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Phenotypic Reversal in *Arabidopsis thaliana*: Sucrose as a Signal Molecule Controlling the Phenotype of Gravi- and Photo-tropism Mutants

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Abstract In experimental work with *Arabidopsis thaliana*, sucrose is routinely used in growth media as an energy source assuring vigorous growth and stable development. We investigated the impact of sucrose on the phenotypic stability of two early tropism mutants of *Arabidopsis* with defects in either the *EHB1* or *AGD12* gene. Database analyses show that both these genes possess in their upstream promoter regions sucrose- and light-responsive elements. We show that exogenously applied sucrose is able to revert the gravitropic and phototropic phenotypes associated with the two mutants. Depending on the specific tropism assays and the mutant employed, sucrose elicits either a change from hypertropism to hypotropism or vice versa from hypotropism to hypertropism. The observations serve as a caveat to view sucrose exclusively under the aspect of energy supply. The capability of sucrose to elicit phenotypic reversals argues strongly for its role as an essential signaling molecule. The association of the tropism genes, *EHB1* and *AGD12*, with several sucrose-responsive elements indicates that these *cis*-acting elements, in conjunction with the requisite transcription factors, constitute very likely the physical basis for the observed sucrose effects.

Keywords *Arabidopsis* · Gravitropism · Phenotypic reversal · Phototropism · Sucrose · Sucrose-responsive elements

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Abbreviations

AGD12	ARF-gap domain12
ARF	ADP-ribosylation factor
ARF-GAP	ARF GTPase-activating protein
C2/CaLB	Protein kinase C conserved region 2/calcium/lipid-binding domain
EHB1	Enhanced bending1
GAP	GTPase-activating protein
NPH3	Non-phototropic hypocotyl3
NPY	Naked pins in YUC mutants
PHOT1	Phototropin1
PHOT2	Phototropin2
PIF	Phytochrome-interacting factor

Introduction

Mutants are routinely employed for the elucidation of metabolic networks and the characterization of signal-transduction chains. A prerequisite for the genetic dissection of signal-transduction chains consists—provided that the requisite mutant genotypes are stable—in the conservation and reproducibility of the associated phenotypes. In experimental work with *Arabidopsis*, sucrose is routinely, though not always, added to the growth medium as an energy source, which assures vigorous growth, particularly when the development occurs in darkness. Because gravitropism and phototropism studies with *Arabidopsis* usually employ seedlings that are raised in darkness, the requisite tropic phenotypes of wild-type cultivars and their derived tropism mutants are in most cases, though by no means always, characterized under conditions when sucrose is present. Any exogenous carbon supply may, however,

entail a massive developmental and metabolic impact (Yaseen and others 2013). Examples for the effects of carbon supply include abiotic stress responses in plants (Gupta and Kaur 2005) and sugar signaling in roots responding to phosphorus deprivation (Hammond and White 2011).

The question of whether or not the presence or absence of sucrose may affect tropic bending, and as a corollary, the phenotype of the requisite mutants, has never been investigated systematically. If sucrose were to have an effect on tropic phenotypes, the indiscriminate use of media with or without sucrose may lead to variable or, in the worst case, even to contradictory phenotypes.

During the past two decades, considerable evidence has accumulated that sugar acts in plants not exclusively as an energy supply but also as a signal molecule. For various plant genes, *cis*-acting elements have been reported in the upstream region of promoters (for reviews see Rolland and others 2006; Rook and others 2006; Smeekens and others 2010; Tognetti and others 2013; Wind and others 2010). We have identified such sugar-responsive elements in the genes *EHB1* and *AGD12* which act early in the signal cascades for gravitropism and phototropism (Dümmer and others 2015; Knauer and others 2011). We have also included the gravitropism genes *NPY1* (Li and others 2011) and the phototropism gene *NPH3* (Motchoulski and Liscum 1999) in our screening for sucrose-responsive elements (Fig. 1). The SURE1 box was described as a general promotor element in many sugar-regulated genes (Grierson and others 1994); the S3S1 element is a combination of SURE1 and sucrose box3 first identified in grape as an element of *VvHT1* coding for a monosaccharide transporter

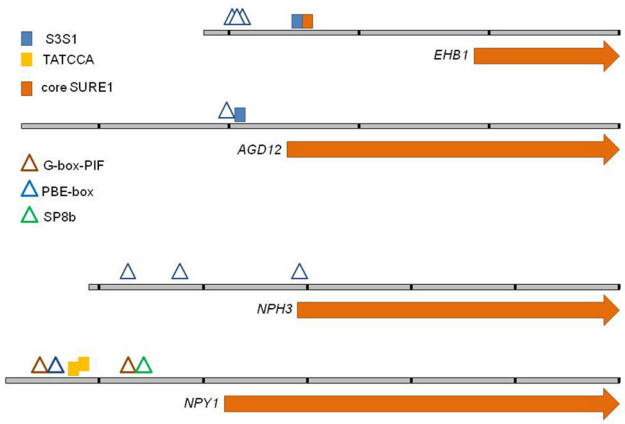

Fig. 1 Sugar- and light-responsive elements in the promoter regions of genes *EHB*, *AGD12*, *NPH3*, and *NPY1* of *Arabidopsis thaliana*. Coding regions are indicated as brown arrows. *Filled squares and circles* sugar-responsive elements. *Open triangles* light-responsive elements (Leivar and Monte 2014). The *tick marks* on the thin *gray lines* indicate 1000 base pairs. Note the different scales for the upper two and lower two genes

(Çakir and others 2003) and the TATCCA-motif, which is a core element of the major sugar-response sequence (Lu and others 1998). Because phototropism and gravitropism can be modulated by irradiation (Molas and Kiss 2009), we additionally included in our in silico analysis light-responsive elements (Fig. 1). Evidently, the mentioned tropism genes possess a variety of sucrose- and also light-responsive elements (Fig. 1). The presence of these *cis*-acting elements suggests that the expression of the requisite genes could possibly be modulated by exogenous sucrose and also by light. This finding opens up the possibility that the phenotypes of tropism mutants depend on and are modulated by the exogenous stimuli, sucrose and light.

