# The Role of Brassinosteroids in Shoot Gravitropism[1][C][W]

**Filip Vandenbussche, Dmitry Suslov, Liesbeth De Grauwe, Olivier Leroux, Kris Vissenberg, and Dominique Van Der Straeten***

Department of Physiology, Laboratory of Functional Plant Biology (F.V., L.D.G., D.V.D.S.) and Department of Biology, Research Group Pteridology (O.L.), Ghent University, B–9000 Ghent, Belgium; Department of Biology, Plant Growth and Development, University of Antwerp, 2020 Antwerpen, Belgium (D.S., K.V.); and Faculty of Biology and Soil Science, Department of Plant Physiology and Biochemistry, Saint Petersburg State University, 199034 Saint Petersburg, Russia (D.S.)

In the current model of gravitropism, negative gravitropic (upward) growth of the shoot of a dicotyledonous plant involves sedimentation of starch-containing plastids (statoliths) in the endodermis (starch sheath). Here, we show that high levels of brassinosteroids (BRs) counteract gravitropic growth, while BR deficiency enhances gravitropism irrespective of the presence of a functional starch sheath. These data support a role for BRs as negative regulators of the shoot graviresponse.

Growth direction of plants is primarily determined by light and gravity (Oyama et al., 1997; Kim et al., 2011). Germinating seedlings are often buried in soil or mulch, hence deprived of light, and solely dependent on gravity perception for growth orientation. In the current widely accepted model, negative gravitropism in the shoot is triggered by the sedimentation of amyloplasts in endodermis cells, followed by an increased auxin level at the lower side (in the direction of the gravity vector). This side consequently elongates more than the upper side, causing bending of the hypocotyl or stem (Morita and Tasaka, 2004; Esmon et al., 2006). To resist to the gravitational force, downstream regulation of cell wall remodeling and cell wall mechanics are thought to provide the necessary structural support (Hoson et al., 2005, 2010). Mutants with a defect in the formation of an endodermal layer, such as *scarecrow* (*scr*)/*shoot gravitropism1*, or with reduced starch production, such as *phosphoglucomutase* (*pgm*), have a defective graviresponse (Kiss et al., 1989; Fukaki et al., 1998). Studies of starch-deficient mutants have revealed that the presence of starch in the amyloplasts is not absolutely essential for a gravitropic response. The load of starch in the amyloplasts increases the weight of the plastid, which facilitates its sedimentation and hence the plant's response to gravistimulation. Therefore, starch is rather a factor enhancing gravitropism that is indispensable for a full response (Kiss et al., 1989).

Gravitropism in dark-grown Arabidopsis (*Arabidopsis thaliana*) seedlings can be evaluated in different ways. In a first approach, seedlings grown on vertically placed petri dishes, are subjected to a reorientation of 90°, a switch from vertical to horizontal position of the hypocotyl-root axis. Consequently, the plants detect their new orientation relative to the gravity vector and reorient shoot and root growth accordingly. This causes the hypocotyl to bend upwards, resulting in a reorientation angle that increases from 0° at time 0 h to about 70° after 15 h (Nakamoto et al., 2006). In a second approach no turning is involved, but seedlings are grown in darkness on horizontally placed petri plates for a few days and the orientation of the plants is evaluated (Shin et al., 2009). This can be formulated by expressing the angle of deviation from the vertical, or by counting the number of seedlings that do not loose gravitropism (Shin et al., 2009). The first assay copies a natural situation of soil disturbance, such as plowing or landslides. However, the majority of plants will rarely experience such a reorientation during their lifetime, which makes the second assay ecologically more significant.

Using the second assay (horizontally placed petri dishes, without displacement), we have studied the effect of BRs on the growth orientation of seedlings. In the absence of Suc in the medium, treatment of wild-type seedlings with exogenous BRs reduced the number of seedlings standing on the medium (Fig. 1A). This effect was concentration dependent, reaching its maximum at 100 nM epibrassinolide (EBL; Fig. 1B). The response toward BRs was seen from germination onward (Supplemental Movies S1 and S2) and consistent throughout further growth. In the presence of 1% Suc, the BR effect was strongly inhibited (Fig. 1, A and B). Glc mimicked the Suc effect, whereas mannitol did not, suggesting a role for the monosaccharides of which Suc is composed, while excluding an effect induced by nonmetabolizable sugar alcohols such as mannitol

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* Corresponding author; e-mail dominique.vanderstraeten@ugent.be.

[C] Some figures in this article are displayed in color online but in black and white in the print edition.

