cells. Bar = 20 µm.
ablation studies have shown that the columella cells with the highest amyloplast sedimentation velocities provided the greatest contribution to graviresponse (Blancaflor et al., 1998). Other research has shown that graviresponsiveness is correlated with the rate and magnitude of plastid sedimentation (MacCleery and Kiss, 1999). In general, plastid sedimentation seems to precede any kind of gravitropic response (reviewed in Sack, 1997).

In the present study, qualitative observations of root cap columella cells revealed that starchy mutant plastids became sedimented to the distal cell wall with increased gravitational acceleration (Figs 5, 7). Even in the WT, further sedimentation of plastids was observed with increasing gravitational acceleration. The quantitative results show that 5 g was sufficient to bring the starchy mutant plastids to the same position of WT amyloplasts at 1 g (Fig. 6), and this coincides with the acceleration necessary to bring the response of starchy roots to a WT level of response at 1 g. In addition, in both WT and starchy mutant plants, the increased graviresponsiveness of the seedlings is correlated with increased sedimentation of the plastids. Thus, this data suggests that the plastids, even the starchy plastids, can play a role in gravity sensing.

Nevertheless, the data from the present study can still be viewed to be compatible with a protoplast-based mechanism (for discussion, see Wayne and Staves, 1996). According to this view, the amyloplasts in the columella cells are ‘ballast’ to the entire weight of the protoplast. However, the results of this study show yet another striking correlation between plastid sedimentation and increased sensitivity to gravity.

Furthermore, one of the few methods truly to separate the effects of the weight of amyloplasts from the total weight of the protoplast is magnetophoresis, and results of studies utilizing this technique show that plastid movement induces localized curvature (Kuznetsov and Hasenstein, 1996, 1997; Weise et al., 2000). Thus, it is believed that this study along with a previous magnetophoresis study (Weise et al., 2000) and the great preponderance of evidence linking plastids to the early events of gravitropism (reviewed in Sack, 1997; Kiss, 2000) supports a statolith-based hypothesis for gravity sensing in plants.

**Differences in gravisensitivity in roots versus shoots**

Throughout these experiments, there was a difference between shoots and roots in their response to hypergravity. For instance, orientation studies indicate that 5 g is sufficient to bring the response of the starchy mutant roots to the level of response of the WT roots at 1 g, whereas 10 g was required to bring the response of the starchy mutant hypocotyls to the level of response of the WT hypocotyls at 1 g. During curvature studies, the response of starchy mutant roots was enhanced at 5 g, while 10 g reduced the response of roots and 12 g inhibited graviresponse in roots of all strains, including the WT. This suggests an inhibition of the gravity sensing mechanism in roots at accelerations of 10 g or greater. In contrast, the graviresponse of all hypocotyls are enhanced in gravitropism at a 10 g acceleration. In comparison to the response at 10 g, hypocotyl curvature is reduced at a 12 g acceleration. Thus, there is a difference in sensitivity between the roots and hypocotyls as reported earlier (Volkmann and Tewinkel, 1996).

The difference in gravisensitivity between roots and shoots suggests that some aspect of the gravity sensing
or signal transduction mechanism is unique to each cell type. The proposed sites of gravisensing in hypocotyls (endodermal cells) and roots (columella cells) differ in several ways. For example, columella cells are highly polarized and have dense cytoplasm in comparison to the highly vacuolate endodermal cells (Kiss et al., 1996; Weise and Kiss, 1999). There is also a difference in the arrangement of the cytoskeleton between columella and endodermal cells. The cytoskeleton in columella cells consists of diffuse actin microfilaments while the cytoskeleton in endodermal cells appears to consist of larger actin cables, although these cells have been largely unstudied (Baluska and Hasenstein, 1997; Driss-Ecole et al., 2000).

Transduction of the signal through the cytoskeleton to the plasma membrane may occur as the amyloplasts sediment to the new lower cell wall (Sievers et al., 1989; Baluska and Hasenstein, 1997). Thus, in root cap columella cells at 10 g, the cytoskeleton may be disrupted.
and, although plastid sedimentation occurs, the signal cannot be transduced through the cytoskeleton. At 12 g, graviresponse is abolished, suggesting complete disruption of the cytoskeleton. This would explain the inhibition of curvature in the roots of all strains at higher gravitational accelerations. In contrast to roots, the graviresponse of hypocotyls was enhanced at 10 g, and inhibition began at 12 g. Previous research shows that actin microfilaments are closely associated with amyloplasts, and cytoskeletal disrupting agents nullify the ability of roots and hypocotyls to respond to gravity (Baluska and Hasenstein, 1997). Thus, the difference in sensitivity between the roots and shoots may be explained through different cytoskeletal arrangements within the gravity-perceiving cells.

The effects of hypergravity on plant development and cell polarity

The polarity of root cap columella cells and the ultrastructural integrity of the cell was maintained during the hypergravity acceleration. The nucleus was consistently located in the proximal portion of the cell, regardless of the gravitational acceleration. Likewise, lipid bodies, mitochondria and Golgi bodies also were unaffected by hypergravity. Thus, the plastids appeared to be the only organelle that increased their sedimentation with an increased gravitational acceleration. The cell area of hypergravity grown seedlings was not significantly different from the cell areas of seedlings grown at 1 g. Therefore, low doses of hypergravity do not appear to affect cell area. It was shown that area was proportional to volume fraction (Guisinger and Kiss, 1999), so it can be inferred that cell volume was not affected by hypergravity.

Conclusions

Hypergravity improved the gravitropic capability in both WT and starch-deficient mutants of Arabidopsis. In roots, an acceleration of 5 g was required for the restoration of the starchless mutant to WT level of gravitropism, and in hypocotyls, 10 g was needed to restore gravitropism in the mutant. The differences between roots and hypocotyls may be due to differences in cytoskeletal organization in their respective gravity-perceiving cells. Improvement in gravitropism was associated with increased sedimentation of plastids in all strains of seedlings investigated. Thus, this study provides yet another striking correlation between plastid movement and graviperception in plants.

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References