Studies on the effects of sucrose on elongation growth and tropisms are scattered in the requisite literature. For example, increasing the sucrose concentration in a range from 0 to 2 % correlated with a parallel increase of the length of *Arabidopsis* roots and also their gravitropic responsiveness (Saether and Iversen 1991). Likewise, also the elongation growth of hypocotyls of *Arabidopsis* seedlings is controlled by exogenous sucrose. At concentrations ranging from 1 to 30 mM, sucrose slightly stimulated hypocotyl elongation and diminished it at 100 mM; in contrast, rootlet elongation was continuously stimulated in the same concentration range (Kircher and Schopfer 2012).

We demonstrate in this work that sucrose plays an equally important role in determining the tropic phenotype of the mutants *ehb1* (Knauer and others 2011) and *agd12* (Jensen and others 2000). The loss-of-function mutant *ehb1* (enhanced bending 1) was characterized as a tropism mutant exhibiting a dual phenotype that implies hypergravitropism and also hyperphototropism (Knauer and others 2011). Our more recent investigations showed furthermore that the *ehb1* mutation lowers the absolute gravitropic threshold of roots as determined with a clinostat-centrifuge (Dümmer and others 2015) and greatly affects the establishment of photogravitropic equilibrium (Dümmer and others 2015).

The proteins EHB1 and ARF-GAP (ARF GTPase-activating protein; gene *AGD12*, Jensen and others 2000) share an identical N-terminal C2-domain that includes 2 Ca^{2+}-binding sites (Fig. 1; Knauer and others 2011; Nalefski and Falke 1996). The fact that mutant *ehb1-2* possesses a hypertropic phenotype suggests that EHB1 acts in the wild-type as a negative regulator. Because EHB1 interacts in yeast-two-hybrid experiments with the phototropism-signal protein NPH3, the negative action of EHB1 very likely consists of blocking the positive transducing element NPH3 (Knauer and others 2011). In contrast to mutant *ehb1-2*, mutant *agd12* possesses a hypotropic phenotype (Dümmer and others 2015), an observation that suggests that ARF-GAP (protein coded by *AGD12*) functions as a positive regulator in gravi- and photo-tropism (Knauer and

others 2011). Because ARF-GAP interacts with a small ARF-type GTPase, which in turn plays a prominent role in plant gravitropism through vesicle trafficking (for review see Molendijk and others 2004), the *ehb1-2* mutant might lack an inhibitory element which competes with ARF-GAP for a common receptor structure located within the activated small G-protein. The proposition that EHB1 and ARF-GAP compete for the same binding sites explains the hypertropic phenotype of the loss-of-function mutant *ehb1-2* (Dümmer and others 2015; Knauer and others 2011).

During the course of these studies, it became apparent that the various phenotypes associated with *ehb1-2* under a variety of gravitropic and phototropic stimuli critically depended on the presence or absence of sucrose in the growth medium. This observation prompted us to systematically compare the requisite phenotypes in the presence or absence of sucrose in the culture medium. Our results show that sucrose is not only capable of neutralizing a mutant phenotype but also of reverting the phenotype into its opposite. Sucrose can thus cause a reversion from hypertropism to hypotropism and vice versa, that is, from hypotropism to hypertropism. The various experimental conditions with which we tested gravitropism or phototropism were developed in the context of clarifying various aspects of tropic bending and were thus originally unrelated to the subject of the influence of sucrose. Although we focus in this work on the phenomenon of sucrose-induced phenotypic reversal, the physiological aspects of our tropism analyses are described in detail elsewhere (Dümmer and others 2015).

Materials and Methods

Plant Material and Growth Conditions

As the wild-type of *Arabidopsis thaliana* (L.) Heynh., we employed the ecotype *Columbia*. In addition, we used the Salk mutant SAIL 385_C07 (*ehb1-2*) in the isogenic *Columbia* background (*Columbia-3*; stocknr. 8846). The *ehb1-2* mutant displays anomalous gravitropism and phototropism (Dümmer and others 2015; Knauer and others 2011). The second mutant line used in this work was deficient for AGD12 (Salk_0363506), which was compared with the isogenic wild-type *Columbia* (*Col-8*; stocknr. N60000). Seeds were obtained from the ABRC (Alonso and others 2003). *Arabidopsis* seeds were surface-sterilized by rinsing them in 3 % (v/v) sodium hypochloride solution. For all experiments that were done to study the effect of exogenous sucrose on gravitropism and phototropism, seeds were sown on half-strength Murashige and Skoog salts medium (Sigma, St. Louis) containing 0.9 % (w/v) phytagel in rectangular culture plates (100 × 100 mm, 2 cm height; Sarstedt, Nümbrecht, Germany). The growth medium was either without sucrose or enriched with 1 % (w/v) sucrose. Plates were inoculated with 3 parallel rows of 30 seeds each and subsequently maintained for 2 days in darkness at 5 °C to break dormancy; before the start of the actual experiment they were placed at 22 °C for 7 h under white overhead light (10 μmol m^{-2} s^{-1}) to induce germination. After irradiation, the plates were transferred for 3 days into a darkroom at 22°. During this time, the plates were placed vertically such that the germinating, etiolated seeds grew upward on the surface of the solid medium forming this way straight hypocotyls and rootlets. Subsequently, the plates with the seedlings were transferred to the irradiation or gravitropism boxes (see below).

Sequence Analyses

The DNA sequences of various genes involved in gravi- and photo-tropism of *Arabidopsis thaliana* were obtained from the *Arabidopsis* Information Resource (TAIR). The screening and identification of sucrose- and light-responsive elements was done using Vector NTI 10 (Invitrogene, Carlsbad, CA).