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(Fig. 1C). Keeping in mind that Suc-derived Glc can be used as a building block for starch, a potentiating factor in the graviresponse (Kiss et al., 1989), we assessed whether starch accumulation is related to the observed BR response. We first tested the effect of endogenous BRs by treatment of wild type and the starchless mutant *pgm* with brassinazole (Brz), a compound that blocks BR biosynthesis. On medium without Suc, only 15% of the *pgm* mutants are standing, indicating loss of gravitropism, as opposed to 85% for the Columbia-0 (Col-0) wild type (Fig. 2A). In the presence of 1% Suc, this difference remains. Remarkably, treatment with Brz increased the number of standing seedlings to 100% in the wild type and to over 80% in the *pgm* mutants, irrespective of the presence of Suc. It should be noted, however, that in the presence of Brz, *pgm* mutants were standing slanted, with a larger deviation from the vertical than that in the wild type (Supplemental Fig. S1B). A similar result was obtained when testing the *scr* mutant, lacking the starch sheath, except that the Brz effect was less pronounced as compared to *pgm* (Fig. 2B).

Second, to determine whether the presence of BRs affects starch accumulation in statoliths, a lugol stain was performed on wild-type seedlings grown in presence or absence of a BR signal. Starch accumulation was visible as brown spots, most conspicuously around the stele (in the endodermis), irrespective of the presence of exogenous Suc in untreated controls (Fig. 2C). Reducing endogenous BR levels by Brz treatment of the wild type did not affect starch accumulation (Fig. 2C). However, ectopic accumulation of starch granules was visible in cell layers outside of the endodermis in the presence of 1% Suc, in all treatments. This points to a higher overall starch level. Interestingly, simultaneous treatment with Suc and EBL caused a stronger overaccumulation of starch, as evidenced by intense amyloplast staining in cell layers beyond the endodermis, particularly in the cortex (Fig. 2C; Supplemental Fig. S2). Overall, the data in Figure 2C support the fact that EBL does not reduce gravitropism by limiting starch accumulation. Starch overaccumulation as in *starch excess* mutants leads to enhanced shoot gravitropism (Vitha et al., 2007), which is the contrary of what is observed here in BR-treated seedlings (Figs. 1 and 2C). Since Brz rescued the agravitropic phenotype of the starchless *pgm* mutant, the effect on starch accumulation was evaluated in the mutant. A lugol stain did not reveal accumulation of starch in Brz-treated *pgm* (Fig. 2C), indicating that the recovery of gravitropic growth by the absence of a BR signal is not dependent on starch accumulation. Only on media with exogenous Suc, starch accumulation in statoliths extended beyond the upper region of the hypocotyl and was visible in the endodermis in the middle of the hypocotyl in wild-type plants. Again, ectopic starch accumulation was seen in this region when also exogenous BRs were present (Supplemental Fig. S2).

