J. Plant Biol. (2014) 57:255-263
DOI 10.1007/s12374-014-0096-z

ORIGINAL ARTICLE

Effects of Secondary Mutation in *det2-1* on Root Growth and Development in Arabidopsis

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Received: February 27, 2014 / Accepted: July 8, 2014
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Abstract Brassinosteroids (BRs) are plant steroidal hormones that regulate a wide range of developmental processes. Most BR mutants display impaired growth and responses to developmental and environmental stimuli. Here, we found a BR-deficient mutant *det2-1* that displayed exceedingly short roots and agravitropic growth, which were not present in other BR mutants. By back-crossing *det2-1* with the wild type, we isolated a secondary mutation named *det2-1 phenotype modifier 1* (*dpm1*) and demonstrated that those aberrant phenotypes in the original *det2-1* were independent of the BR deficiency. Phenotypic analysis showed that impaired root growth of *dpm1* appeared in BR-deficient condition, but not in a normal condition. In the light condition, the mutant showed enhanced shoot growth which was suppressed in the *det2-1* background. Starch granules in the columella cells of the root tip were highly accumulated and expanded in *dpm1*. Agravitropic roots and the expanded starch granules of *dpm1* could not be recovered by BR. Taken together, these results suggest that *DPM1* is required for gravitropic growth, and that its functions on root and shoot growth are BR-dependent.

Keywords: *Arabidopsis thaliana*, brassinosteroids, *det2-1*, gravitropism, Root growth, Statolith

Introduction

Brassinosteroids (BRs) are plant growth regulators that control various aspects of plant growth and development. BR has its own signaling machinery of which core components, from the membrane-localized BRI1 receptor to nuclear transcription factors BZR1/BZR2, have been completely characterized (Wang et al. 2001; He et al. 2005; Yin et al. 2005; Kim et al. 2009). Using this signaling machinery, BR transduces internal and external signals to the growth and developmental processes in plants. BRs manipulate many physiological processes such as tissue elongation, seed germination, vascular differentiation, flowering and photomorphogenesis by directly regulating cell proliferation and expansion or by interacting with other signaling pathways (Chory et al. 1991; Steber and McCourt 2001; Caño-Delgado et al. 2004; Domagalska et al. 2007; Park et al. 2010; González-García et al. 2011; Oh et al. 2012; Bai et al. 2012). BR-insensitive and -biosynthetic mutants show growth defects in overall plant developmental stages resulting in dwarf phenotypes (Chory et al. 1991; Clouse et al. 1996; Azpiroz et al. 1998; Li et al. 2001).

Exogenous application of BRs has been performed to study the physiological functions of this growth regulator. One of these functions is the effect of BR on the root growth. At low concentrations, exogenous applications of BR have been known to promote root growth by promoting cell elongation. However, the root growth was inhibited when a high concentration of BR was applied, suggesting that the effect of BR on root elongation is highly dependent on the concentration of BR applied (Müssig et al. 2003; Kim et al. 2007). The application of BR biosynthetic inhibitors resulted in the inhibition of the root elongation along with reduced shoot size, demonstrating that endogenous BR is required for normal root growth (Asami and Yoshida 1999; Tanaka et al. 2003; Hartwig et al. 2012).

In addition to promoting root elongation, BR has positive effects on the root gravitropic response. Root gravitropism consists of two major steps. The first step is the gravity sensing

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process, which is mediated by starch-enriched amyloplasts in columella cells (Morita 2010). The second step is differential growth, which is mediated by differential auxin distribution (Boonsirichai et al. 2002). In contrast to the effect on the shoot gravitropic response (Vandenbussche et al. 2011, 2013), BR stimulates gravitropic curvature of the roots (Kim et al. 2000; Li et al. 2005; Kim et al. 2007). This stimulation is mediated by BR promotion of polar auxin transport (Li et al. 2005). It has been suggested that the effects of BR on the gravitropic response are distinct from its effect on the root elongation (Kim et al. 2007).

The *det2-1* mutant has been generated by ethyl methanesulfonate mutagenesis and identified by the screening of plants showing a de-etiolated morphology when grown in the dark (Chory et al. 1991). Biochemical analysis demonstrated that *det2-1* has defects in the BR biosynthetic pathway (Fujioka et al. 1997). Since the functions of *DET2* in the BR biosynthetic pathway have been elucidated, the *det2-1* mutant has been used extensively in the BR research field. Typically, *det2-1* displays small, dark green leaves and prolonged vegetative growth phase compared to the wild type. In addition, it shows reduced root growth and gravitropic response (Chory et al. 1991; Li et al. 2005). These phenotypes can be rescued by the exogenous application of BR, demonstrating BR is involved in these processes.