Light Sources and Irradiation Measurements

The light sources for the phototropism experiments were two slide projectors (Prado Universal 31047; Leitz, Wetzlar, Germany) in combination with two heat-absorbing filters (KG1, 5 mm; Schott Glaswerke, Mainz, Germany). Monochromatic light was obtained with interference filters (466 nm, type IL, 10-12 nm half band width; Schott). Fluence rates were controlled by a resistor attached to the slide projectors and/or by neutral density filters (type NG, Schott). Fluence rates were determined with a UV-enhanced photodiode (Messkopf BN-9102-4; Gigahertz-Optik, Puchheim, Germany) and a calibrated readout instrument (Optometer P-9201; Gigahertz-Optik). The two slide projectors were also employed for gravitropism experiments in which horizontal seedlings were irradiated bilaterally with white incandescent light. The symmetric irradiation allowed gravitropic bending without eliciting phototropism. For generating fluence rate-response curves for phototropism such as shown in Fig. 2a, a threshold box (95.5 cm long × 17.5 cm deep × 14.5 cm high) was employed, which contained 10 adjacent compartments that provided unilateral light for the *Arabidopsis* seedlings (details in Galland 2002; Knauer and others 2011). The light from the light sources was partially reflected by beamsplitters (60 % transmittance, 40 % reflectance; Pörschke, Hoechst, Germany) which were centered in front of the Petri dishes at a 45° angle relative to the horizontally

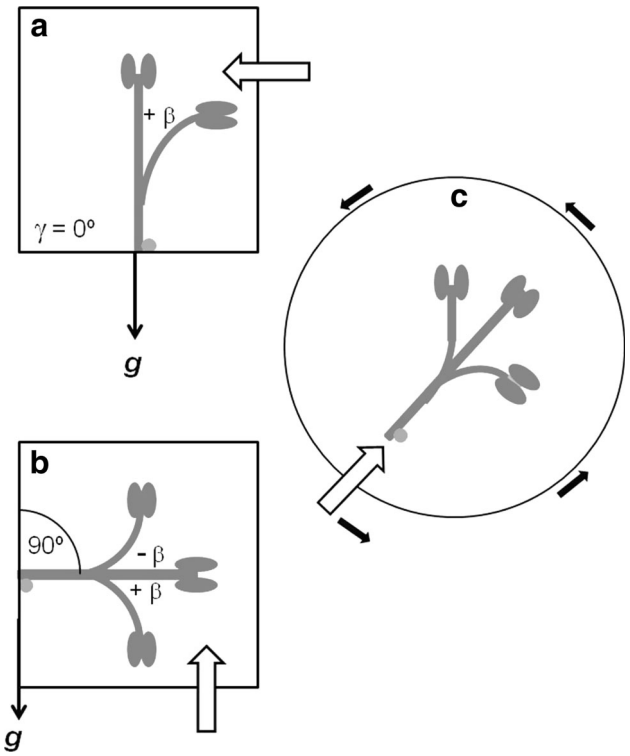

Fig. 2 Experimental setup for determining the phototropism and photogravitropic equilibrium of hypocotyls reached after 24 h of unilateral irradiation with blue light (466 nm). **a** Standard experiment with vertical hypocotyls, that is, initial inclination angle $\gamma = 0°$; unilateral irradiation from the side (*open arrow*). **b** Initial inclination angle $\gamma = 90°$; unilateral irradiation from below (*open arrow*). At photon-fluence rates compensating gravitropism, the hypocotyls maintain the initial inclination angle γ, that is, they stay horizontal. At photon-fluence rates above the compensation point, positive phototropism prevails and the bending angles become positive ($+\beta$); below the compensation point negative gravitropism prevails ($-\beta$). γ = inclination angle of the hypocotyls at the onset of the irradiation. At the compensating photon-fluence rates, the bending angles β stay zero. **c** The seedlings and also the light source (*open arrow*) are clinostatted "head-over" at 1 r.p.m; *filled arrows* indicate the clinostat rotation of the entire setup including seedlings, lamp and irradiation box

incident light. A second type of threshold box that was mounted in a metal frame could be tilted along with its light source by a variable inclination angle γ (for example, 90° as in Fig. 2b). The etiolated, 3-day-old seedlings grew on the vertical surface of the solid growth medium in rectangular Petri dishes that were placed vertically into the 10 compartments of the boxes. In this way, seedlings could be accommodated either upright (that is, parallel to the plumbline, Fig. 2a) or else at angles tilted 90° relative to the gravity vector (Fig. 2b; details in Galland 2002). A third threshold box could be rotated around its long axis such that the *Arabidopsis* seedling was clinostatted continually "headover" at 1 r.p.m. (Figure 2c; Grolig and others 2000; Galland 2002).

Determination of Bending Angles

Culture plates containing bended seedlings were photographed with a CCD camera (model Fine PixS2Pro, Fuji Photo Film Co., LTD, Tokyo, Japan). Bending angles were determined semiautomatic by analyzing the photographically documented bending angles using the Image Tool software (http://compdent.uthscsa.edu/dig/download.html). Subsequently, mean values, SE, were calculated for each experiment. The significance of differences between wild-type and mutant seedlings was confirmed by using one-way ANOVA, *F* test (calculated by Microsoft Excel), and Bonferroni correction.

Results

To assess the effect of exogenous sucrose on gravitropism and phototropism, and specifically, the tropic phenotypes of mutants *ehb1-2* and *agd12*, we employed a variety of test conditions. Gravitropism was tested by placing etiolated seedlings horizontally (inclination angle = 90°) or else vertically "headover" (inclination angle 180°). Phototropism was tested in three different modes depicted in Fig. 2: (i) Seedlings were irradiated with unilateral blue light (466 nm) as shown in Fig. 2a. (ii) Seedlings were placed horizontally and were irradiated from below (Fig. 2b). (iii) Seedlings were unilaterally irradiated with blue light that was impinging on the tip of the root while they were clinostatted (Fig. 2c). The various gravitropic and phototropic stimuli lasted for 24 h. For all of these experimental conditions, the ensuing bending angles were determined for seedlings grown on media with or without sucrose.

To assess the effect of exogenous sucrose on the effectiveness of gravitropic bending, etiolated seedlings of *Arabidopsis* were placed horizontally in darkness, and the gravitropic bending angles of the hypocotyls and roots were determined after 24 h. The data for the wild-type strain *Columbia* and the tropism mutant *ehb1-2* are shown in Fig. 3. It became apparent that sucrose increased the gravitropic bending of hypocotyls of *Columbia* irrespective of whether or not the seedlings bent in darkness or in the presence of bilaterally applied white light, which by itself did not elicit a phototropic response (Fig. 3a, black and gray columns). In darkness, the presence of sucrose magnified the gravitropic response of *Columbia* hypocotyls about 2.3-fold. In contrast, exogenous sucrose stimulated the gravitropic bending of hypocotyls of *ehb1-2* only 1.5-fold. The stimulation by sucrose was evident even when gravitropism occurred in the presence of bilateral light. However, in this case, the sucrose-stimulated bending was much more prominent in *Columbia* (2.4-fold) than in *ehb1-*

