Brassinosteroid Regulates Seed Size and Shape in Arabidopsis[1][W][OPEN]

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Seed development is important for agriculture productivity. We demonstrate that brassinosteroid (BR) plays crucial roles in determining the size, mass, and shape of Arabidopsis (*Arabidopsis thaliana*) seeds. The seeds of the BR-deficient mutant *de-etiolated2* (*det2*) are smaller and less elongated than those of wild-type plants due to a decreased seed cavity, reduced endosperm volume, and integument cell length. The *det2* mutant also showed delay in embryo development, with reduction in both the size and number of embryo cells. Pollination of *det2* flowers with wild-type pollen yielded seeds of normal size but still shortened shape, indicating that the BR produced by the zygotic embryo and endosperm is sufficient for increasing seed volume but not for seed elongation, which apparently requires BR produced from maternal tissues. BR activates expression of *SHORT HYPOCOTYL UNDER BLUE1*, *MINISEED3*, and *HAIKU2*, which are known positive regulators of seed size, but represses *APETALA2* and *AUXIN RESPONSE FACTOR2*, which are negative regulators of seed size. These genes are bound in vivo by the BR-activated transcription factor BRASSINAZOLE-RESISTANT1 (BZR1), and they are known to influence specific processes of integument, endosperm, and embryo development. Our results demonstrate that BR regulates seed size and seed shape by transcriptionally modulating specific seed developmental pathways.

Seed development in flowering plants is a complicated process controlled by a complex network. The double fertilization in the embryo sac generates two zygotic products, the embryo and endosperm. The embryo gives rise to the daughter plant while the endosperm provides nutrients for embryo during embryogenesis and germination (Lopes and Larkins, 1993; Faure et al., 2002). Seed coats, which enclose the embryo and endosperm, are differentiated from maternally derived integuments (Haughn and Chaudhury, 2005), and the final seed size and weight are coordinately determined by the growth of the endosperm, embryo, and integument (Berger et al., 2006; Zhou et al., 2009).

The molecular mechanisms of seed development have been studied extensively, and many genes involved in seed development have been identified. These include *SHORT HYPOCOTYL UNDER BLUE1* (*SHB1*), *HAIKU1* (*IKU1*), *MINISEED3* (*MINI3*), and *HAIKU2* (*IKU2*), which promote the development of endosperm and embryo and positively regulate the seed size (Garcia et al., 2003; Luo et al., 2005; Zhou et al., 2009; Wang et al., 2010). IKU1, MINI3, and IKU2 successively act in the same pathway that is required to expand the seed cavity and to induce the endosperm growth in the early phase of seed development (Luo et al., 2005). IKU1 directly interacts with MINI3 (Wang et al., 2010). SHB1 is a positive regulator of seed development and may activate *MINI3* and *IKU2*. SHB1 has also been shown to enhance the embryo cell proliferation and expansion through an unknown IKU2-independent pathway (Zhou et al., 2009). By contrast, APETALA2 (AP2) inhibits integument growth and the development of embryo and endosperm and reduces the seed size. AP2 determines seed weight and seed size by coordinating the growth of endosperm, embryo, and maternal integuments (Jofuku et al., 2005; Ohto et al., 2005, 2009). In addition, *AUXIN RESPONSE FACTOR2* (*ARF2*) determines seed mass and seed size through regulating integument growth. Deficiency of ARF2 results in the dramatically increased seed size and weight due to the extra cell division in the integuments, which leads to the formation of enlarged seed coats (Schruff et al., 2006). The cytochrome P450 CYP78A5 (KLUH [KLU]) has been shown to act as a maternal regulator of seed size by stimulating the integument cell proliferation (Ito and Meyerowitz, 2000; Adamski et al., 2009). Additional factors, including FERTILIZATION INDEPENDENT SEED2 (FIS2), AINTEGUMENTA (ANT), RETARDED GROWTH OF EMBRYO1 (RGE1), ARABIDOPSIS HIS KINASE2 (AHK2), AHK3, and AHK4,

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and CYTOKININ INDEPENDENT1 (CKI1), regulate the seed weight and size through epigenetic regulation of endosperm development and paternal imprinting, regulation of embryo proliferation, endosperm, and integument development (Sun et al., 2010a).

Brassinosteroid (BR), a kind of steroid hormone, plays important roles in plant growth and development, such as stem elongation, leaf expansion, vascular differentiation, stress tolerance, senescence, and stomatal development (Clouse and Sasse, 1998; Gudesblat et al., 2012; Kim et al., 2012; Wang et al., 2012). An important role for BR in plant reproductive growth and seed development has been suggested by the studies of BR-deficient and -insensitive mutants of Arabidopsis (*Arabidopsis thaliana*), pea (*Pisum sativum*), tomato (*Solanum lycopersicum*), and rice (*Oryza sativa*; Fujioka and Yokota, 2003; Nomura et al., 2007; Ye et al., 2010). In Arabidopsis, the weak BR-deficient mutant *de-etiolated2* (*det2*; Fujioka et al., 1997) has lower fertility compared with the wild type (Ye et al., 2010), and the strong BR-deficient mutants *dwarf4* (*dwf4*) and *constitutive photomorphogenesis and dwarfism* (*cpd*) are completely male sterile (Choe et al., 1998; Ye et al., 2010). Rice mutants with defects in BR biosynthesis or signaling also show reduced seed length (Hong et al., 2005; Tanabe et al., 2005; Morinaka et al., 2006). Overexpression of a BR biosynthetic gene in rice increases seed filling and seed size (Wu et al., 2008). The BR-deficient mutant *lk* of pea has irregularly shaped seeds (Nomura et al., 2007). BR acts through the cell surface receptor BRASSINOSTEROID INSENSITIVE1 (BRI1; Kim et al., 2009) and transcription factors BRASSINAZOLE-RESISTANT1 (BZR1) and BRI1-EMS-SUPPRESSOR1 (BES1; Wang et al., 2002; Yin et al., 2002; He et al., 2005) to control a large number of BR-responsive genes (Sun et al., 2010b; Yu et al., 2011). Although it has been reported that BR affects seed size and shape (Hong et al., 2005; Takahashi et al., 2005; Tanabe et al., 2005; Morinaka et al., 2006), the molecular mechanism of BR regulation of seed development has remained unclear.

To understand the mechanisms by which BR controls seed size and shape, we analyzed the seed development in BR-deficient and -insensitive mutants and the effects of BR on genes known to control various aspect of seed development. Our studies indicated that BR increased seed size by affecting the integument, endosperm, and embryo development, and BR-activated BZR1 directly regulated several genes known to control the seed size. We further showed that the seed shape would be determined largely by BR signal of maternal tissues, whereas BR produced by the embryo and endosperm seemed sufficient to increase seed size, providing evidence for a mode of localized actions of BR in seed development.

RESULTS

BR-Deficient and -Insensitive Mutants Have Small Seeds

To evaluate the effects of BR on seed growth, we analyzed the size and weight of seeds of the BR-deficient mutant *det2* and the BR receptor mutant *bri1-5* (a weak allele of *bri1* mutant; Fujioka et al., 1997; Wang et al., 2001). The mature dry seeds of *det2* and *bri1-5* were smaller than wild-type seeds (Fig. 1, A–E), and the embryos from mature *det2* seeds were significantly smaller than those of the wild type (Fig. 1, F and G). The *det2* seeds were 17% lighter than the wild type (ecotype Columbia [Col-0]; Fig. 1H), and the seeds of *bri1-5* were about 10% lighter than the wild-type seeds (ecotype Wassilewskija [Ws]; Fig. 1H). The seeds of *det2* and *bri1-5* had shorter length but slightly bigger width compared with the wild-type controls (Fig. 1, I–J). The seeds of *bzr1-1D*, a gain-of-function mutant of the *BZR1* gene, were not bigger or heavier than the seeds of wild type (Fig. 1, A, C, H, I, and K); the *bzr1-1D* mutant also showed a short-hypocotyl phenotype, which is thought to be due to feedback inhibition of BR biosynthesis (Wang et al., 2002; He et al., 2005). These results suggested that BR positively regulated seed size and was involved in seed shape determination.