In this report, we show that the original *det2-1* mutant has a secondary modifier in addition to the *DET2* locus. We isolated the mutation and found that it caused short and agravitropic roots, as well as an enriched and expanded domain of starch granules in the root caps of the original *det2-1*. However, the isolated mutant plant showed normal root growth, but enhanced hypocotyl and petiole elongation, which were not shown in the original *det2-1*. These results suggest that the mutation affects the root and the shoot growth in a BR-dependent manner, and influences the gravitropic response and differentiation of the columella cells of the root.

Fig. 1. Phenotypic differences between *det2-1* and other BR mutants. (A) Twelve d-old and (B) 5 weeks-old the wild type (WT) and homozygous *bri1-116*, *dwf4* and *det2-1*. Seedlings were grown on vertical plates under continuous light and transferred to soil. Scale bar = 10 mm.

Results

det2-1 Shows Short and Agravitropic Roots Independent of BR Deficiency

BR is known to be required for normal root growth and promotes root elongation at a certain concentration (Hacham et al. 2011; Kim et al. 2007; Müssig et al. 2003). The BR-deficient mutant *det2-1* shows growth defects of the entire organ and failure of etiolation (Chory et al. 1991; Fujioka et al. 1997). Grown in the light conditions, *det2-1* and other BR mutants such as *bri1-116* and *dwf4* showed shorter roots than those of the wild type (Fig. 1A). However, the reduced root growth of *det2-1* was more severe than those of *bri1-116* and *dwf4* (Fig. 1A). The size of the vegetative leaves of *det2-1* was smaller than that of wild type but larger than those of *bri1-116* and *dwf4* (Fig. 1B). These results indicate that the deficiency of active BR signaling in *det2-1* is not as great as the deficiencies in *bri1-116* and *dwf4*, and the strong root growth reduction of *det2-1* is not only due to the low level of BR. This suggests that the short root phenotype of *det2-1* is partially independent of BR deficiency and signaling.

In addition to the root growth, the root of *det2-1* was grown agravitropically (Fig. 2A). To test whether this abnormal gravity response was due to the absence of functional *DET2*, a transgenic plant expressing exogenous *DET2* in *det2-1* under the control of the CaMV 35S promoter (*35S-DET2/det2-1*) was generated. The *35S-DET2/det2-1* plant rescued the shoot dwarfism and slightly restored the root growth defect of *det2-1* (Fig. 2A and Supplement Fig. 1). However, the *35S-DET2/det2-1* failed to rescue the agravitropism of the root (Fig. 2A). To test whether BR deficiency in *det2-1* causes the agravitropic response, a *det2-1* seedling was grown on the media containing brassinolide (BL), the most