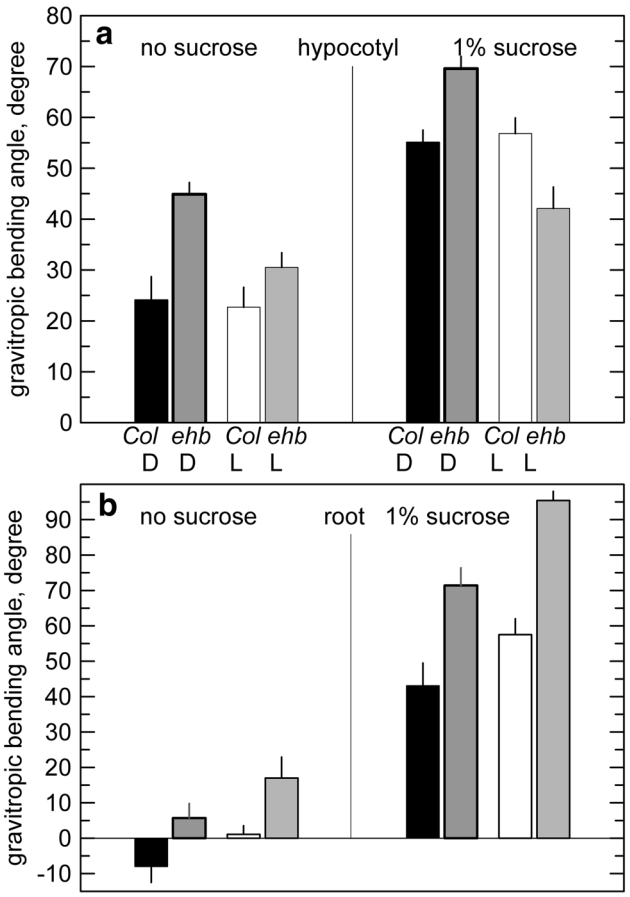

Fig. 3 **a** The effect of sucrose on the gravitropic bending of horizontally inclined hypocotyls of seedlings of *Arabidopsis*. **b** The effect of sucrose on the gravitropic bending of horizontally placed roots. *Col Columbia*: *black* or *white* columns. *ehb*: mutant *ehb1-2*: *dark-gray* columns. *D* seedlings were kept in darkness. *L* seedlings were irradiated bilaterally with white light (1 μmol Wm^{-2} s^{-1}). SE of 30–50 determinations

2 (1.4-fold; Fig. 3a, right). As a result, we noted that the gravitropic phenotype of hypocotyls of *ehb1-2* depends on sucrose, and in addition, also on the presence of light. In darkness, mutant *ehb1-2* displayed a hypergravitropic phenotype irrespective of the presence or absence of sucrose. With bilateral irradiation, however, the hypocotyls of *ehb1-2* displayed a hypogravitropic phenotype provided that sucrose was present. Thus, the capability of exogenous sucrose to revert the phenotype of mutant *ehb1-2* depended on the presence of light.

The phenomenon of sucrose-induced reversal of the *ehb*-phenotype was restricted to hypocotyls. The data displayed in Fig. 3b clearly indicate that the roots of mutant *ehb1-2* maintain their hypergravitropic phenotype irrespective of the presence or absence of sucrose and light.

To find out whether or not the phenotype of mutant *ehb1-2* was specific for bending and not related to altered elongation growth, we determined the effect of exogenous sucrose on the length of hypocotyls and roots of 3-day-old etiolated seedlings. It can be inferred from the data presented in Fig. 4 that exogenous sucrose reduced the hypocotyl length about 2.5-fold (*Columbia*) and 2.2-fold (*ehb1-2*), respectively. In contrast, sucrose stimulated the length of roots about 1.2-fold (*Columbia* and also *ehb1-2*). We conclude that *ehb1-2* displays normal elongation growth and that the mutation affects specifically gravitropic and phototropic bending. As a consequence, *ehb1-2* did not display a sucrose-mediated phenotypic reversal with respect to elongation growth.

The gravitropic enhancement effect of sucrose was also manifested with seedlings of mutant *agd12* that were inclined 90° (Fig. 5a). The enhancement was absent in hypocotyls, but strongly expressed in roots. Without sucrose, hypocotyls of *agd12* manifested a barely hypogravitropic phenotype compared to *Columbia*; with sucrose, however, hypocotyls of *agd12* bent significantly less than *Columbia*. Roots reacted much more strongly to exogenous sucrose than hypocotyls. Roots of *agd12* were clearly hypogravitropic without sucrose, whereas they displayed a slightly hypergravitropic phenotype in the presence of sucrose. Clearly, sucrose induced in roots of *agd12* a substantial phenotypic reversal. The elongation growth remained unaffected by the *agd12* mutation independently of the presence or absence of exogenous sucrose (Fig. 5b). It should be emphasized that exogenous sucrose

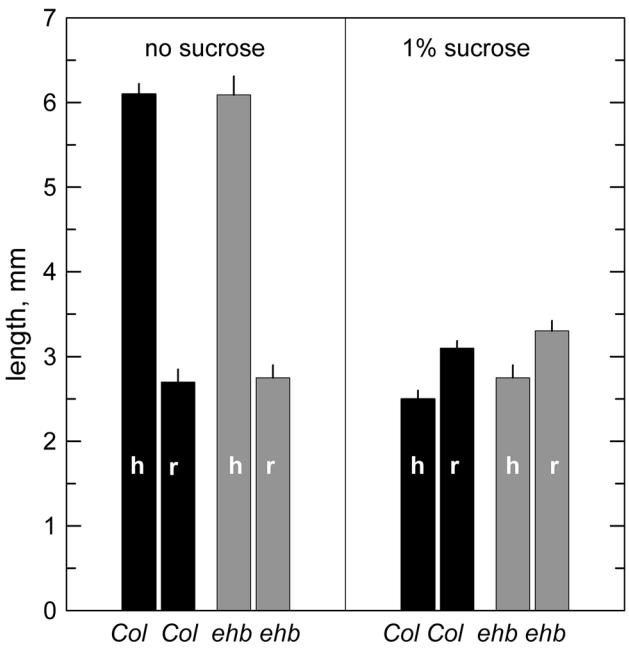

Fig. 4 Length of hypocotyls and roots of etiolated seedlings in dependence of exogenous sucrose. The length was determined three days after seed germination. *h* hypocotyls. *r* root. *Black columns Columbia*. *Gray columns ehb1-2*. *Error bars* SE of 40–60 determinations