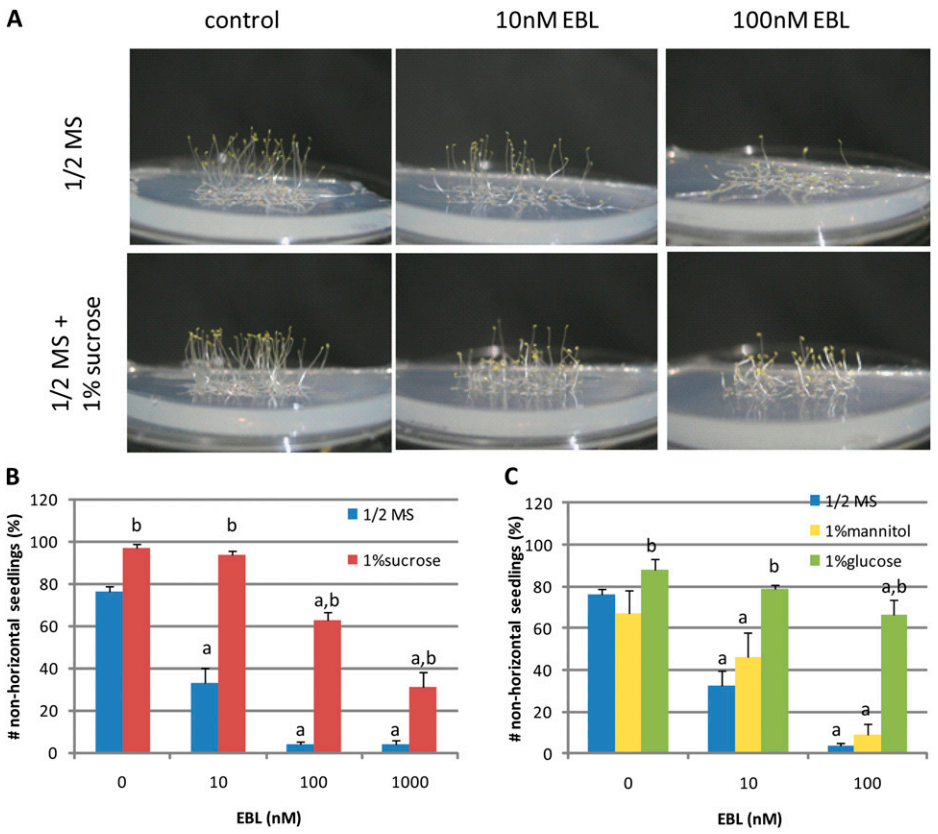


**Figure 1.** BRs and Suc differentially affect gravitropic growth of Arabidopsis seedlings. A, Three-day-old etiolated seedlings grown on one-half-strength Murashige and Skoog medium with or without 1% Suc and different concentrations of 24-EBL. B, Bar graph representing the number of plants that are standing (nonhorizontal) when grown as in A. Values are means of at least four independent biological experiments. Error bars are SEM. a, Statistically different from non-EBL-treated seedlings (Student's *t* test, $P < 0.05$); b, statistically different from seedlings grown without exogenous Suc (Student's *t* test, $P < 0.05$). C, Effect of 1% Glc and 1% mannitol on the BR response. Values are means of at least four independent biological experiments. Error bars are SEM. a, Statistically different from the respective, non-EBL-treated counterpart (Student's *t* test, $P < 0.05$); b, statistically different from the respective counterpart grown without exogenous sugar (Student's *t* test, $P < 0.05$).