To investigate the cellular basis for the altered seed size and shape of *det2*, we examined the cell size and number of the embryonic shoot or hypocotyl and embryonic root apex in mature *det2* and wild-type seeds using scanning electron microscopy. The results showed that the cell length of *det2* hypocotyls was reduced by 23% compared with the wild type, and cell width was not obviously altered (Fig. 1, L). In addition, the numbers of cells in the epidermal cell files from the base of cotyledon junction to the embryonic root apex, and along the axis of cotyledon, were smaller in *det2* mutant than in the wild type (Fig. 1, M). Microscopic analysis further showed that the integument cells of *det2* mutants were significantly shorter than the wild type (Supplemental Fig. S1). These results indicated that the decrease in seed size and seed weight in *det2* was correlated with reduced embryo cell size and number and decreased integument cell length.

The sections of ovaries also showed that the endosperm cellularization of *det2* was delayed. The endosperm cellularization of the wild type began at 5 d after pollination (DAP) when embryos were at the early-heart stage, whereas *det2* endosperm cellularization was initiated at late 6 to 7 DAP, when embryos were close to the middle-heart stage. Endosperm cellularization of *det2* and wild-type seeds was finished at 9 and 7 DAP, respectively (Supplemental Fig. S1). These results indicate that the reduced seed cavity and endosperm volume, delayed embryo development, decreased embryo cell size and number, and reduced integument cell length possibly contributed to the reduced seed size of *det2*.

Tissue-Specific Effects of BR on Seed Size and Shape

Seed is a complex organ that contains both maternal tissues (integument) and zygotic tissues (embryo and endosperm). Control of seed size involves complex interactions among the zygotic embryo and endosperm, the maternally derived seed coat, and the parent plant

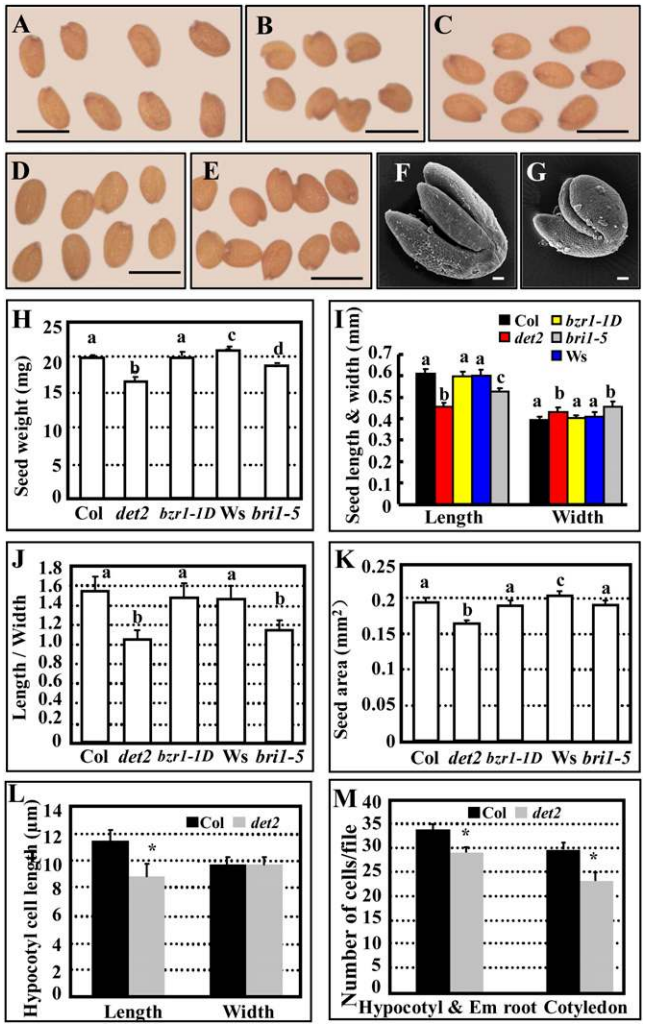

Figure 1. BR-deficient mutants and BR-insensitive mutants have small seeds. Mature seeds of Col-0 (A), *det2* (B), *bzr1-1D* (C), Ws (D), and *bri1-5* (E). Mature embryos of Col-0 (F) and *det2* (G). Seed weight per 1,000 mature dried seeds (H), seed length and seed width (I), the ratio of length to width (J), and seed area (K) of mature dried seeds of Col-0, *det2*, *bzr1-1D*, Ws, and *bri1-5*. Hypocotyl cell length and width of Col-0 and *det2* ($*P < 0.05$). Average cell numbers from three columns in the central region of the hypocotyl (L), plus the embryonic (Em) root (left) and the cotyledon (right) of Col-0 and *det2* ($*P < 0.05$; M). Bars = 750 μm (A–E) and 50 μm (F and G). Values that differ at the 0.05 significance level are labeled with different letters.

(Schruff et al., 2006). To dissect the effects of BR synthesis and signaling in specific tissues on seed size and shape, we performed reciprocal crosses of the wild type with BR-deficient and -insensitive mutants to create seeds that contain wild-type embryo and endosperm but mutant integuments. To avoid the volume effect of siliques to seed shape, we compared the ovaries shape in the very early stages after fertilization. After hand pollination, the ovaries in early-, middle-, and mature-heart stages were cleared and analyzed respectively under differential contrast microscopy. The heart shape embryos indicated the same angles of the photography, especially in middle-heart stage. As shown in Figure 2 and Supplemental Figure S2, *det2*–/–, *dwf4*–/–, and *bri1*–/– ovaries produced by hand pollinating the corresponding pollens to self pistils were smaller and shorter than the wild type. On average, the *det2-1* seeds were 16% lighter than those of the wild type, and the *dwf4* (–/–) and the *bri1-116* (–/–) seeds were 17% and 18% lighter than those of the wild type, respectively. The ovaries from the *det2*, *dwf4*, and *bri1-116* mutant plants pollinated with wild-type pollen, resulting in wild-type embryos and endosperm but mutant integuments, were bigger but of same round shape compared with those from self-pollinated mutant plants (Fig. 2A; Supplemental Fig. S2, A–G). Consistent with these observations of developing ovaries, the *det2* (+/–), *dwf4* (+/–), and *bri1-116* (+/–) mature seeds produced from *det2*, *dwf4*, or *bri1-116* pistils pollinated with wild-type pollens had similar seed weight and seed area as wild-type seeds, which showed rescue of the seed size but not the seed shape by the wild-type alleles from the pollens (Fig. 2, B–E). By contrast, the ovaries and seeds produced by wild-type plants pollinated by the mutant pollens are almost the same size and shape of the wild type, consistent with the recessive nature of these mutations (Fig. 2, A–E). These results indicated that BR produced by the embryo and endosperm increased the seed size but had little effect on seed shape, whereas BR signaling in the integument contributed to the elongated shape of Arabidopsis seeds. The inability of BR produced in the embryo and endosperm to rescue the seed shape suggested that BR had limited mobility, and BR synthesized in these internal seed organs had little effect on the integument cells.