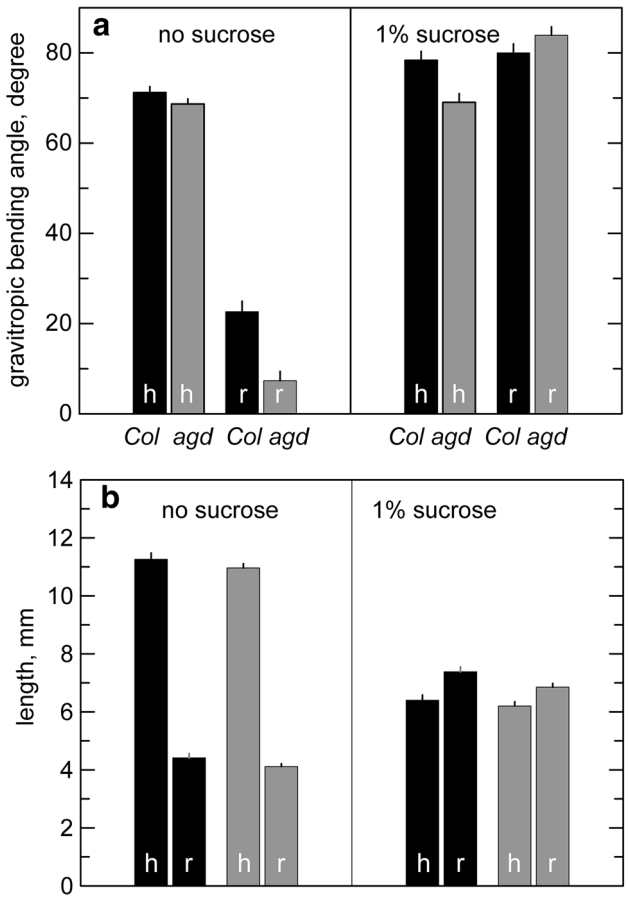

Fig. 5 **a** The effect of sucrose on the gravitropic bending of hypocotyls and roots of 4-day-old seedlings that were inclined 90° (*horizontal*). **b** Length of hypocotyls and roots in dependence of sucrose. Seedlings were grown for 3 days in darkness. *Black columns Columbia*. *Gray columns* mutant *agd12*. *h* hypocotyls. *r* root. *Error bars* SE of 60–90 determinations

diminished the elongation growth of hypocotyls, but stimulated the elongation of roots (Fig. 5b).

Even though the *ehb1-2* mutation affects mainly root gravitropism (Dümmer and others 2015), it modulates also the phototropism of the hypocotyls. We tested, therefore, the phototropism of hypocotyls of *Columbia* and compared it to the responsiveness of mutant *ehb1-2*. The phototropism was either tested in the standard way as shown in Fig. 2a or in a special setup that allowed unilateral irradiation and at the same time slow clinostatization of the seedlings as well as the light source (Fig. 2c; see Materials and Methods). In the clinostat experiments, the irradiation was given from "below" such that the collimated light beam was impinging on the tip of the rootlets (Fig. 2c). This unusual procedure for unilateral irradiation was chosen because it allows maximizing the ensuing phototropic bending.

We obtained for seedlings irradiated in the standard modus (Fig. 2a) the following results: in the absence of sucrose mutant, *ehb1-2* behaved slightly hyperphotogravitropic, that is, the bending angles were only some 2°–12° larger than those of *Columbia* (Fig. 6a, filled symbols). In the presence of sucrose, however, the behavior of *ehb1-2* was reversed and no longer distinguishable from that of *Columbia*. These results show that exogenous sucrose is able to neutralize the phenotype of mutant *ehb1-2*.

The ability of sucrose to elicit a phenotypic reversal was much better manifested when we employed the third irradiation procedure, that is, with clinostatization and unilateral irradiation from below as shown in Fig. 2c. In this case, the following results were obtained: without sucrose

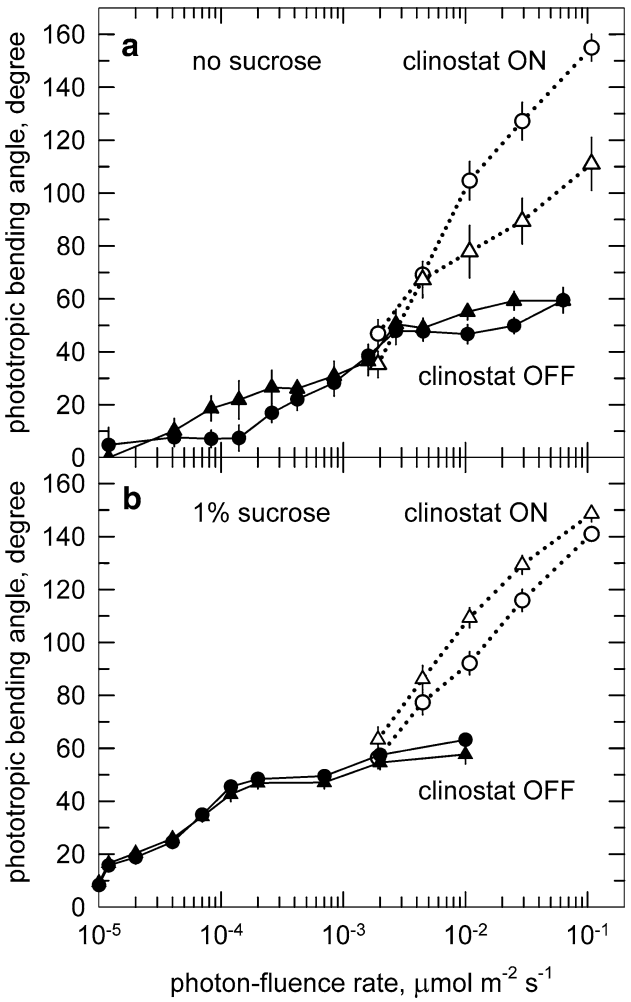

Fig. 6 Photon-fluence rate-response curves for photogravitropic bending of hypocotyls of *Arabidopsis* seedlings. The seedlings were irradiated for 24 h with unilateral monochromatic light as shown in Fig. 2a (without clinostatization) or in Fig. 2c (with clinostatization). **a** Without sucrose. **b** With 1 % exogenous sucrose. *Solid lines* and *filled symbols* no clinostatization. *Dotted lines* and *open symbols* with clinostatization and irradiation from below as shown in Fig. 2c. *Circles Columbia*. *Triangles* mutant *ehb1-2*. *Error bars* SE of 30–50 determinations. Reversal of the phototropic phenotype of *ehb1-2* is manifested only during clinostatization (*dotted lines*)