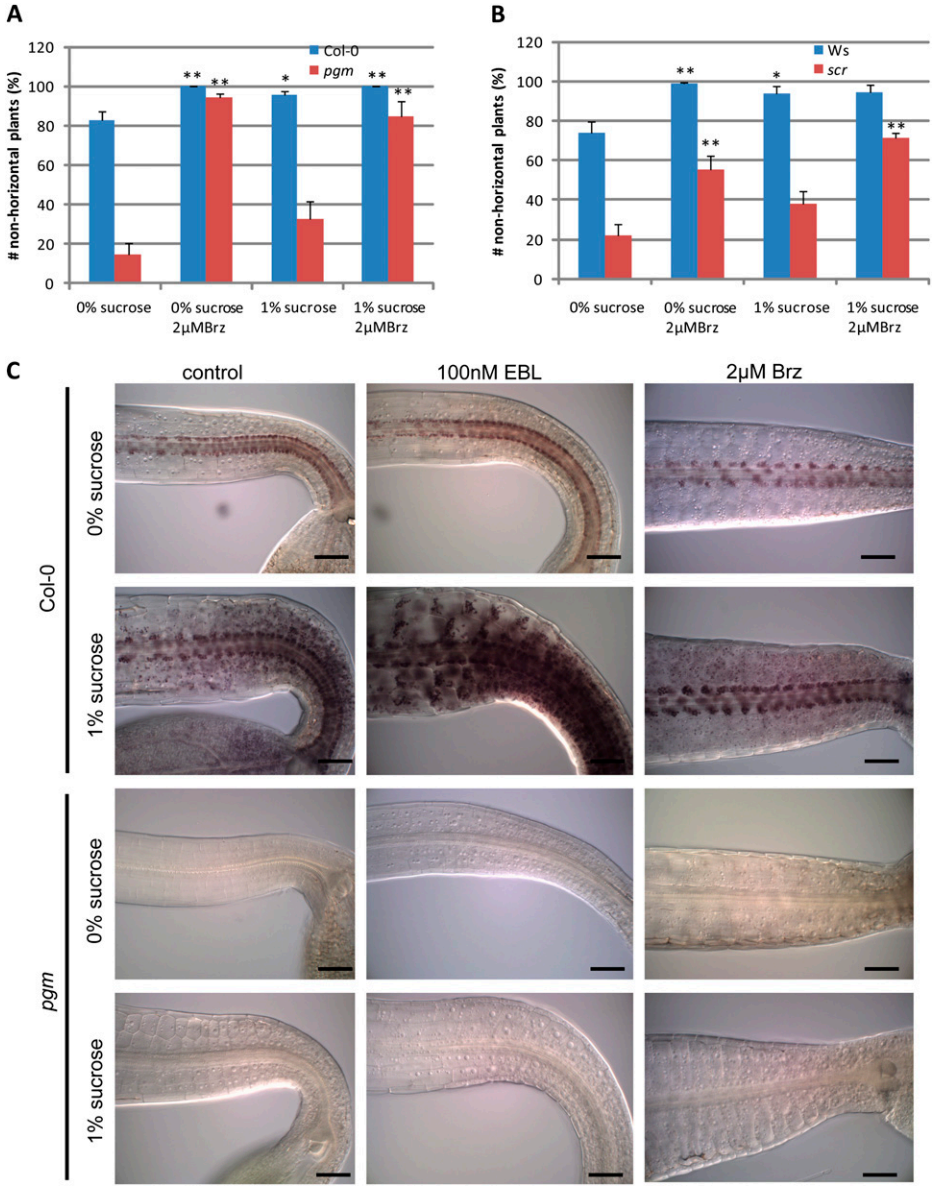


**Figure 2.** BR deficiency positively affects gravitropism independently of the presence of starch-containing statoliths. A and B, Bar graphs representing the number of 3-d-old etiolated wild-type and mutant seedlings standing (nonhorizontal) in the presence or absence of 2 $\mu$M Brz and/or 1% Suc. Values are means of at least four independent biological experiments. Error bars are SEM. Asterisks (**) indicate values statistically different from the non-Brz-treated counterpart (based on Student's *t* test, $P < 0.05$); asterisk (*) indicates values statistically different from the non-Suc-treated counterpart (based on Student's *t* test, $P < 0.05$). A, Wild-type Col-0 versus *pgm* mutants. B, Wild-type Wassilewskija versus *scr* mutants. C, Lugol stain of etiolated Col-0 wild-type and *pgm* mutant seedlings grown in the presence or absence of 1% Suc and/or combined with either 2 $\mu$M Brz or 100 nM 24-EBL. The top portion of the hypocotyl is shown. Photographs were taken at the same magnification. Bars = 100 $\mu$m. [See online article for color version of this figure.]

An increased starch load in statoliths is seemingly in contradiction with the loss of upward growth in BR-treated seedlings, but clearly shows that BR may affect sugar metabolism. This in turn could change the availability of sugar precursors that serve as building blocks for cell wall components. Since BRs are also known to influence the expression of cell wall-related genes (Vert et al., 2005), and resistance to gravity is believed to depend on cell wall properties (Hoson et al., 2010), we tested whether BR induces changes in cell wall mechanics of dark-grown Arabidopsis seedlings. To that end, in vitro extension of hypocotyls from 4- and 5-d-old seedlings was measured by the constant-load method. Creep rate at pH 6 was higher at all loads in hypocotyls of 4-d-old BR-treated seedlings grown in the absence of Suc versus controls, demonstrating the effect of BRs on the wall mechanical properties (Fig. 3A). The BR effect was reversed when 1% of Suc was present. In the latter conditions, we had to use 5-d-old seedlings, to obtain an overall length sufficient for the extensiometry (>8 mm). In 4-d-old BR-treated plants grown with Suc approximately 70% of hypocotyls were too short for securing between the clamps of the extensiometer. However, the remaining 30% of hypocotyls (>8 mm in length) in these BR samples had the same creep rate as the control (results not shown), consistent with the reversal of the BR effect on the wall mechanics in the presence of Suc (Fig. 3A). As shown above, mutation in *pgm* caused loss of gravitropism irrespective of the presence of Suc (Fig. 2) and has been assumed to reduce gravitropic growth by loss of starch accumulation. However, *pgm* plants had more extensible hypocotyls than the wild type at all loads (Fig. 3B), implying that this mutation