BR Treatment Increases Seed Size and Affects Seed Shape

Application of BR to the inflorescence of the wild type and *det2* mutants resulted in enhanced seed weight in both the wild type and *det2* mutant (Fig. 3A). But the seed weight of the *det2* mutant under BR treatment was lower than wild-type seeds without BR treatment, indicating the exogenetic BR could not fully rescue BR deficiency in the *det2* mutant. The seed area of the *det2* mutant was recovered to wild-type level under BR treatment, and there was no significant difference of seed area between the wild type and *det2* mutant after BR treatment (Fig. 3A). For seed shape, ratio of seed length to width of wild-type plant was not changed by BR treatment, and that of *det2* mutant was enhanced (Fig. 3A). The length/width ratio of the *det2* mutant under BR treatment was lower than the wild type, suggesting that BR treatment could not fully rescue seed shape. The explanation of partial rescue would be the efficiency of extrinsic BR treatment. Above all, we still could conclude that BR directly regulated seed size and affected seed shape.

BR Regulates the Expression of Seed Size-Related Genes

Previous studies indicated that seed size is coordinately determined by the growth of the endosperm,

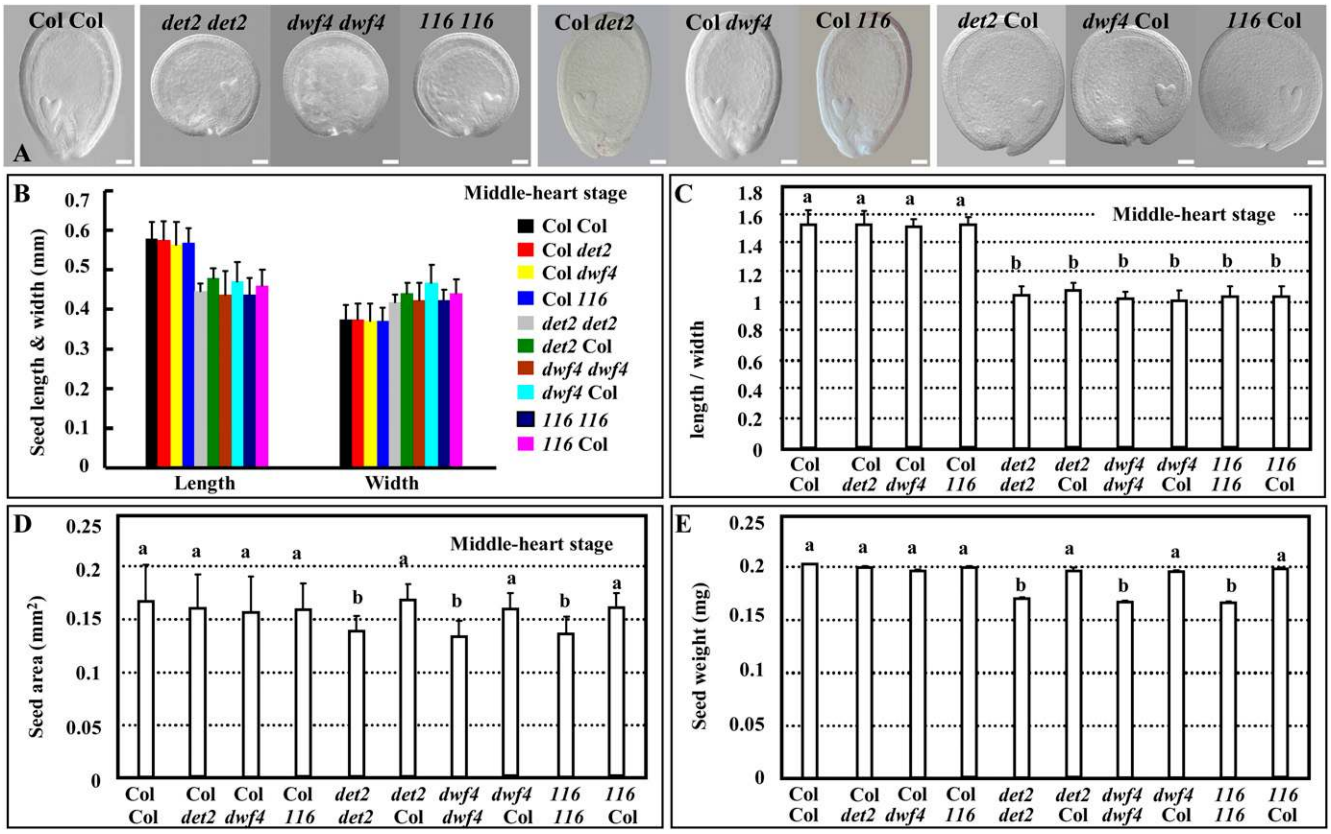

Figure 2. BR regulates seed size and shape. Cleared seeds of the plants from reciprocal crosses between Col-0 and *det2*, *dwf4*, and *bri1-116* (shown *116*) in middle-heart stage were imaged with differential contrast optics. Bar = 60 μm. Col-0 *det2* indicates Col-0 pistils pollinated with *det2* pollen and so on (A). Seed length and seed width (B), the ratio of length to width (C), and seed area (D) from seeds of Col-0 Col-0, Col-0 *det2*, Col-0 *dwf4*, Col-0 *116*, *det2 det2*, *det2* Col-0, *dwf4 dwf4*, *dwf4* Col-0, *bri1-116 bri1-116*, and *bri1-116* Col-0 in middle-heart stage. Col-0 *det2* indicates Col-0 pistils pollinated with *det2* pollen and so on. Seed weight per 1,000 mature seeds from seeds of these reciprocal crosses (E). Values that differ at the 0.05 significance level are labeled with different letters.

embryo, and integument (Berger et al., 2006; Zhou et al., 2009), and several genes involved in these processes have been identified. We thus analyzed whether any of the genes known to regulate seed size or shape were regulated by BR. The expression levels of these genes in the wild type plant and *det2* mutant under BR treatment or brassinazole (BRZ), a triazole compound that specifically blocks brassinolide biosynthesis by inhibiting *DWF4* gene (Asami et al., 2001), were first analyzed. As shown in Figure 3, the expression levels of the *SHB1*, *IKU1*, *MINI3*, *IKU2*, *HEAT SHOCK FACTOR15* (*HSF15*), and *KLU* genes, which were positive regulators of seed size, were significantly increased after 3 h of BR treatment and obviously reduced by 3 h of treatment with the BR biosynthetic inhibitor BRZ in both wild-type and *det2* siliques (Fig. 3, B and C). Then the transcription level of these genes were analyzed in *det2* and *bri1-5* siliques at 4 to 5 d after hand pollination. As shown in Figure 3D, the expression levels of positive regulators of seed size were significantly reduced in the *det2* and *bri1-5* mutants but slightly increased or unaffected in the *bzr1-1D* mutant. The transcript levels of the *AP2* and *ARF2* genes, which were negative regulators of seed size, were decreased by BR treatment and increased by BRZ treatment in wild-type and *det2* siliques (Fig. 3, B and C), as well as enhanced in the *det2* and *bri1-5* mutants but slightly decreased in the *bzr1-1D* mutant (Fig. 3D). In addition, the expressions of *FIS2*, *ANT*, *RGE1*, *AHK1*, *AHK3*, *AHK4*, and *CKI1*, which are all related to seed size, were changed under altered BR levels (Supplemental Fig. S3). These results suggested that BR increased seed size by activating the expressions of many genes that possibly increase seed size and repressing the genes that inhibit seed growth.