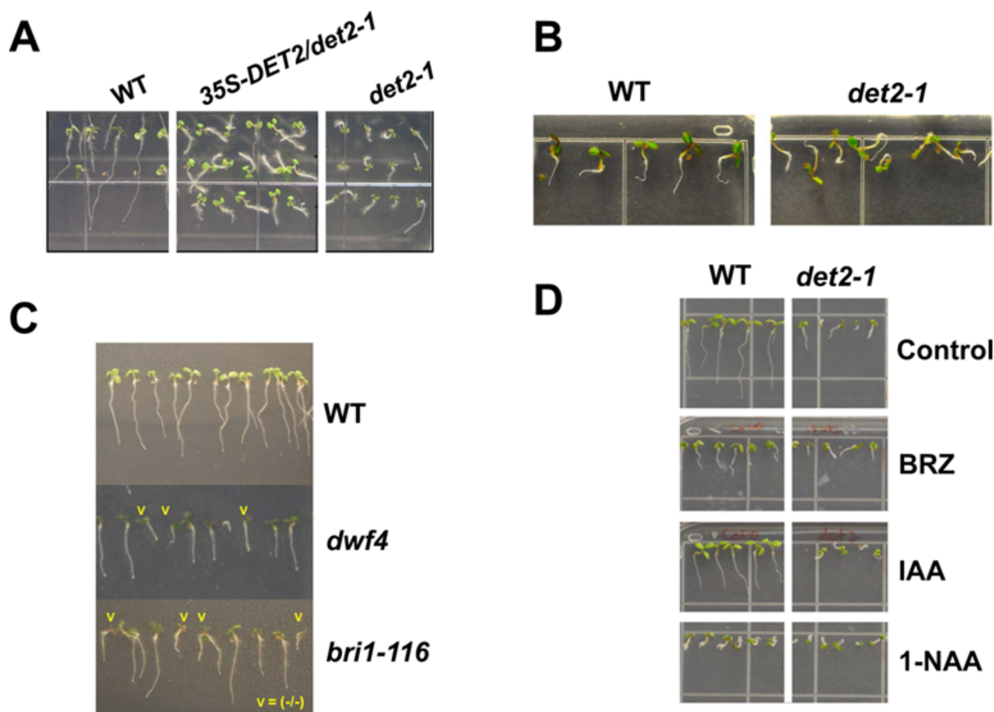


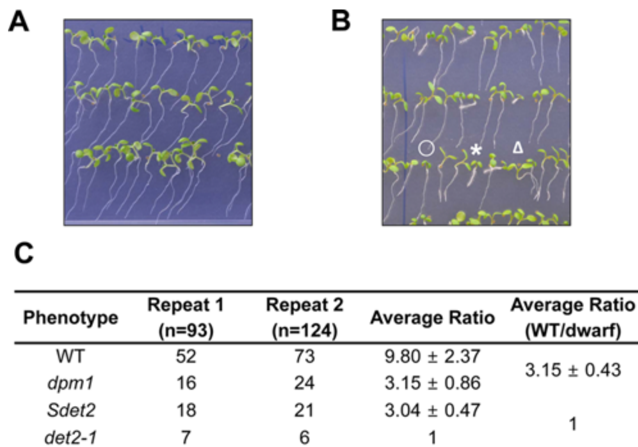
Fig. 2. The agravitropic growth of *det2-1* is independent of BR deficiency. (A) Nine d-old the wild type, *35S-DET2/det2-1* and *det2-1*. (B) Five d-old WT and *det2-1* seedlings grown on medium containing 10 nM BL. (C) Five d-old WT, *dwf4* and *bri1-116*. Homozygous seedlings are indicated by "v". (D) Seven d-old WT and *det2-1* seedling grown on media containing 2 μM of BRZ, 0.1 μM IAA or 1 μM 1-NAA. (A-D) Seedlings were grown on vertical plates under continuous light.

bioactive BR. Although the shoot dwarf phenotype was recovered by the exogenous application of BL, BL failed to rescue the agravitropic growth of *det2-1* (Fig. 2B). In addition, *dwf4* and *bri1-116* showed normal gravitropic root growth (Fig. 2C). A BR biosynthetic inhibitor, brassinazole (BRZ), was applied to reduce the endogenous level of BRs (Asami and Yoshida 1999). BRZ treatment caused root growth inhibition, but not agravitropic growth in the wild type (Fig. 2D). These results suggest that the severe root growth defect and the agravitropic response of *det2-1* are partially independent of the functions of BR.

Auxin is a major plant hormone that regulates growth and the gravitropic response of roots (Fu and Harberd 2003; Swarup et al. 2001; Kim et al. 2007). We speculated that the phenotypes of *det2-1* are related to auxin. To test this hypothesis, indole-3-acetic acid (IAA) was administered to *det2-1*. The exogenous application of IAA could not rescue the short and the agravitropic roots of *det2-1* (Fig. 2D). To test whether *det2-1* has a defect in an auxin transporter, a lipophilic auxin, 1-naphthaleneacetic acid (1-NAA), which does not require auxin transporters for its action, was administered to *det2-1*. Similar to IAA, 1-NAA could not rescue the agravitropism of *det2-1* (Fig. 2D). These results suggest that the root growth defect and the agravitropic phenotypes of *det2-1* are not due to a deficiency in auxin or its transporters.

Secondary Mutation in *det2-1* Affects Root Growth and Gravitropism

The BR-independent root growth and gravitropic phenotype of *det2-1* led us to speculate on the presence of a second modifier in *det2-1*. To test whether *det2-1* has an additional mutation in the genome, we backcrossed *det2-1* with a wild type plant. All of the resulting F1 seedlings grown on vertical plates showed wild type-like root and shoot phenotypes with normal gravitropic growth (Fig. 3A). After the self-pollination of the F1 plants, we generated F2 plants. Interestingly, the resulting F2 seedlings showed four distinctly segregated phenotypes (Fig. 3B and Supplement Fig. 2). Some of the wild type-like plants grew in random directions, which was rarely seen in the F1 seedlings, indicating that this phenotype is recessive. In *det2-1*-like dwarf plants, some seedlings showed normal gravitropic growth and longer roots than those of other *det2-1*-like dwarf seedlings. We separated seedlings into four types, the wild type, the segregated wild type showing agravitropic roots, the original *det2-1* and the segregated *det2-1* showing partly rescued root growth with normal gravitropism. Here, we named the segregated *det2-1* as *sdet2* and the segregated wild type-like



Phenotype	Repeat 1 (n=93)	Repeat 2 (n=124)	Average Ratio	Average Ratio (WT/dwarf)
WT	52	73	9.80 ± 2.37	3.15 ± 0.43
dpm1	16	24	3.15 ± 0.86	
Sdet2	18	21	3.04 ± 0.47	1
det2-1	7	6	1	

Fig. 3. The original *det2-1* has a secondary modifier that is a recessive trait. The original *det2-1* was backcrossed with Col-0 WT. The resulting F1 plants were used to generate F2 plants by self-pollination. Seven d-old (A) F1 and (B, C) F2 seedlings grown on vertical plate under continuous light. (B) Representatives of the original *det2-1*, *sdet2* and *dpm1* are indicated by asterisk, opened circle and triangle, respectively. (C) Segregation ratio of WT, *dpm1*, *sdet2* and *det2-1*in F2 generation. Seedlings excluding non-germinated seeds were segregated based on the phenotypes. Average ratio to *det2-1* was calculated from two biological repeats with standard error.