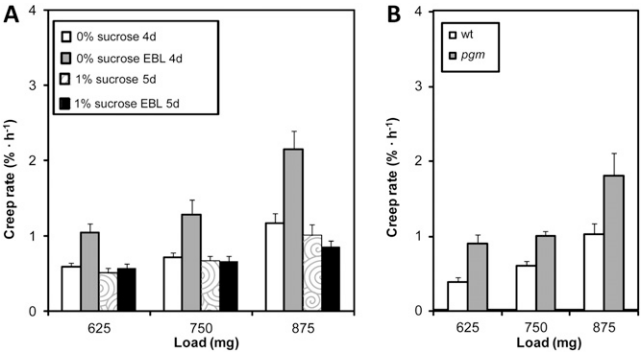


**Figure 3.** Cell wall mechanics of frozen/thawed hypocotyls of dark-grown seedlings in various conditions. A, In vitro extension (expressed as creep rate % $h^{-1}$) of hypocotyls from 4-d-old wild-type Col-0 plants grown without Suc (0% Suc) ± 100 nM 24-EBL and from 5-d-old plants grown with 1% Suc ± 100 nM EBL. Error bars represent SEM ($n \geq 10$). B, In vitro extension of hypocotyls from 5-d-old wild-type Col-0 (wt) and *pgm* plants grown with 1% Suc. Error bars represent SEM ($n = 8$).

may affect cell wall synthesis, and hence wall strength, in addition to starch accumulation. Our observations indicate that the defective sugar metabolism, caused by either *pgm* mutation or BR treatment both induce a loss of gravitropic response. This suggests a correlation between the loss of gravitropism and the cell wall mechanical properties. The role of enzymes involved in sugar metabolism, such as PGM, during gravitropic responses may thus be on two fronts: starch synthesis and cell wall modification.

Cell wall extensibility could be less important for negative gravitropism when plants have some support to grow on. To determine whether the BR-induced loss of upward growth is influenced by the presence of a physical support, wild-type plants were grown on vertically standing plates in darkness, thus allowing them to lean on the medium. On the second day after germination, plates were rotated over 90°, while keeping them vertically oriented. The reorientation of hypocotyls was followed over 24 h. On average, exogenous BR did not change the reorientation rate, nor the average angle of curvature at the end of the time course (Fig. 4). By contrast, in the presence of Suc, or in case of BR deficiency caused by Brz treatment, an enhancement of the reorientation rate and average angle of the hypocotyls after 24 h were observed (Fig. 4). The effects of Brz and Suc were additive. Together, these data suggest that loss of upward growth caused by weaker cell walls in the presence of BR can be overcome by providing a physical support for the growing seedlings.

Seedling shoots need several prerequisites for their upward growth: they should be strong enough to support their own weight, be able to detect gravity, and adapt their orientation accordingly. A lot of effort has gone to the elucidation of mechanisms of gravity perception and growth reorientation (Morita and Tasaka, 2004). We have shown that BRs are negative regulators of shoot gravitropism, but do not reduce starch synthesis. In addition, BR deficiency counteracts gravitropic defects in the absence of a functional starch sheath, such as in *pgm* and *scr* mutants. Hence, BRs likely do not interfere with gravity perception, but rather act downstream in the pathway of graviresponse. The gravitropic orientation is strongly controlled by auxins; thus, BRs may be involved in regulation of gravitropism through interaction with auxins. Both hormones have a large overlap in induction of target genes (Nemhauser et al., 2004; Vert et al., 2005), which points to a coordinated regulation of responses. However, whereas auxins are considered beneficial for gravitropism, our data indicate that BRs reduce gravitropic growth. In this case auxin and BRs could counteract one another. A similar interplay has been found in a suppressor screen for the *auxin response factor7* (*arf7*) mutation. Using the plate reorientation assay, it was shown that a reduced level of BRs overcomes the defective gravitropism caused by *arf7* mutation (Nakamoto et al., 2006). The exact mechanism is not known, but may involve the control of the repressor ARF2, which is a known target for the BR-regulated BRASSINOSTEROID INSENSITIVE2 kinase (Vert et al., 2008). ARF2 could inhibit transcription of ARF7-regulated promoters.

The biomechanical properties of seedling hypocotyls have been given little attention with respect to the graviresponse at earth gravity. Nevertheless, reports have shown that resistance to hypergravity involves rigidification of the cell wall (Hoson et al., 2005, 2010), while microgravity results in more extensible cell walls by xyloglucan breakdown (Soga et al., 2002). We have found that BR treatment stimulates extension of frozen/thawed Arabidopsis hypocotyls at pH 6, which is indicative of changed cell wall