Previous genome-wide studies have identified direct target genes of the BR-signaling transcription factors BZR1 and BZR2/BES1 (Wang et al., 2002; Yin et al., 2002); the data suggest that those important seed development-regulating genes mentioned above (*SHB1-MINI3-IKU2*, *AP2*, and *ARF2*) are direct targets of BZR1 but not BES1 (Sun et al., 2010b; Yu et al., 2011), so we further performed BZR1 chromatin immunoprecipitation (ChIP) followed by quantitative PCR (qPCR) analysis to confirm the direct involvement of BZR1 in the regulation of

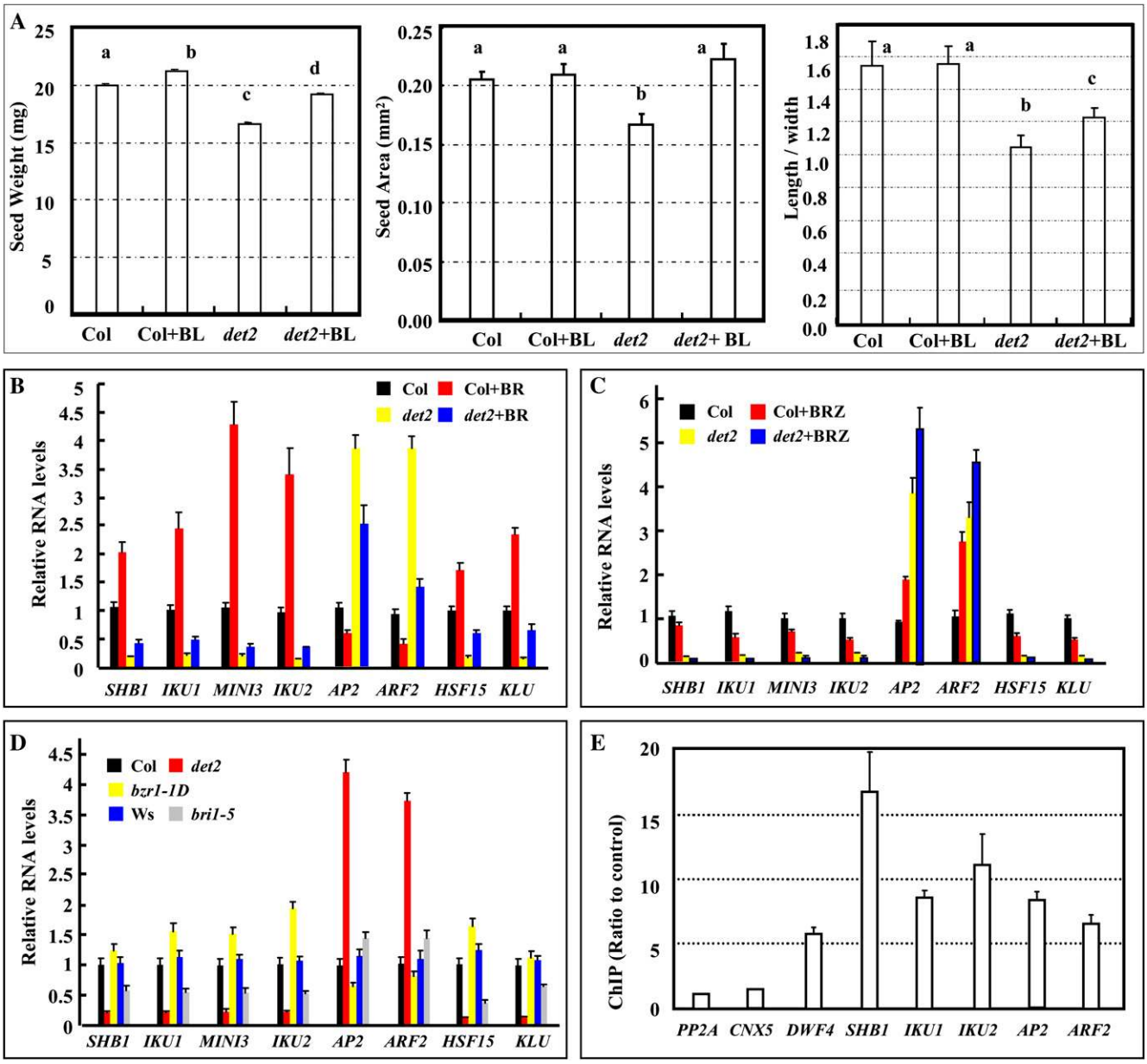

Figure 3. BR increases seed size and affects seed shape, and BR regulates the expression levels of seed size-related genes through directly binding to their promoters by BZR1. A, Statistics of seed weight, seed area, and the ratio of seed length to width indicated that BR treatment resulted in Col-0 seeds with enhanced weight and det2 seeds with rescued seed area and partially rescued seed weight/ratio of seed length to width. Values that differ at the 0.05 significance level are labeled with different letters. The inflorescence of Col-0 and det2 mutants was dipped with 10 μM 24-epibrassinolide (BR) or mock solution once a day. The inflorescence was dipped 10 s and then grew normally. B, Expression levels of the above-mentioned genes in siliques 4 to 5 d after hand pollination of Col-0 with mock solution or 10 μM 24-epibrassinolide (BR) treatment and *det2* with mock solution or 10 μM 24-epibrassinolide (BR) treatment for 3 h as determined by quantitative RT-PCR analysis. C, Expression levels of the above-mentioned genes in siliques 4 to 5 d after hand pollination of Col-0 with mock solution or 1 μM BRZ treatment and *det2* with mock solution or 1 μM BRZ treatment for 3 h as determined by quantitative RT-PCR analysis. D, Expression levels of *SHB1*, *IKU1*, *MINI3*, *IKU2*, *AP2*, *ARF2*, *HSF15*, and *KLU* in siliques 4 to 5 d after hand pollination of Col-0, *det2*, *bzr1-1D*, Ws, and *bri1-5* as determined by quantitative RT-PCR analysis. E, ChIP analysis revealed that BZR1 binds to the promoters of *SHB1*, *IKU1*, *IKU2*, *AP2*, and *ARF2*. Means were calculated from three biological samples, and each biological sample was examined in triplicate. Error bars indicate SD.

seed development-related genes. The results showed that in vivo BZR1 bound to the promoters of *SHB1*, *IKU1*, *IKU2*, *AP2*, and *ARF2* (Fig. 3E). Although MINI3 was not a direct target of BZR1, its interacting protein IKU1 (Wang et al., 2010) was a direct target of BZR1 (Fig. 3E). It has been reported that SHB1 regulates the expression of *MINI3* (Zhou et al., 2009), so it was deduced that BZR1 indirectly regulated *MINI3* through *IKU1* or *SHB1*. Additional genes involved in regulating seed size, including *KLU*, *HSF15*, *FIS2*, *RGE1*, *ANT*, *AHK1*, *AHK3*, *AHK4*, and *CKI1* (Sun et al., 2010b), were all confirmed to be direct targets of BZR1 by ChIP-qPCR detection (Supplemental Fig. S3). These results suggested that BZR1 directly mediated BR regulation of genes involved in seed development.

MINI3 and *SHB1* Act Downstream of BR in Regulating Seed Size

To further investigate whether the reduced expression of *SHB1*, *IKU1*, *MINI3*, and *IKU2* in the *det2* mutant were responsible for the small-seed phenotype, we analyzed the genetic interactions of these genes with *det2-1*. Overexpression of *MINI3* in the *det2* mutant resulted in obviously heavier seeds, although the seed length, width, ratio of length to width, and seed area were not significantly affected (Fig. 4, A–E). Overexpression of *MINI3* in wild-type plant also resulted in heavier seeds, while other seed traits were unaffected (Supplemental Fig. S4; Supplemental Table S1). These results suggested that *MINI3* possibly acted downstream of BR and mediated BR regulation of seed weight but not seed shape.