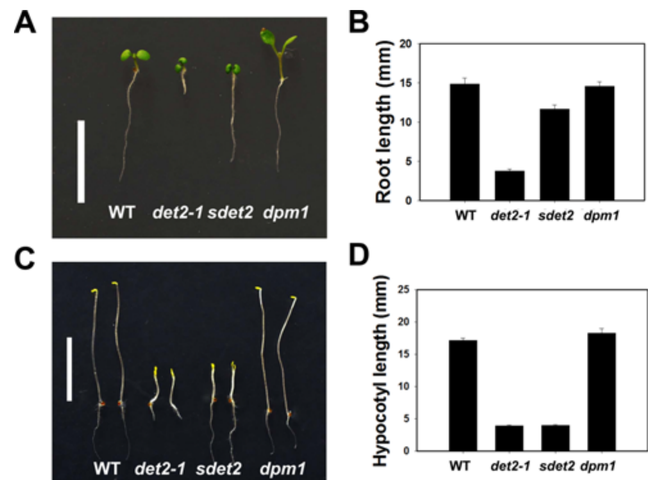


Fig. 4. Comparison of the original *det2-1*, *sdet2* and *dpm1* with the wild type. Phenotypes of (A, B) light-grown and (C, D) dark-grown the wild type, *det2-1*, *sdet2* and *dpm1*. Seedlings were grown under continuous light for 7 d. (A, C) Seedlings representing each genotype are shown. Bars = 10 mm. (B) Primary root length of light-grown seedlings. Two biological repeats showed similar results. Error bars mean standard error (n ≥ 16). (D) Hypocotyl length of dark-grown seedlings. Two biological repeats showed similar results. Error bars mean standard error (n ≥ 15).

agravitropic plant as <u>d</u>*et2-1* <u>p</u>*henotype* <u>m</u>*odifier 1*, or *dpm1*. In F2 generation, the segregation ratios of the wild type to *dpm1* and *sdet2* to the original *det2-1* show a Mendelian segregation pattern (Fig. 3C and Supplement Fig. 2). In addition, the ratio of the normal to dwarf plants is 2.72:1. These results suggest that *dpm1* mutation and the dwarfism caused by *det2-1* are recessive traits and monogenic.

dpm1 is Hypersensitive to BR Deficiency Causing Root Growth Reduction

To study physiological functions of the mutants in detail, we analyzed phenotypes of homozygous *dpm1* and *sdet2* in the F3 generation. To check whether the short root phenotype of the original *det2-1* is caused by the *dpm1* mutation, the root length of *sdet2* was measured and compared with the original *det2-1*. The root of *sdet2* was shorter than that of the wild type, indicating that BR deficiency causes root growth reduction (Figs. 4A and 4B). However, the root of *sdet2* was significantly longer than that of the original *det2-1* and similar to other BR mutants, demonstrating that the exaggerated short root of the original *det2-1* is caused by the *dpm1* mutation (Figs. 4A and 4B and Fig. 1A). Interestingly, although the root growth defect of the original *det2-1* was caused by the *dpm1* mutation, no significant differences in the root length between *dpm1* and the wild type were observed (Figs. 4A and 4B). This reveals that *dpm1* is hypersensitive to the BR-deficient condition with respect to root elongation. The petiole and hypocotyl of *dpm1* were longer than those of the wild type (Fig. 4A). However, the phenotypes were suppressed in the original *det2-1* mutant, suggesting that the enhanced shoot growth of *dpm1* is dependent on BR.

It has been reported that dark-grown *det2-1* has de-etiolated phenotypes such as a shortened hypocotyl and opened cotyledon (Chory et al. 1991). To see whether the *dpm1* mutation affects the de-etiolated phenotypes of the original *det2-1*, *sdet2* and *dpm1* plants were grown in the dark along with the wild type and the original *det2-1*. *sdet2* displayed the same normal de-etiolated phenotypes as the original *det2-1*, except for the root length (Fig. 4C). In the dark, the hypocotyl length of *dpm1* was comparable to that of the wild type (Figs. 4C and 4D).