mutant, *ehb1*-2 displayed less phototropic bending (hypophototropism) than *Columbia* (Fig. 6a). In contrast, in the presence of sucrose, *ehb1-2* displayed now a slight hyperphototropic phenotype (Fig. 6b). The latter results were, to say the least, surprising and unexpected. We thus note that in the presence of clinostatization and irradiation from below the phenotype of *ehb1-2* is qualitatively the opposite of that obtained for seedlings that were not clinostatted (Fig. 6 filled symbols).

We tested the effect of sucrose on photogravitropic equilibrium in another set of experiments that allowed a more precise and quantitative assessment of the phototropic fluence rate required to counterbalance the gravitropic stimulus (Fig. 2b). In these experiments, the seedlings were placed horizontally and were then irradiated unilaterally for 24 h with blue light (466 nm) as shown in Fig. 7a (inset). This setup allows the determination of the fluence rate required to compensate the ensuing gravitropic stimulus (Galland 2002). Depending on the applied irradiances, either gravitropism (low fluence rates) or positive phototropism (elevated fluence rates) dominates. When we generated this way fluence rate-response curves for the photogravitropic equilibrium, we found that mutant *ehb1-2* displayed above 10^{-4} µmol m^{-2} s^{-1} a hyperphototropic phenotype on medium without sucrose (Fig. 7a). When, however, medium with sucrose was employed, the fluence rate-response curves of *ehb1-2* changed dramatically, and the mutant phenotype reverted now to hypophototropism (Fig. 7b). Although the balancing fluence rate (that is, 0° bending) of *ehb1-2* occurred without sucrose near 10^{-4} µmol m^{-2} s^{-1}, it occurred in the presence of sucrose at 10^{-3} µmol m^{-2} s^{-1}. The phenotypic change of *ehb1-2* occurred thus not only in comparison to *Columbia* but also in absolute terms. It was furthermore noted in control experiments that the gravitropic phenotype of *ehb1*-2 was sucrose-dependent. Without sucrose, *ehb1-2* bent twice as much as *Columbia* (Fig. 7a, open symbols); with sucrose, however, it bents gravitropically less than *Columbia* (Fig. 7b, open symbols).

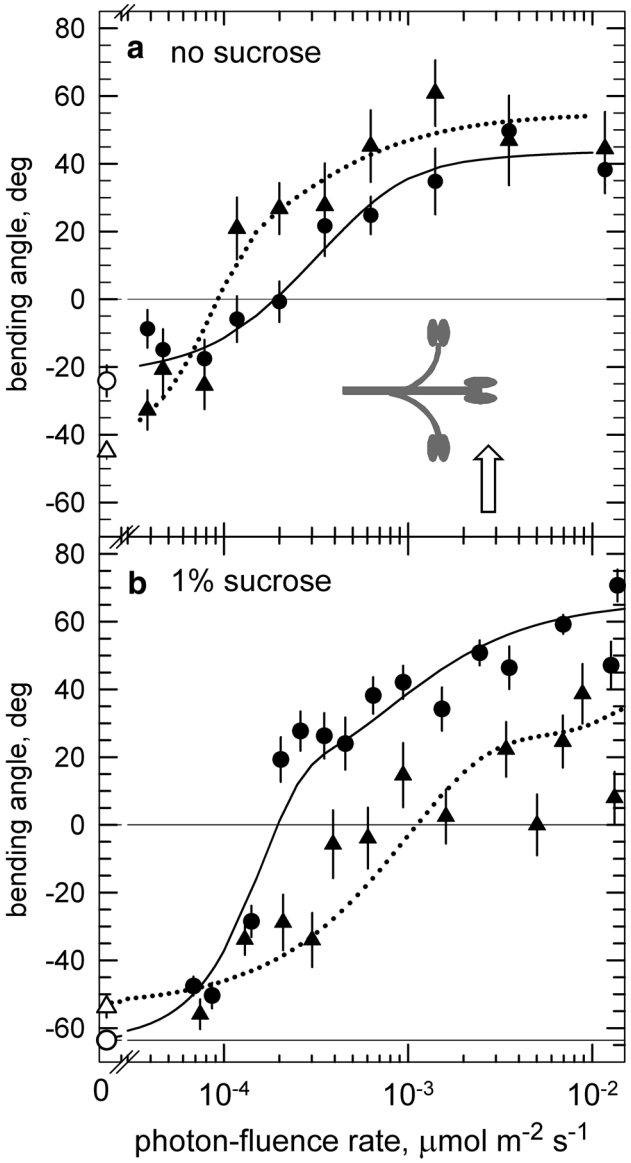

Fig. 7 The effect of sucrose on the photogravitropic equilibrium of hypocotyls of *Arabidopsis*. Seedlings were horizontally placed and irradiated for 24 h with monochromatic light (466 nm) as shown in the inset of graph 7a (*vertical open arrow = blue light*). **a** No sucrose. **b** 1 % sucrose. *Circles Columbia*. *Triangles* mutant *ehb1-2*. Negative values on the ordinate indicate negative gravitropism (upward bending); positive values indicate positive phototropism (downward bending toward the blue light). The open symbols at zero fluence rate represent control experiments for gravitropism in darkness with seedlings that were initially inclined horizontally. *Error bars* SE of 30–50 determinations