The *shb1-Dominant* (*shb1-D*) mutant produces heavier seeds (Zhou et al., 2009). We crossed *shb1-D* with *bri1-5*, which are both in the Ws background. Analysis of more than 10 *bri1-5 shb1-D* double mutant individuals showed increased seed mass and seed area compared with those of *bri1-5* or the wild type and no obvious difference when compared with *shb1-D* (Fig. 4, F–I). The seed length of *bri1-5 shb1-D* was larger than that of *bri1-5* and shorter than *shb1-D*, while the seed width of *bri1-5 shb1-D* was larger than either *bri1-5* or *shb1-D* (Fig. 4G). The ratio of length to width of *bri1-5 shb1-D* was not obviously different from that of *bri1-5* and was significantly smaller than that of Ws or *shb1-D* (Fig. 4H). These results showed that *shb1-D* can partially restore the seed weight and seed size of the *bri1-5* mutant, consistent with *SHB1* acting downstream of BR in promoting seed size. However, BR's effect on seed shape was independent of *SHB1*.

Genetic studies by crossing the *shb1*, *mini3*, and *iku2* mutants with *bzr1-1D* mutants were performed to further test whether *SHB1*, *MINI3*, and *IKU2* acted downstream of BZR1 and BR signal. The mass and area of seeds produced from *shb1 bzr1-1D*, *mini3 bzr1-1D*, and *iku2 bzr1-1D* were all significantly lower than those of *bzr1-1D* mutants and had no obvious difference from those of *shb1*, *mini3*, and *iku2* mutants (Fig. 5, A, B, and E). The other seed traits, including the ratio of length to width, in *shb1 bzr1-1D*, *mini3 bzr1-1D*, and *iku2 bzr1-1D* were also similar to that of *shb1*, *mini3*, and *iku2* mutants, respectively (Fig. 5,C and D). These genetic results were consistent with *SHB1*, *MINI3*, and *IKU2* acting downstream of BZR1.

AP2 and *ARF2* Are Negative Regulators of Seed Size Acting Downstream of BR

It is reported that AP2 negatively regulates seed size and mass. The weak (*ap2-5*) and strong (*ap2-6*) allele of *AP2* mutants produced larger seeds (Jofuku et al., 2005; Ohto et al., 2005). Double mutants of *det2* with *ap2-5* and *ap2-6* were generated and analyzed to investigate whether the increased expression of *AP2* in *det2* was responsible for the small-seed phenotype of *det2*. As shown in Figure 6, mutations in *AP2* suppressed the small-seed phenotype of *det2*. Seed mass and seed area of *ap2-5 det2* was significantly larger than that of the *det2* mutant and similar to the *ap2-5* mutant (Fig. 6, B and E). The *ap2-5 det2* seeds had notably increased length and width but unchanged ratio of length to width compared with *det2* (Fig. 6, C and D). Seeds from *ap2-6 det2* were dramatically heavier and larger than *det2* and were not obviously different from those of *ap2-6* (Fig. 6, A, B, and E). By contrast, the seed shape of *ap2-6 det2* was similar to *det2* but different from *ap2-6*. The seed length and width of *ap2-6 det2* were notably increased, but their ratio remained the same compared with *det2*. The seed length of *ap2-6 det2* was shorter than that of *ap2-6*, but the seed width was increased compared with that of *ap2-6*. There was no significant difference in seed shape between *ap2-6 det2* and *det2* (Fig. 5, C and D). These results indicated that seed weight and seed size of the *ap2 det2* double mutant were similar to the single *ap2* mutants, whereas the seed shape of double mutants was similar to *det2* but notably different from *ap2*, suggesting that *AP2* acted downstream of *DET2* and played a negative role in BR regulation of seed size. On the other hand, BR-mediated seed shape regulation was independent of *AP2*.

To investigate whether *AP2* acted downstream of *BZR1* and BR signal, we performed double mutation analysis combining *ap2-6* with *bzr1-1D* mutants. Seeds produced from *ap2-5 bzr1-1D* (Huang et al., 2012) and *ap2-6 bzr1-1D* double mutants were significantly heavier and larger than those of *bzr1-1D* mutants and were not obviously different than those of *ap2-5* and *ap2-6* mutants, respectively (Fig. 5, A, B, and E). Other seed traits, such as seed length and width and the ratio of length to width, in *ap2-5 bzr1-1D* and *ap2-6 bzr1-1D* double mutants resembled those of *ap2-5* and *ap2-6* mutants (Fig. 5, C and D). These data were consistent with *AP2* acting downstream of *BZR1*.

It has been shown previously that the embryos of *ap2* mutants have larger cell size and a greater number of cells (Jofuku et al., 2005; Ohto et al., 2005). Analysis showed that the embryo cell length of *ap2-6 det2* was significantly larger than that of the *det2* mutant and