To determine whether *dpm1* displays enhanced root growth reduction under BR-deficient conditions, *dpm1* seedlings were grown on a medium containing propiconazole (PPZ), a BR biosynthetic inhibitor (Sekimata et al. 2002; Hartwig et al. 2012), and their root growth was observed and compared with those of the wild type. PPZ treatment inhibited the root growth in both the wild type and *dpm1* (Fig. 5). However, the roots of *dpm1* were much shorter than those of the wild type when grown on a medium containing PPZ, indicating that *dpm1* is hypersensitive to PPZ (Fig. 5). These results suggest that *dpm1* is a conditional mutant of which root growth is synergistically retarded when the endogenous BR level is low.

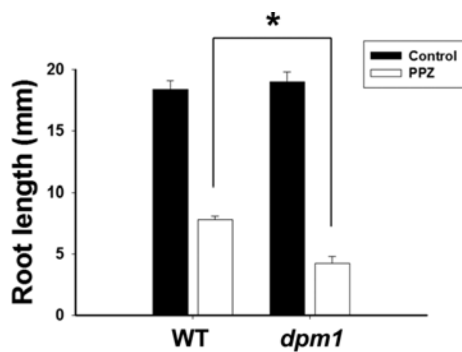


Fig. 5. Effects of *dpm1* on root growth are dependent on BR deficiency. The wild type (WT) and *dpm1* seedlings were grown on media containing without (black column) or with (white column) 2 μM PPZ. Seven d after planting, the length of primary roots were measured. Three biological repeats showed similar results. Asterisk shows significant differences between the WT and *dpm1* treated with PPZ by t-test ($P<0.001$). Error bars mean standard error ($n \geq 8$).

DPM1 is Required for the Gravitropism and Differentiation of Statolith-producing Cells

To verify whether *dpm1* gives rise to the agravitropic roots of the original *det2-1*, the growth phenotypes of the wild type, the original *det2-1*, *sdet2* and *dpm1* were observed. When grown in the light on a vertical plate, the roots of *dpm1* and the original *det2-1* grew in random directions, but the roots of the wild type and *sdet2* grew downward, demonstrating that the agravitropic roots of the original *det2-1* resulted from the *dpm1* mutation (Fig. 6). To test whether the agravitropic roots of *dpm1* are affected by BR or auxin treatment, *dpm1* was grown on media containing BL or IAA. Neither BL nor IAA could rescue the agravitropic root of *dpm1*, similar to the original *det2-1* (Fig. 6 and Figs. 2B and 2D). Similar to the light-grown seedlings, dark-grown *dpm1* seedlings displayed agravitropic growth (Supplement Fig. 3).

The first step of gravitropic response is the gravity sensing, the site of which is mainly located in the columella tissue of the root cap (Hashiguchi et al., 2013). In this step, amyloplasts in the columella cells function as statoliths to sense gravity. To check whether *dpm1* disrupts the gravity sensing machinery, we checked the organization of the statoliths in the *dpm1* root tips by Lugol staining. The statoliths were localized in the basal sides of the columella cells in the wild type (Fig. 7). In contrast, the statoliths of *dpm1* and the original *det2-1* displayed expanded domains from the columella cells to the lateral side of root cap (Fig. 7). In addition, the mutants were highly accumulated and filled with starch granules, so the pattern of statoliths localized to the lower sides of cells was rarely observed. Although the content of the statoliths in the tips of the *sdet2* roots was higher than in the wild type, this pattern of statoliths observed in *dpm1* and the original *det2-1* was not present in *sdet2* (Fig. 7). In addition to the content and distribution of statoliths, the shapes of the root tips of *dpm1* and the original *det2-1* were different from those of the wild type (Fig. 7). The tips of *dpm1* and the original *det2-1* roots were round in shape, as compared to those of the wild type. However, this root tip shape was not displayed in *sdet2*. PPZ treatment induced the accumulation of statoliths in the root tips of the wild type (Fig. 8). BR treatment reduced the statolith content in columella cells of the wild type, suggesting that BR inhibits starch granule accumulation in the columella cells (Fig. 8). However, neither BR nor PPZ affected the expanded