Discussion

Table 1 summarizes the various phenomena of sucrose-induced phenotypic reversals manifested in the mutant lines *ehb1-2* and *agd12*. We use the terms *hyper-* and *hypo-gravitropism* and *hyper-* and *hypo-phototropism* only for the mutant strains and with reference to their behavior relative to that of the wild-type cultivar, *Columbia*. To indicate the fact that exogenous sucrose can stimulate the various tropisms of *Columbia*, we use the symbol "+++." The responses highlighted in bold indicate a sucrose-induced phenotypic reversal. For example, roots of mutant *ehb1-2* without sucrose were hypergravitropic compared to *Columbia*; exogenous sucrose rendered them, however, normally gravitropic, that is, their responsiveness was indistinguishable from that of *Columbia* (Table 1). An opposite sucrose effect was, however, observed with respect to the phototropism of hypocotyls of mutant *ehb1-2*: in this case, exogenous sucrose reversed the phenotype

Table 1 Summary of the effects of exogenous sucrose on gravitropism and phototropism of *Columbia* and mutants *ehb1-2* and *agd12*

Strain	Gravitropism no sucrose	Gravitropism 1 % sucrose	Phototropism no sucrose	Phototropism 1 % sucrose
Columbia				
Hypocotyl	gravitropic	gravitropic +++	phototropic	phototropic +++
Root	gravitropic	gravitropic +++		
ehb1-2				
Hypocotyl	hypergravitropic in darkness	hypergravitropic in darkness	hyperphototropic no clinostat	**hypophototropic** no clinostat
	hypergravitropic in bilateral light	**hypogravitropic** in bilateral light	**hypophototropic** with clinostat	hyperphototropic with clinostat
			hyperphototropic tested as in Fig. 2b	**hypophototropic** tested as in Fig. 2b
Root	hypergravitropic darkness or light	hypergravitropic darkness or light		
agd12				
Hypocotyl	normal	hypogravitropic		
Root	hypogravitropic	**hypergravitropic**		

The phenotypic reversals that are caused by the presence or absence of sucrose are highlighted (bold)

of *ehb1-2* from hyperphototropic to hypophototropic (Table 1, no clinostat).

Our observation that the addition of 1 % sucrose to the culture medium increased the gravitropic bending (Figs. 3, 5, 7) is in line with the requisite literature. For example, increasing the sucrose concentration in a range from 0 to 2 % correlated with a parallel increase of the length of *Arabidopsis* roots and their gravitropic responsiveness (Saether and Iversen 1991). Likewise, also the elongation growth of hypocotyls and roots of seedlings of *Arabidopsis* are controlled by exogenous sucrose. At concentrations ranging from 1 to 100 mM, sucrose slightly stimulated hypocotyl elongation up to 30 mM and diminished it at 100 mM, whereas rootlet elongation was stimulated two-fold (Kircher and Schopfer 2012). Our results (Figs. 4, 5) are in line with these observations.

Because EHB1 and ARF-GAP (*AGD12*) both possess identical binding sites for calcium, it is assumed that the Ca^{2+}-activated proteins compete with each other for docking unto NPH3 and possibly NPY or a similar protein (Li and others 2011) operating in the gravitropic chain. Because EHB1 does not possess any known catalytic activity, it might function as a negative regulator by competing with the positive regulator ARF-GAP, which possesses GTPase activity that leads in turn to GTP hydrolysis of an activated small G-protein (ARF type). The small G-protein mediates vesicular trafficking (Molendijk and others 2004) and relocation of PIN3 proteins (Friml and others 2002). A sucrose-induced enhancement of gravitropic bending could occur in two ways: (i) via an increase in the formation of starch containing amyloplast-statoliths, a phenomenon ubiquitous among plants (for example, Song and others 1988; Vandeputte and Delcour 2010) and (ii) via an decreased expression of EHB1, which leads to enhanced ARF-GAP activity and thus to an decreased pool of activated G-protein (Fig. 7). It must be emphasized in this context that bending implies differential growth. As a consequence, it is not exclusively the pool size of activated G-protein that determines the effectiveness of bending but rather the gradient of activated G-protein across the bending organs. It is evident from Fig. 1 that more sucrose-responsive elements are associated with the gene *EHB1* than with *AGD12*. This finding makes it likely that *EHB1* and *AGD12* respond differentially to the presence of exogenous sucrose.

To explain the inhibitory effect, which bilateral irradiation exerts on gravitropism, we assume that light enhances the function of EHB1. Because *EHB1* possesses three light-responsive elements and *AGD12* only one (PEB-box, a PIF3-binding element; Zhang and others 2013), the two genes are probably differentially expressed, a constellation that presumably leads to a relative overexpression of the competitor EHB1, which should cause a diminution of the pool of activated G-proteins.

Sucrose-Responsive Elements: The Cause for Phenotypic Reversal

Sucrose-sensitive metabolic networks can operate via sucrose-specific *cis* elements and a variety of associated sucrose-sensitive transcription factors such as MYB75/PAP1 and bZIP11 (for reviews see Tognetti and others 2013; Smeekens and others 2010; Wind and others 2010). Examples for sucrose-specific gene expression include the

patatin gene, which possesses in the upstream region of the promoter the sucrose-responsive elements SURE1 (AATAGAAAA) or SURE2 (AATACTAAT) (Grierson and others 1994). We assume that the enhanced bending, which is caused in seedlings of *Arabidopsis* by exogenous sucrose, is not exclusively due to the extra energy supply but also due to the presence of specific *cis*-acting sucrose-responsive elements and corresponding transcription factors as described above. The genes *EHB1* and *AGD12* both possess an S3S1 element ($AAATCA(N)_XATAGAAA$) (Rook and others 2006) and share with *NPY1* and *NPH3* TEF elements (CATAAT, Fig. 1; Rook and others 2006). These findings make it thus comprehensible that the correlated gene expressions become amenable to the regulation by exogenous sucrose. Because sugar-specific response elements operate in combination with several other response elements that are associated with phytohormones, stress and light, the mode of action of sucrose-responsive elements becomes context dependent (Rook and others 2006; Smeekens and others 2010). Even in our gravitropism and phototropism experiments, we encountered such context dependence. For example, the phenotypic reversals that are caused by the loss of EHB1 are different for dark and light conditions (Fig. 3) or, in the case of phototropism, for non-clinostatted and clinostatted seedlings (Fig. 5). This complex phenotypic behavior is in line with the presence of light-responsive elements that we identified upstream of the coding regions (Fig. 1). The light-responsive elements that are associated with the analyzed tropism genes include the PBE-box (for PIF-binding E-box: CATGGT, or CATGTG, or CACATG), the G-box(CACGTG), and SP8b (TACTATT) all of which interact with PIFs (phytochrome-interacting factors) and which are thus required for the orchestration of a great number of light-regulated genes (Leivar and Monte 2014).