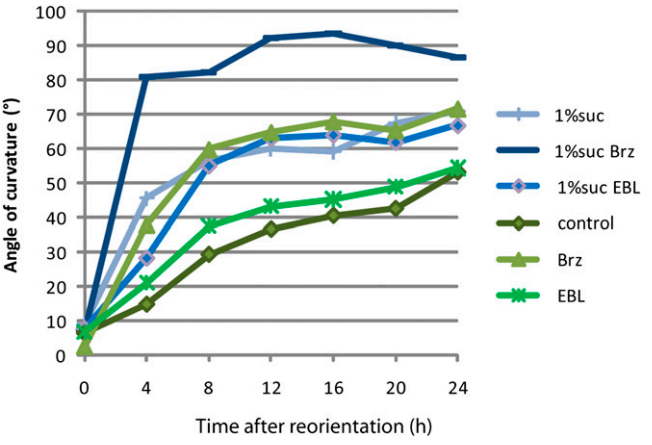


**Figure 4.** Response of wild-type Col-0 seedlings after growth and reorientation on vertically standing plates. Seedlings were grown in darkness. On day 2 after germination, plates were rotated and the average reorientation angle of the hypocotyl was calculated. Data are mean values of at least five seedlings. Seedlings were grown on one-half-strength Murashige and Skoog media without (control) or with 1% Suc (1%suc), 2 μM Brz, or 100 nM 24-EBL. 90° corresponds with the new direction of the gravity vector.

properties. This effect is consistent with the BR-induced expression of the wall-related genes (Vert et al., 2005; Nemhauser et al., 2006). It is likely that at least part of the loss of negative gravitropism caused by BRs is mediated by loss of cell wall rigidity. Indeed, when BR-treated seedlings are used in a reorientation assay, thus allowed to lean against the medium as a support, they are capable of reorienting like untreated controls (Fig. 4). In addition, inhibition of BR synthesis by Brz, or the presence of Suc, enhanced the graviresponse in these conditions, supporting our findings on horizontal plates (Figs. 1 and 4). However, presence of Suc also leads to higher starch content and hence likely an improved graviperception. Interestingly, *pgm* starchless mutants had less rigid cell walls as well. Thus, their agravitropic phenotype may result not only from a reduced starch load of the amyloplasts interfering with gravity perception, but also from a defect in the cell wall rendering their hypocotyls too weak to support their own weight. The overaccumulation of starch in wild-type seedlings in the presence of levels of BRs that severely inhibit gravitropism (Fig. 2C; Supplemental Fig. S2), may be a part of the mechanism by which the plant tries to overcome the defect. Ectopic accumulation of starch in BR-treated plants indicates a profound change in sugar metabolism. This change may have consequences not only for the wall mechanics (Fig. 3A), but also for osmoregulation in the hypocotyls, as many osmolytes accumulating in the central vacuole of a plant cell derive from sugars. Both cell wall mechanics and osmotic concentration of cellular contents affect turgor (Ray et al., 1972). In plant organs containing mostly primary cell walls such as Arabidopsis hypocotyls it is turgor that may be crucial for defining their mechanical strength (Ryden et al., 2003) and, hence, their ability to support their own weight. Further studies are needed to clarify the relative contribution of the modified starch accumulation, the wall mechanics, and, possibly, osmoregulation in the defective graviresponse of BR-treated plants.

## PLANT MATERIALS AND CHEMICALS

Col-0, Wassilewskija, *pgm* (N210), and *scr* (N8539) lines were acquired at the European Arabidopsis Stock Centre (Nottingham, UK). One-half-strength Murashige and Skoog medium (Duchefa) was prepared as described before (Vandenbussche et al., 2007). EBL and mannitol were from Sigma, Brz was purchased from TCI, Suc from VWR, and Glc from Merck.

## HYPOCOTYL GRAVITROPISM ASSAY

Seeds were surface sterilized as described before (Vandenbussche et al., 2007) and sown on plates containing the respective media. The plates were kept for 48 h in darkness at 4°C and then transferred to white light and 22°C for 6 h to stimulate germination. The plates were wrapped in two layers of aluminum foil and kept in darkness at 22°C. After 4 d, the percentage of seedlings lying flat on the medium was determined. Determination of the deviation from the vertical was done as follows. A photograph of plants was taken from above after 4 d of growth. The projection of the distance between the root-shoot junction and the shoot meristem was determined on this picture using Image J analysis software (National Institutes of Health). Afterward, the seedlings were carefully laid flat without disturbing the plant's shape, and photographed. The real distance between the root-shoot junction and the shoot meristem was measured on this photograph. The latter value serves as the hypotenuse, while the projected value represents the opposite side to an angle $\alpha$ in a rectangular triangle (Supplemental Fig. S1A). The angle $\alpha$ that describes the deviation of the position of the shoot apical meristem from the vertical can thus be determined as the arcsine of the projected value (the opposite $x$) divided by the real value (hypotenuse $z$).