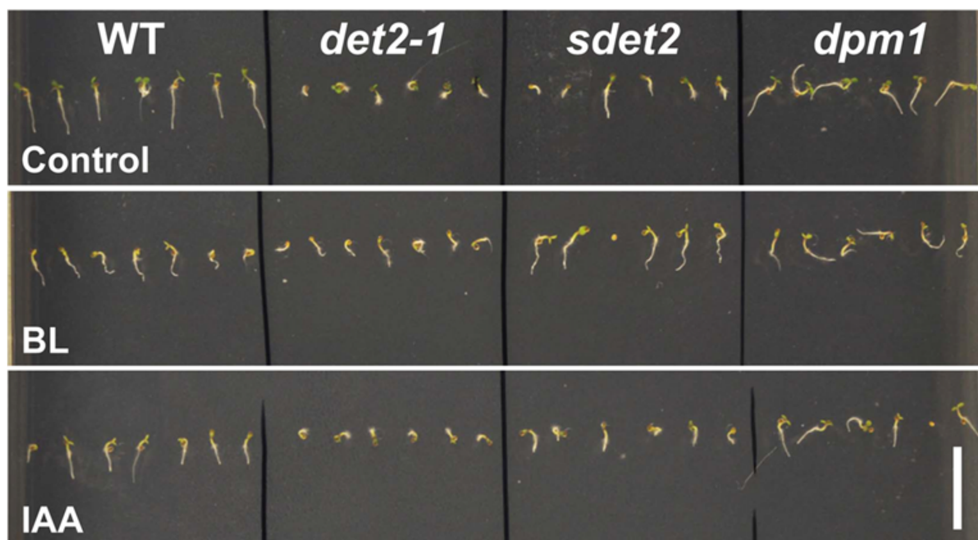


Fig. 6. Effects of BR and auxin on agravitropic growth of *dpm1*. The wild type (WT), *det2-1*, *sdet2* and *dpm1* seedlings were grown on media containing without or with 1 nM BL or 10 nM IAA. Four d after planting, photographs of seedlings were taken. Bars = 10 mm.

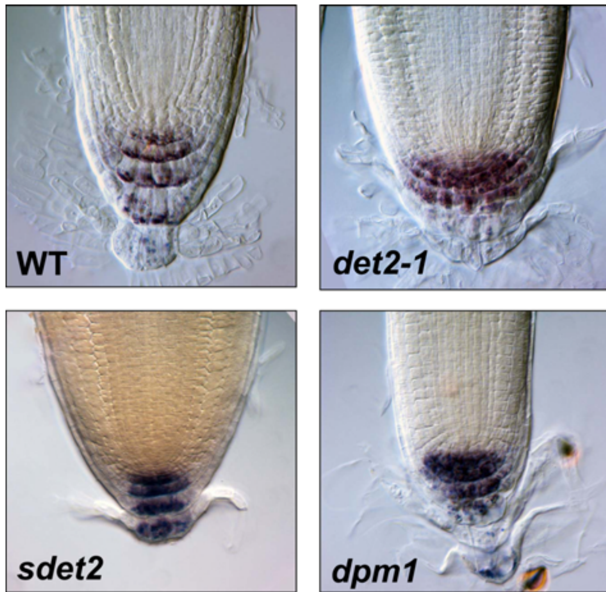


Fig. 7. Starch granule accumulation in the root caps of wild type (WT), *det2-1*, *sdet2* and *dpm1*. Starch granules in columella cells were stained using Lugol solution. Twelve d-old seedlings were used for Lugol staining. Stained root tips were treated with chloral hydrate prior to observation under differential interference contrast (DIC) microscope.

pattern of statoliths in columella cells and the round shapes of root tips of *dpm1* (Fig. 8). These results indicate that *dpm1* has defects in the gravitropic response and the organization of statoliths that are independent of BR.

Discussion

Secondary mutations have been reported in other mutants such as *ctr1-1* and *phyB-1* (Fukaki et al. 1997; Shin et al. 2013). These secondary mutations impede accurate understanding of the biological processes in which the mutations affect. By comparing the phenotypes of BR mutants, we found that the original *det2-1* mutant has a recessive secondary phenotypic modifier (*dpm1*), which caused strong root growth inhibition, agravitropic root growth and accumulated starch granules in the columella cells of the original *det2-1*, and those phenotypes are not related to BR deficiency. Without *dpm1*, *sdet2* showed slightly reduced root elongation, normal gravitropic root growth, dwarf shoots, dark green leaves and a de-etiolated phenotype (Fig. 4). Thus, these phenotypes that appeared in *sdet2* might be caused solely by *det2-1* mutation.

The generation of secondary modifiers has been used to identify novel factors that have functional relationships with the original mutants in forward genetics studies (Yin et al. 2002; Silverstone et al. 1997). The *dpm1* mutant that we isolated from the original *det2-1* displayed normal root elongation compared to the wild type (Figs. 4A and 4B). However, when *dpm1* was presented in a *det2-1* background (in case of the original *det2-1*) or grown in a medium containing a BR biosynthetic inhibitor, the root was much shorter than that of wild type (Figs. 4B and 5). This suggests that DPM1 has positive roles in the root growth and the influences of its deficiency on root growth are enhanced in BR-deficient conditions. One of the possible mechanisms