From our previous observations and the present study, we thus conclude that besides gravity light also serves as a major sensory input for EHB1 and AGD12. The close association of light- and sugar-response elements in the promoter-upstream regions of *EHB1* and *AGD12* allows for a complex modulation of gravitropic bending through irradiation and sucrose supply. We conclude from the presented data that the sucrose-responsive elements and their associated transcription factors mitigate the phenomenon of sucrose-mediated reversal of mutant phenotypes. Even though we describe this novel phenomenon in the context of gravitropism and phototropism, we argue that the phenomenon should be ubiquitous, because the sucrose-responsive elements are widespread (Rook and others 2006). As a consequence, sucrose-mediated phenotypic reversal may occur even in completely different contexts, provided that the requisite genes and mutants are associated with sucrose-responsive elements. The combined arguments strengthen our and other authors' notion (Kircher and Schopfer 2012; Rook and others 2006; Tognetti and others 2013) that sucrose operates in various metabolic and developmental networks as a very crucial signal molecule. As a general caveat, we like to add that mutant phenotypes that have been determined by employing sucrose-enriched media may take on very different appearances when tested on media lacking sugar.

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References

Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R (2003) Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science 301:653–657

Çakir B, Agasse A, Gaillard C, Saumonneau A, Delrot S, Atanassova R (2003) A grape ASR protein involved in sugar and abscisic acid signaling. Plant Cell 15:2165–2180

Dümmer M, Forreiter C, Galland P (2015) Gravitropism in *Arabidopsis thaliana*: root-specific action of the *EHB* gene and violation of the resultant law. J Plant Physiol. doi:10.1016/jplph.2015.09.008

Friml J, Wiśniewska J, Benkova E, Mendgen K, Palme K (2002) Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. Nature 415:806–809

Galland P (2002) Tropisms of *Avena* coleoptiles: sine law for gravitropism, exponential law for photogravitropic equilibrium. Planta 215:779–784

Grierson C, Du JS, de Torres Zabala M, Beggs K, Smith C, Holdsworth M, Bevan M (1994) Separate cis sequences and trans factors direct metabolic and developmental regulation of a potato tuber storage protein gene. Plant J 5:815–826

Grolig F, Eibel P, Schimek C, Schapat T, Dennison DS, Galland P (2000) Interaction between gravitropism and phototropism in sporangiophores of *Phycomyces*. Plant Physiol 123:765–776

Gupta AK, Kaur N (2005) Sugar signaling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. J Biosci 30:761–776

Hammond JP, White PJ (2011) Sugar signaling in root responses to low phosphorus availability. Plant Physiol 156:1033–1040

Jensen RB, Lykke-Andersen K, Frandsen GI, Nielsen HB, Haseloff J, Jespersen HM, Mundy J, Skriver K (2000) Promiscuous and specific phospholipid binding by domains in ZAC, a membrane-associated *Arabidopsis* protein with an ARF GAP zinc finger and a C2 domain. Plant Mol Biol 44:799–814

Kircher S, Schopfer (2012) Photosynthetic sucrose acts as cotyledon-derived long-distance signal to control root growth during early seedling development in *Arabidopsis*. Proc Nat Acad Sci USA 109:11217–11221

Knauer T, Dümmer M, Landgraf F, Forreiter C (2011) A negative effector of blue light-induced and gravitropic bending in *Arabidopsis thaliana*. Plant Physiol 156:439–447

Leivar P, Monte E (2014) PIFs: systems integrators in plant development. Plant Cell 26:56–78

Li Y, Dai X, Cheng Y, Zhao Y (2011) *NPY* genes play an essential role in root gravitropic responses in *Arabidopsis*. Mol Plant 4:171–179

Lu CA, Lim EK, Yu SM (1998) Sugar response sequence in the promoter of a rice α-amylase gene serves as a transcriptional enhancer. J Biol Chem 273:10120–10131

Molas ML, Kiss JZ (2009) Phototropism and gravitropism in plants. Adv Bot Res 49:1–34

Molendijk AJ, Ruperti B, Palme K (2004) Small GTPases in vesicle trafficking. Curr Oinion Plant Biol 7:694–700

Motchoulski A, Liscum E (1999) *Arabidopsis* NPH3: a NPH1 photoreceptor-interacting protein essential for phototropism. Science 286:961–964

Nalefski EA, Falke JJ (1996) The C2 domain calcium-binding motif: structural and functional diversity. Protein Sci 5:2375–2390

Rolland F, Baena-Gonzalez E, Sheen J (2006) sugar sensing and signaling in plants. Conserved and novel mechanisms. Annu Rev Plant Biol 57:675–709

Rook F, Hadingham SA, Li Y, Bevan MW (2006) Sugar and ABA response pathways and the control of gene expression. Plant Cell Environ 29:426–434

Saether N, Iversen TH (1991) Gravitropism and starch statoliths in an *Arabidopsis* mutant. Planta 184:491–497

Smeekens S, Ma J, Hanson J, Rolland F (2010) sugar signals and molecular networks controlling plant growth. Curr Opin Plant Biol 13:274–279

Song I, Lu CR, Brock TG, Kaufman PB (1988) Do starch statoliths act as the gravisensors in cereal grass pulvini? Plant Physiol 86:1155–1162

Tognetti JA, Pontis HG, Martínez-Noël (2013) Sucrose signaling in plants. A world yet to be explored. Plant Signal Behav 8:e23316-4

Vandeputte GE, Delcour JA (2010) From sucrose to starch granule to starch physical behaviour: a focus on rice starch. Carbohydr Polymers 58:246–266

Wind J, Smeekens S, Hanson J (2010) Sucrose: metabolite and signaling molecule. Phytochemistry 71:1610–1614

Yaseen M, Ahmad T, Sabiok G, Standardi A, Hafiz IA (2013) review: role of carbon sources for in vitro plant growth and development. Mol Biol Rep 40:2837–2849

Zhang Y, Mayba O, Pfeiffer A, Shi H, Teppermann JM, Speed TP, Speed TP, Quail PH (2013) A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in *Arabidopsis*. PLoS Genet 9:e1003244