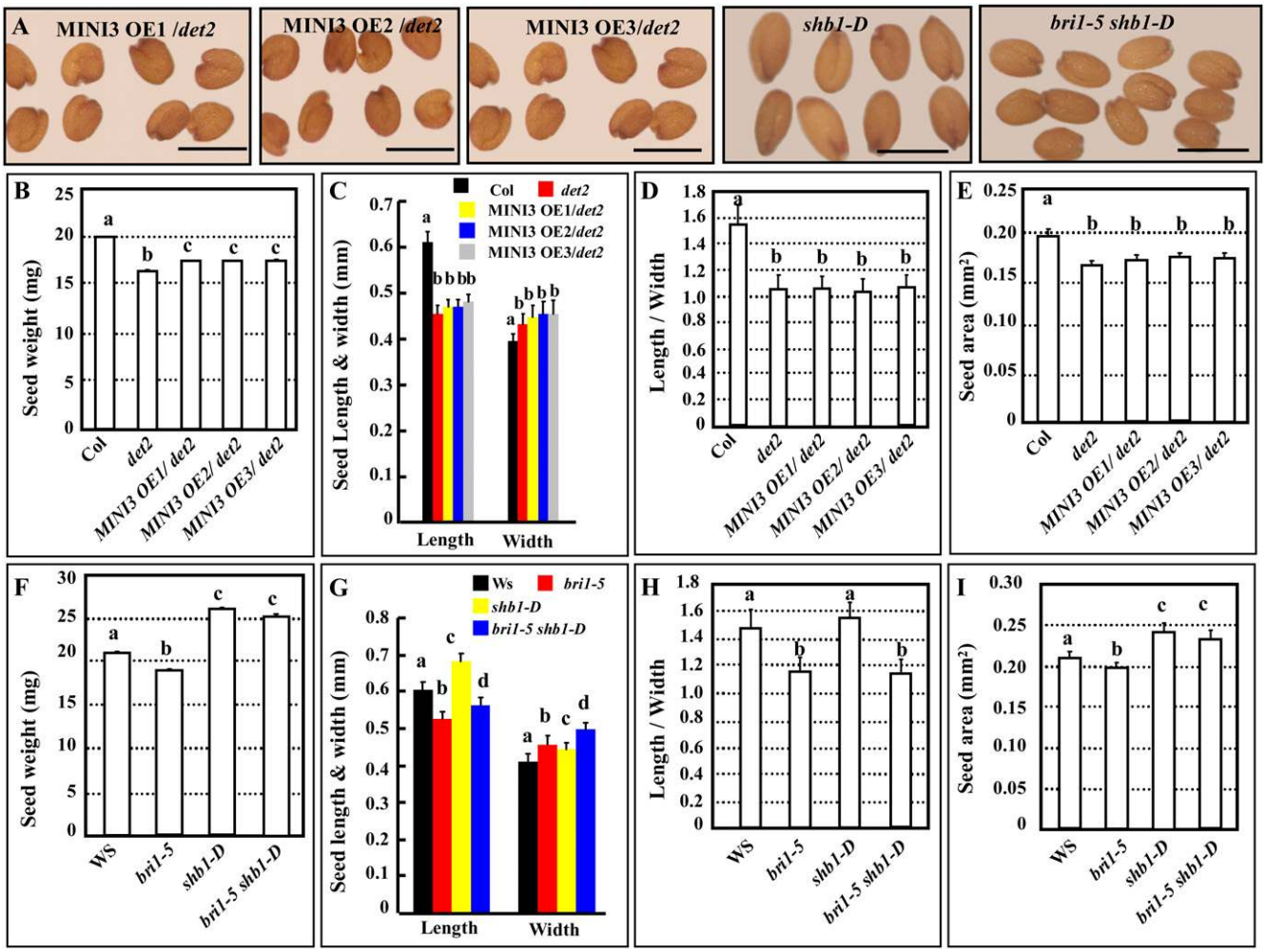

Figure 4. Increased *MINI3* expression can partially restore the seed phenotypes of the *det2* mutant, and *shb1-D* also can partially restore the seed phenotypes of the *bri1-5* mutant. A, Overexpression of *MINI3* in *det2* mutants produces larger seeds compared with the *det2* mutant. Mature dried seeds from MINI3 overexpression (OE) 1*/det2*, MINI3 OE 2*/det2*, MINI3 OE 3*/det2*, *shb1-D*, and *bri1-5 shb1-D* were shown. Col-0 and *det2* and Ws and *bri1-5* data are shown in Figure 1, and all the seeds came from parallel experiments. Bar = 750 μm. Seed weight per 1,000 mature dried seeds (B), seed length and seed width (C), the ratio of length to width (D), and seed area of mature dried seeds (E) of Col-0, *det2*, MINI3 OE 1*/det2*, MINI3 OE 2*/det2*, and MINI3 OE 3*/det2* were shown. Seed weight per 1,000 mature dried seeds (F), seed length and seed width (G), the ratio of length to width (H), and seed area of mature dried seeds (I) of Ws, *bri1-5*, *shb1-D*, and *bri1-5 shb1-D* were shown. Values that differ at the 0.05 significance level are labeled with different letters.

similar to the *ap2-6* mutant, but there was no obvious difference in cell width between *ap2-6 det2* double mutants and *ap2-6* mutants (Supplemental Figs. S1 and S5). The embryo shoot and embryo root apex or cotyledon of *ap2-6 det2* double mutants produced more cells than *det2* mutants, and the cell number of *ap2-6 det2* double mutants was similar to that of the *ap2-6* mutants (Supplemental Figs. S1 and S5). These results indicated that *AP2* mutation rescued the phenotypes of smaller cell size and fewer cell numbers of the *det2* mutant, suggesting that the increased expression of *AP2* in *det2* contributed to the small-seed phenotype and that *AP2* was involved in BR regulation of embryo cell size and number.

AP2 affects seed cavity, endosperm size, and endosperm cellularization (Ohto et al., 2009). Our observation showed that the seed cavity and endosperm size of *ap2-6 det2* was larger than those of *det2* at the same stage and was similar to that of *ap2-6*. The seed development of *ap2-6* and *ap2-6 det2* started more slowly than the wild type in the first 5 d, but the growth speed increased soon after, giving rise to a cavity size similar to the wild type after 7 DAP. The final embryo sizes of *ap2-6* and *ap2-6 det2* were bigger than those of the wild type (Supplemental Fig. S6). Similar to *ap2-6*, *ap2-6 det2* initiated endosperm cellularization when embryos were at the middle-heart stage at approximately 6 DAP and completed cellularization

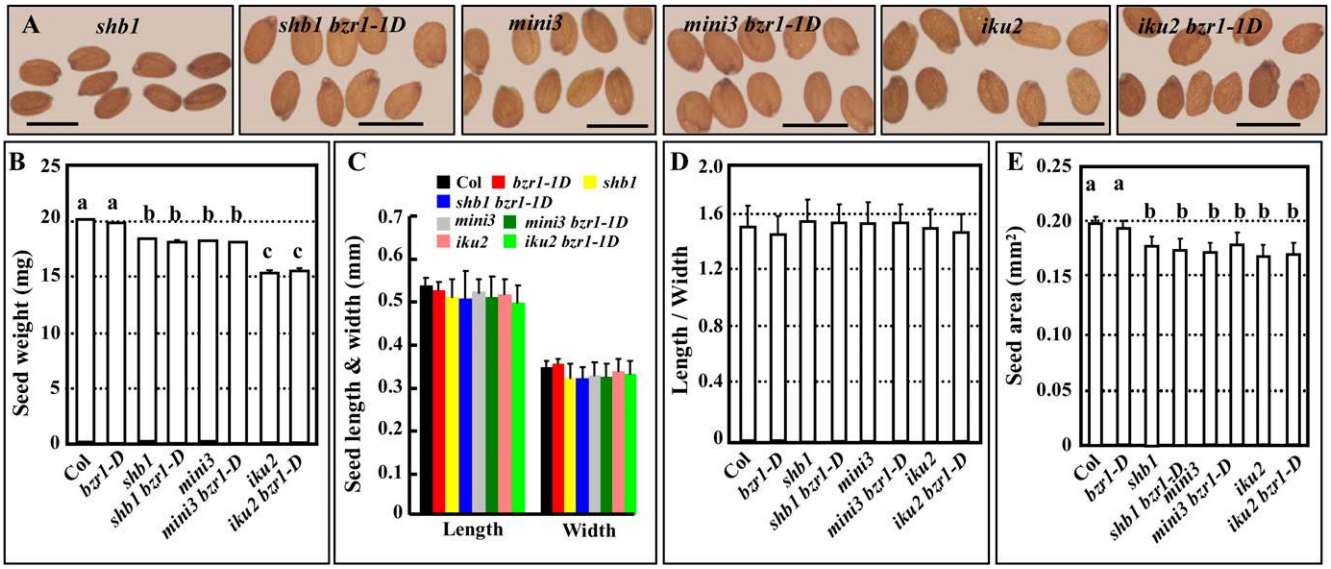

Figure 5. *SHB1*, *MINI3*, and *IKU2* act downstream of *BZR1*. A, Mature dried seeds of *shb1*, *shb1 bzr1-1D*, *mini3*, *mini3 bzr1-1D*, *iku2*, and *iku2 bzr1-1D* were shown. Col-0 and *bzr1-1D* data are shown in Figure 1, and all the seeds came from parallel experiments. Bar = 750 μm. Seed weight per 1,000 mature dried seeds (B), seed length and seed width (C), the ratio of length to width (D), and seed area of mature dried seeds (E) of Col-0, *bzr1-1D*, *shb1*, *shb1 bzr1-1D*, *mini3*, *mini3 bzr1-1D*, *iku2*, and *iku2 bzr1-1D*. Values that differ at the 0.05 significance level are labeled with different letters.

when embryos were at the late-heart stage at approximately 7 DAP (Supplemental Fig. S6). These results indicated that *AP2* was epistatic to *det2* and played an important role in BR regulation of seed cavity, endosperm, and embryo development.

ARF2 is a negative regulator of seed size and mass. Loss-of-function mutant *arf2-8* has increased seed size (Schruff et al., 2006). We crossed *arf2-8* with *det2* to test whether the increased expression of *ARF2* in *det2* was responsible for the smaller seed size of *det2*. As shown in Figure 7, the seed weight and seed area of *arf2-8 det2* were notably larger than those of *det2* or the wild type and not obviously different to *arf2-8*. The seed length of *arf2-8 det2* was larger than that of *det2* but shorter than that of *arf2-8*, and the seed width was increased compared with *det2* or *arf2-8* (Fig. 7C). There was no significant difference in the ratio of length to width compared with *det2*; however, the ratio was significantly lower than the wild type or *arf2-8* (Fig. 7D). These results indicated that the seed weight and seed size of *arf2-8 det2* double mutants resembled *arf2-8* phenotypes, and *ARF2* acted downstream of *DET2* as a negative regulator of seed size. On the other hand, BR regulation of seed shape was independent of *ARF2*.