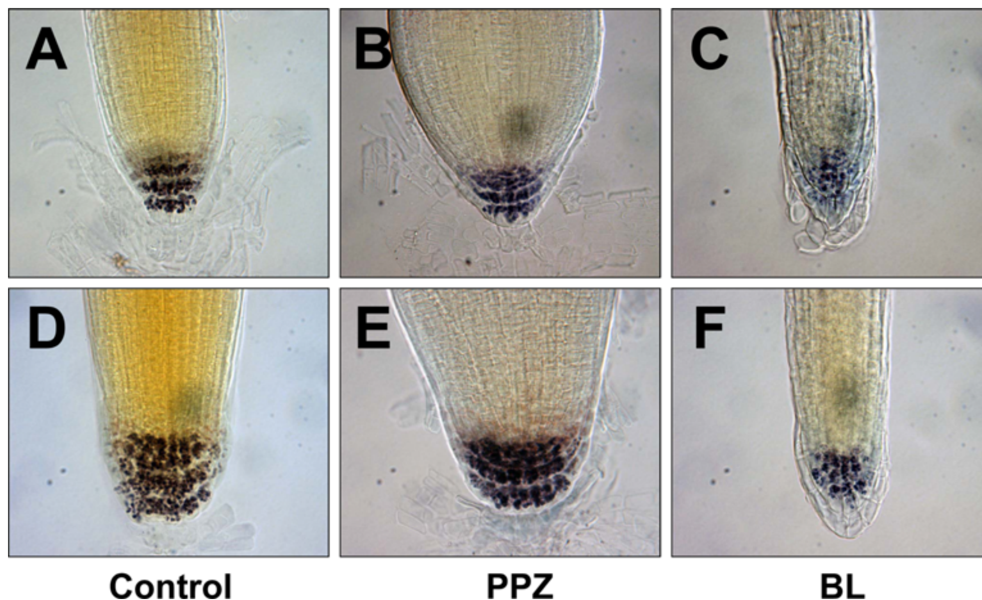


Fig. 8. Effect of BR on starch granule accumulation in the root cap of *dpm1*. The wild type (A-C) and *dpm1*(D-F) seedlings were grown on control media (A, D) or media containing 2 μM of PPZ (B, E) or 0.1 μM of BL (C, F) for 9 d. Seedlings were stained with Lugol solution. Stained root tips were treated with chloral hydrate prior to observation under DIC microscope.

that would explain this result is that the positive function of DPM1 on root growth is only activated in BR-deficient conditions. Under normal conditions, BR might repress DPM1's function on root growth. Another possibility is that DPM1 has homologs that are functionally redundant to *dpm1* and BR is required for the activation of the redundant function of the homologs.

It is known that BR promotes not only root elongation but also gravitropic response. Our results show that the synergistic effect between *det2-1* and *dpm1* mutation is mainly occurred in roots. It is possible that DPM1 may specifically function in BR-mediated root development. In particular, agravitropic roots were shown regardless of BR deficiency while short roots of *dpm1* was observed only in BR deficient condition (Fig. 2D and Fig. 6) . Thus, it seems that the function of DPM1 is closely linked to root growth, but not gravitropic response, regulated by BR signaling.

We isolated *dpm1* from the original *det2-1* by crossing with the wild type. The segregation ratios of *dpm1* to the wild type and *det2-1* are close to 1:3 and 3:1, respectively (Fig. 3C). This means *dpm1* mutation displays single recessive trait following Mendelian segregation ratio and is not linked to *det2-1* mutation. These results suggest that the mutation is not located on chromosome 2 on which *DET2* is located.

A notable phenotype of *dpm1* is agravitropic growth. When grown on a vertical plate, *dpm1* seedlings grew in random directions (Fig. 6 and Supplemental Fig. 2). This abnormal growth was independent of light conditions. Auxin is a major hormone that regulates the gravitropic response. Many of auxin's transport and signaling mutants are reported as agravitropic mutants. AUX1 is an auxin influx carrier, and its mutation causes agravitropic growth. The agravitropic growth of the *aux1* mutant is rescued by 1-NAA treatment (Swarup et al. 2001). However, agravitropic root growth caused by *dpm1* was not rescued by 1-NAA treatment (Fig. 2D). Some of the AUX/IAA mutants such as *axr2* and *axr3* also show agravitropic root growth, but the phenotypes of *axr2* and *axr3* are different from that of *dpm1* (Leyser et al. 1996; Timpte et al. 1994). For instance, *axr2-1* and *axr3-1* display reduced hypocotyl elongation in the dark while *dpm1* showed no significant differences (Fig. 4C and 4D) In addition, *dpm1* was sensitive to 1-NAA for auxin-induced root growth inhibition (Fig. 2D). Therefore, it seems that *dpm1* has a mutation that is different from known agravitropic auxin mutants and is independent of auxin transport and signaling. However, given that many auxin-related mutants display abnormal columella layer as well as agravitropic response, possible relationship between DPM1 and auxin in root development should be further investigated.