For the reorientation assay of Arabidopsis Col-0 wild-type seedlings, seedlings were first grown for 48 h in infrared light on vertically standing square plates. The plates were then rotated by 90° and the growth of the seedlings was followed by time-lapse imaging for 24 h using an infrared-enabled camera system (Vandenbussche et al., 2010). The reorientation angle of curvature of the hypocotyls was defined as 0° at the start of the experiment and 90° when reaching the new vertical.

## LUGOL STAIN

One-hundred milliters of iodine solution was made using 5 g of $I_2$ and 10 g KI. Seedlings were submerged in 150 mg/mL iodine solution for 5 min and washed in distilled water for 1 h. They were mounted on microscope slides in chloral hydrate solution (2.5 g of chloral hydrate per mL of 30% [v/v] glycerol; Supplemental Fig. S2A). For Figure 2, seedlings were submerged for 10 min and mounted immediately thereafter in a solution containing one-tenth (v/v) of the aforementioned iodine solution, and nine-tenths (v/v) of the aforementioned chloral hydrate solution.

For lugol staining on sections (Supplemental Fig. S2B), seedlings were fixed under vacuum at room temperature for 2 h in freshly prepared fixative consisting of 4% (w/v) formaldehyde and 1% (w/v) glutaraldehyde in phosphate-buffered saline buffer (prepared from a 10× stock solution of 1.37 M NaCl, 27 mM KCl, 100 mM $Na_2HPO_4$, and 20 mM $KH_2PO_4$, pH 7.4). After washing in the same buffer, samples were dehydrated in a graded ethanol series, and infiltrated with low-viscosity resin (Spurr, 1969) at 4°C. Polymerization was performed at 70°C for 16 h in flat-bottom capsules. Two-micrometer-thick sections were made with a Reichert Jung ultracut E ultramicrotome using glass knives, and mounted on Vectabond-coated slides. Staining was done with a 0.5% iodine (w/v) + 1% KI (w/v) solution

in water for 5 min. Slides were rinsed twice with water before mounting. Photographs were taken on a Zeiss Axiovert inverted microscope (Carl Zeiss) using 20× Plan Apochromat objectives with differential interference contrast function.

## HYPOCOTYL EXTENSION EXPERIMENTS

In vitro extension of frozen/thawed Arabidopsis hypocotyls was measured with a custom-built constant-load extensiometer described in Suslov and Verbelen (2006). A 5-mm-long hypocotyl segment (starting from 1.5 mm below the cotyledons) was secured in the extensiometer and preincubated in a 20 mM MES-KOH buffer, pH 6.0, in the relaxed state for 2 min, after which it was extended in the same buffer under a constant load.

In vitro extension of hypocotyl segments was recorded for 15 min. Its amplitude was measured during the intervals 0 to 5 s (initial deformation), 5 s to 5 min, 5 to 10 min, and 10 to 15 min after loading. The average creep rate was calculated during the interval 5 to 15 min after loading in relative units (% $h^{-1}$) using the formula $(\ln L_{15\text{min}} - \ln L_{5\text{min}})/T \times 100\%$ adapted from Green (1976) and Thompson (2008). Here $L_{15\text{min}}$ and $L_{5\text{min}}$ indicate the length of an extending hypocotyl segment at 15 min and 5 min after loading, respectively, $T$ is the time during which the average creep rate is calculated (10 min). $L_{5\text{min}}$ is the sum of the length of the hypocotyl segment before stretching (5 mm) plus initial deformation plus creep 5 s to 5 min. Accordingly, $L_{15\text{min}}$ is calculated as $L_{5\text{min}}$ plus creep 5 to 10 min plus creep 10 to 15 min.

### Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Determination of the deviation from the vertical of hypocotyls from 3-d-old etiolated seedlings grown on horizontal plates.

**Supplemental Figure S2.** Lugol staining of wild-type Col-0 seedlings grown in darkness in the presence or absence of 1% Suc and/or 100 nM EBL.

**Supplemental Movie S1.** Three-day time-lapse image sequence of growth of untreated wild-type Col-0 seedlings in darkness and in the absence of Suc.

**Supplemental Movie S2.** Three-day time-lapse image sequence of growth of wild-type Col-0 seedlings treated with 100 nM EBL in darkness and in the absence of Suc.