DISCUSSION

Seed size is a major agricultural trait, and our study demonstrated an important role of BR in promoting seed development. Our studies showed that deficiency of BR biosynthesis and signal transduction led to smaller seed size and abnormal seed shape in Arabidopsis. The effects of BR on seed size and seed shape were attributed to BR actions in the embryo/endosperm and maternal tissues, respectively. While BR was known to increase cell size and cell division in general, our molecular analysis provided evidence that BR modulated specific developmental regulators of seed development. Thus, our study linked BR signal to developmental pathways that controlled seed size and shape. This knowledge not only provided insight into the molecular mechanism of hormonal regulation of seed development but also potential means for improving grain yield.

BR Plays Crucial Roles in Regulating Seed Size and Seed Mass

The positive effect of BR on seed growth has been observed in BR-deficient mutant and BR-overproducing rice plants (Wu et al., 2008). Our analysis of Arabidopsis BR mutants showed similar requirements of BR for normal seed development. The BR-deficient mutant *det2* and BR-insensitive mutant *bri1* both had reduced seed size, and BR treatment obviously increased the *det2* seed weight, area, and ratio of seed length to width, although the enhanced seed weight and length/width ratio are still not as normal as the wild type (Fig. 3A). We further showed that the reduced seed size of *det2* was mainly due to reduced embryo cell size and cell number and smaller integument cell size (Fig. 1). In flowering plants, the embryo and the endosperm, which are enclosed by maternal seed integument, are derived from double fertilization, and the development of these three components are finely coordinated (Garcia et al., 2003). Our studies suggested that BR regulated seed size

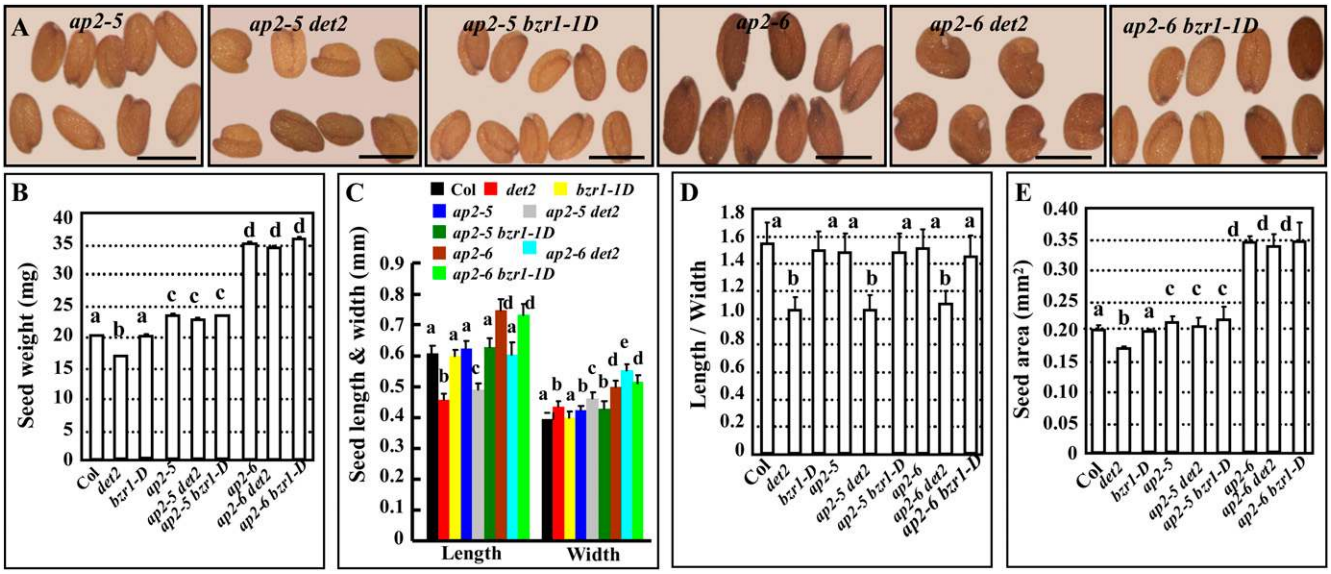

Figure 6. The *AP2* mutation in *det2* mutants suppressed the small seed phenotype of *det2*, and *AP2* acts downstream of *BZR1*. A, Mature dried seeds from Col-0, *det2*, *bzr1-1D*, *ap2-5*, *ap2-5 det2*, *ap2-5 bzr1-1D*, *ap2-6*, *ap2-6 det2*, and *ap2-6 bzr1-1D* were shown. Col-0, *det2*, and *bzr1-1D* data are shown in Figure 1, and all the seeds came from parallel experiments. Bar = 750 μm. Seed weight per 1,000 mature dried seeds (B), seed length and seed width (C), the ratio of length to width (D), and seed area of mature dried seeds (E) of Col-0, *det2*, *bzr1-1D*, *ap2-5*, *ap2-5 det2*, *ap2-5 bzr1-1D*, *ap2-6*, *ap2-6 det2*, and *ap2-6 bzr1-1D*. Values that differ at the 0.05 significance level are labeled with different letters.

by coordinating the growth of the embryo and integument through independent mechanisms that depend on BR produced in each tissue. Pollinating BR-deficient or -insensitive mutants with wild-type pollen, which resulted in a functional wild-type embryo in the mutant integument, rescued the size but not shape of the seed, suggesting that BR produced in the embryo and endosperm were sufficient for increasing seed size, but the elongated seed shape required BR production and signaling in the integument. This observation also suggested that BR could not move far from the source cells that synthesize it. Consistent with such independent action of BR in embryo/endosperm on seed size and BR action in the integument determining seed shape, altering several BR-regulated genes suppressed the small-seed phenotype of *det2* but not the seed shape. Our results suggested that BR regulated seed size and seed shape through independent actions of distinct downstream genes acting separately in the embryo/endosperm and the integument.

BR Activates the *SHB1-MINI3-IKU2* Pathway and Represses *AP2* and *ARF2* to Increase Seed Size, though Directly Binding Their Promoters by BZR1

BZR1 is a very important transcription factor in the BR signaling transduction pathway, and *bzr1-1D* is a BR signal-enhanced mutant. There is no significant difference in seed weight, area, and shape between *bzr1-1D* and the wild type. We statistically analyzed *bzr1-1D* seed phenotypes in different grow places and found that the *bzr1-1D* seed area and weight might be slightly lower than the wild type under poor conditions in some instances, which could be explained by feedback inhibition to BR biosynthesis genes (He et al., 2005), too many seeds per silique (Huang et al., 2012), or the putative function of BZR1/BES1 in seed development (Yu et al., 2011). We did not analyze *bes1-D* in this work because many important seed development-regulating genes, such as *SHB1-MINI3-IKU2*, *AP2*, and *ARF2*, were not identified as direct target genes of BES1. BES1 possibly regulates seed development through targeting other seed development-regulating genes, such as *KLU* and *AHK4*, which are the target genes of both BZR1 and BES1 (Sun et al., 2010b; Yu et al., 2011), and regulates their expressions, which might be worth investigation in the future.