As *dpm1* has disorganized and accumulated statolith contents (Fig. 7), agravitropic growth of *dpm1* might be due to disruption of the gravity sensing step or an early phase of gravity signal transduction in the mutant. ARG1 and its homolog are known to function in gravity signaling pathway (Fukaki et al. 1997; Guan et al. 2003). Modifier screening of *arg1* mutant that enhance the gravitropic defect of the mutant identified that *mar1* and *mar2*, components of the translocon of outer membrane of chloroplasts (TOCs) complex, play a role in gravity signaling pathway (Stanga et al. 2009). Although *arg1*, *mar1* and *mar2* single mutants showed normal gravitropic growth, *mar1-1 arg1*-2 and *mar2-1 arg1*-2 double mutants displayed agravitropic growth similar to *dpm1* (Fig. 6). In addition to agravitropic growth, morphologies of their root tips are analogous with *dpm1* (Fig. 7 and 8D). Therefore, it is likely that *dpm1* is a mutant of which gene encodes a key signaling component of ARG1 and TOC complex-mediated gravity signal transduction rather than ARG1, MAR1 or MAR2 itself. Identification of DPM1 using map-based cloning and genetic interaction with mutants showing agravitropic response will shed light on the understanding of gravity signal transduction pathway and the root physiology regulated by BR and/or auxin.

Materials and methods

Plant materials and Growth Conditions

Arabidopsis thaliana ecotype Columbia-0 was used as the wild type in this study. Surface sterilized seeds were incubated at 4°C for 2 d. Seeds were then planted on 0.8% agar (Phytoblend, Caissons labs) containing 0.5 × Murashige and Skoog (MS)-salt medium (PhytoTechnology Laboratories) and 1% (w/v) sucrose. Seedlings were grown in the growth chamber under continuous light at 22±1°C. For etiolated conditions, plates were wrapped with aluminum foil and incubated in the growth chamber. Soil-grown plants were grown in a greenhouse with a 16-h light/8-h dark cycle at 22-24°C.

For hormones and inhibitor treatment, seeds were germinated and grown on hormone- or inhibitor-containing medium. For root and hypocotyl length measurements, plates with 7-d-old seedlings were photographed.

Construction of *35S-DET2/det2-1*

DET2 ORF was amplified as a *Sma*I-*Sac*I fragment by PCR using forward primer (5'-CCCCGGGAGTTGATTCTGCCCTTATTAG-3') and reverse primer (5'-CGAGCTCAACTTTCTCAATTCTCAG-TACAC-3'). PCR products were inserted into a pGEM-T Easy vector (Promega) for sequencing. Sequence confirmed *DET2* fragments were cloned into corresponding site of pBI121 expression vector (Clontech). The construct was introduced into *Agrobacterium tumefaciens* strain GV3101 (pMP90). The construct was transformed into *det2-1* plant by the floral dip method (Clough and Bent 1998). Transformants were selected in kanamycin-containing LB medium.

To confirm overexpression of *DET2*, total RNA was extracted from seedlings of selected plants using TRI reagent (Sigma) according to the manufacturer's manual. First-strand cDNAs were synthesized using M-MLV reverse transcriptase (Promega). cDNAs were amplified using same primers used for the cloning. As a loading control, *UBQ5* cDNA was amplified using a forward primer (5'-GACCATAACC-

CTTGAGGTTGAATC-3') and a reverse primer (5'-AGAGAG-AAAGAGAAGGATCGATC-3').

Lugol Staining

Seedlings were grown in vertical plates. The root segment are stained in Lugol solution (Sigma). Stained roots were washed with water and cleared with chloral hydrate solution (chloral hydrate/glycerol/water = 8:1:3) prior to observation under differential interference contrast (DIC) microscopy using a Leica DC 500 microscope.

Acknowledgments

This work was supported by the Young Investigator Research Program of Chung-Ang University 2010 year and the Chung-Ang University Research Scholarship Grants in 2012. We thank Tae-Wuk Kim (Hanyang University, Seoul, Korea) for helpful suggestions.

Authors' Contributions

CHP and SKK designed the experiments and wrote the manuscript. CHP analyzed data. CHP, JHY, JEL and MKK constructed and generated the transgenic plant. CHP, MSJ, SCP and SKK revised the manuscript. All authors have agreed to the contents of the manuscript and declare no conflicting interests.

Supporting Information

Fig. S1. The dwarf phenotype of *det2-1* is rescued by ectopic expression of *DET2*.
Fig. S2. Segregation of *dpm1* and *sdet2* in F2 generation.
Fig. S3. Agravitropic growth of *dpm1* in the dark.

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