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## LITERATURE CITED

**Esmon CA, Tinsley AG, Ljung K, Sandberg G, Hearne LB, Liscum E** (2006) A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. Proc Natl Acad Sci USA **103:** 236–241

**Fukaki H, Wysocka-Diller J, Kato T, Fujisawa H, Benfey PN, Tasaka M** (1998) Genetic evidence that the endodermis is essential for shoot gravitropism in *Arabidopsis thaliana*. Plant J **14:** 425–430

**Green PB** (1976) Growth and cell pattern formation on an axis: critique of concepts, terminology, and models of study. Bot Gaz **137:** 187–202

**Hoson T, Matsumoto S, Soga K, Wakabayashi K** (2010) Cortical microtubules are responsible for gravity resistance in plants. Plant Signal Behav **5:** 752–754

**Hoson T, Saito Y, Soga K, Wakabayashi K** (2005) Signal perception, transduction, and response in gravity resistance: another graviresponse in plants. Adv Space Res **36:** 1196–1202

**Kim K, Shin J, Lee SH, Kweon HS, Maloof JN, Choi G** (2011) Phytochromes inhibit hypocotyl negative gravitropism by regulating the development of endodermal amyloplasts through phytochrome-interacting factors. Proc Natl Acad Sci USA **108:** 1729–1734

**Kiss JZ, Hertel R, Sack FD** (1989) Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. Planta **177:** 198–206

**Morita MT, Tasaka M** (2004) Gravity sensing and signaling. Curr Opin Plant Biol **7:** 712–718

**Nakamoto D, Ikeura A, Asami T, Yamamoto KT** (2006) Inhibition of brassinosteroid biosynthesis by either a dwarf4 mutation or a brassinosteroid biosynthesis inhibitor rescues defects in tropic responses of hypocotyls in the Arabidopsis mutant nonphototropic hypocotyl 4. Plant Physiol **141:** 456–464

**Nemhauser JL, Hong FX, Chory J** (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell **126:** 467–475

**Nemhauser JL, Mockler TC, Chory J** (2004) Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. PLoS Biol **2:** E258

**Oyama T, Shimura Y, Okada K** (1997) The *Arabidopsis* HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. Genes Dev **11:** 2983–2995

**Ray PM, Green PB, Cleland R** (1972) Role of turgor in plant-cell growth. Nature **239:** 163–164

**Ryden P, Sugimoto-Shirasu K, Smith AC, Findlay K, Reiter WD, McCann MC** (2003) Tensile properties of *Arabidopsis* cell walls depend on both a xyloglucan cross-linked microfibrillar network and rhamnogalacturonan II-borate complexes. Plant Physiol **132:** 1033–1040

**Shin J, Kim K, Kang H, Zulfugarov IS, Bae G, Lee CH, Lee D, Choi G** (2009) Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome-interacting factors. Proc Natl Acad Sci USA **106:** 7660–7665

**Soga K, Wakabayashi K, Kamisaka S, Hoson T** (2002) Stimulation of elongation growth and xyloglucan breakdown in *Arabidopsis* hypocotyls under microgravity conditions in space. Planta **215:** 1040–1046

**Spurr AR** (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res **26:** 31–43

**Suslov D, Verbelen JP** (2006) Cellulose orientation determines mechanical anisotropy in onion epidermis cell walls. J Exp Bot **57:** 2183–2192

**Thompson DS** (2008) Space and time in the plant cell wall: relationships between cell type, cell wall rheology and cell function. Ann Bot (Lond) **101:** 203–211

**Vandenbussche F, Habricot Y, Condiff AS, Maldiney R, Van der Straeten D, Ahmad M** (2007) HY5 is a point of convergence between cryptochrome and cytokinin signalling pathways in *Arabidopsis thaliana*. Plant J **49:** 428–441

**Vandenbussche F, Petrásek J, Zádníková P, Hoyerová K, Pesek B, Raz V, Swarup R, Bennett M, Zazímalová E, Benková E, et al** (2010) The auxin influx carriers AUX1 and LAX3 are involved in auxin-ethylene interactions during apical hook development in *Arabidopsis thaliana* seedlings. Development **137:** 597–606

**Vert G, Nemhauser JL, Geldner N, Hong FX, Chory J** (2005) Molecular mechanisms of steroid hormone signaling in plants. Annu Rev Cell Dev Biol **21:** 177–201

**Vert G, Walcher CL, Chory J, Nemhauser JL** (2008) Integration of auxin and brassinosteroid pathways by Auxin Response Factor 2. Proc Natl Acad Sci USA **105:** 9829–9834

**Vitha S, Yang M, Sack FD, Kiss JZ** (2007) Gravitropism in the starch excess mutant of *Arabidopsis thaliana*. Am J Bot **94:** 590–598