Our results showed that the expression of many genes involved positive regulation of seed size, especially the components in the SHB1-MINI3-IKU2 pathway, were activated by BR, mostly through direct regulation by BZR1. Genetics interactions provided further evidence that *SHB1*, *MINI3*, and *IKU2* genes were positive factors for BR regulation of seed size. Overexpression of *MINI3* and dominant mutation of *SHB1* partly suppressed the small-seed phenotype of *det2*. Consistent with a role downstream of BR, the *shb1*, *mini3*, or *iku2* mutants showed normal expressions of the BR biosynthetic genes *DWF4* and *CPD* (Supplemental Fig. S7). The suppression of the seed size but not seed shape of *det2* by *MINI3* overexpression and *shb1-D* was consistent with previous studies showing that the *SHB1-MINI3-IKU2* pathway regulated endosperm

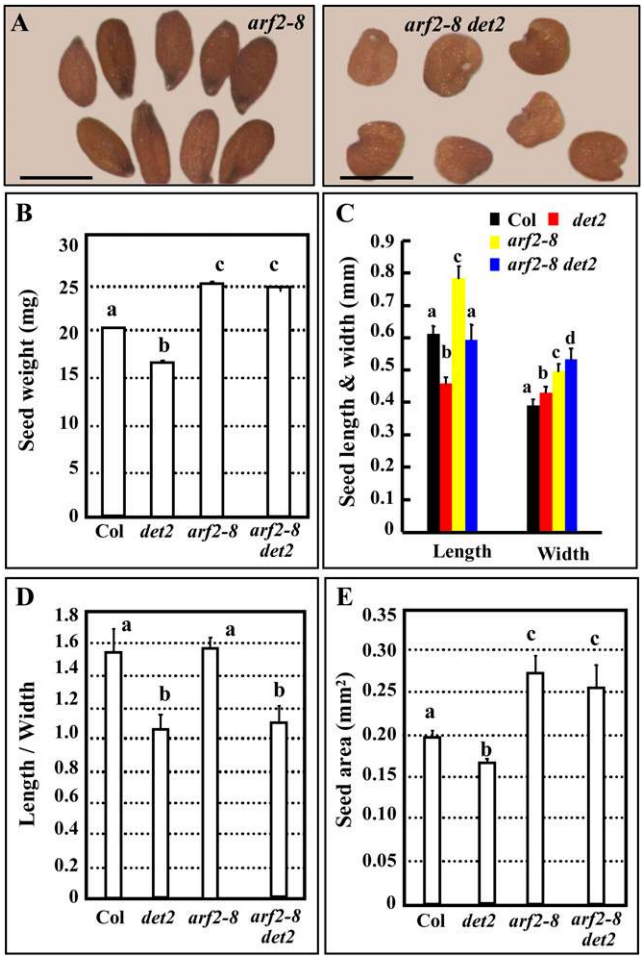

Figure 7. The *ap2-8 det2* double mutants displayed *arf2-8* phenotypes. A, Mature dried seeds from *arf2-8* and *arf2-8 det2* were shown. Col-0 and *det2* data are shown in Figure 1, and all the seeds came from parallel experiments. Bar = 750 μm. Seed weight per 1,000 mature dried seeds (B), seed length and seed width (C), the ratio of length to width (D), and seed area of mature dried seeds (E) of Col-0, *det2*, *arf2-8*, and *arf2-8 det2*. Values that differ at the 0.05 significance level are labeled with different letters.

and embryo but not integument development to affect seed size (Zhou et al., 2009). Our molecular experiments indicated that BZR1 bound to the promoter region of *SHB1*, which might, in turn, activate the expressions of *MINI3* and *IKU2*. BZR1 also binds to the promoter region of *IKU1*, which can interact with MINI3 at the protein level (Wang et al., 2010). The expression of *MINI3* and *IKU2* is altered in *iku1* and *mini3* (Luo et al., 2005), indicating BZR1 might target and regulate IKU1 and thus affect MINI3, which led to *IKU2* activation too. The molecular and genetic data together supported that the SHB1-MINI2-IKU2 pathway acted downstream of BR signaling to positively regulate embryo development and seed size (Fig. 8).

Our studies showed that BR also suppressed the expression of genes that negatively regulated seed size, including *AP2* and *ARF2*. These two genes were direct targets of BZR1 and both of them expressed in high level in *det2* and *bri1* mutants (Fig. 3D). The loss-of-function mutations of *AP2* and *ARF2* increased seed size and suppressed the small-seed phenotype of *det2*, indicating that the elevated expression levels of these genes contributed to the small-seed phenotype of *det2*.

We confirmed that AP2 expression was repressed by BR signaling (Huang et al., 2012), and our results showed that AP2 might also regulate BR synthesis or signaling. The expression levels of the BR biosynthetic genes *DWF4* and *CPD* were decreased in the *ap2* mutants (Supplemental Fig. S7). Due to feedback regulation, expression levels of *CPD* and *DWF4* were increased in

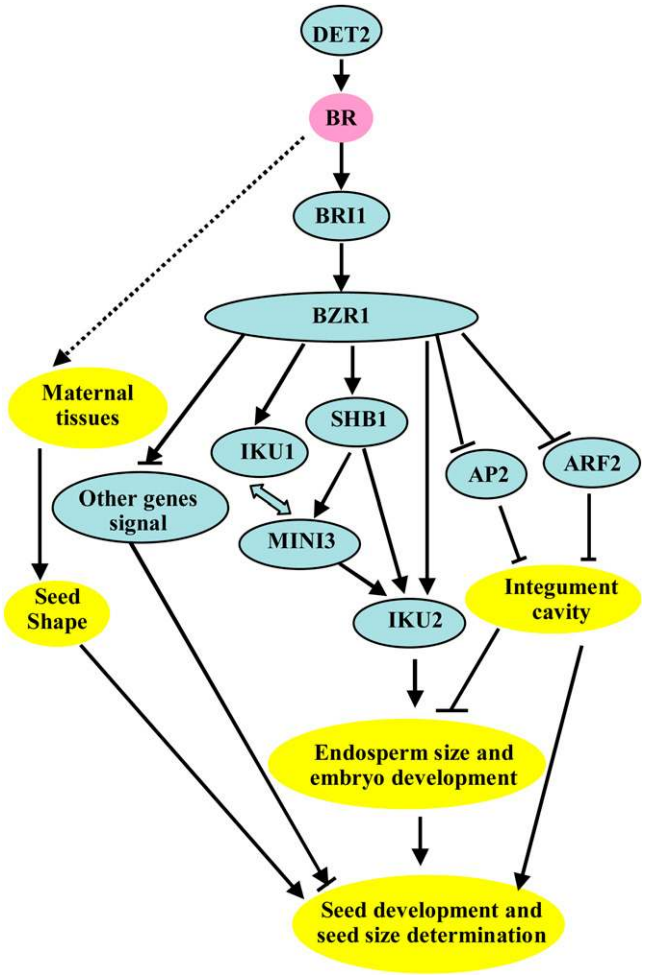

Figure 8. Hypothetical model for BR-regulated seed development. BR regulates embryo and endosperm development to determine seed size through binding to the promoter region of *SHB1* by BZR1, which further regulates the expression of *MINI3* and *IKU2*, or through BZR1 binding to the promoter region of *IKU1*, which interacts with MINI3 to regulate the expression of *MINI3* and *IKU2*, or alternatively, through BZR1 binding to the promoter of *IKU2*, and regulates the expression of *IKU2*. BR regulates integuments, endosperm, and embryo development through BZR1 binding to the *AP2* promoter and then affects seed size. BR also modulates the integument development through BZR1 binding to the promoter of *ARF2*.

det2 and decreased in *bzr1-1D*. The *ap2* mutations reduced *CPD* and *DWF4* expression in the *det2* background but not in the *bzr1-1D* background (Supplemental Fig. S7). AP2 might negatively regulate BR signaling. The reduced expression of BR biosynthetic genes in the *ap2* mutant could be due to feedback inhibition by enhanced BR signal. Alternatively, AP2 might positively regulate BR biosynthetic genes directly and thus would form another feedback loop, as BR inhibition of AP2 expression would lead to reduced *CPD* and *DWF4* expression again. In contrast to AP2, ARF2 seems not to affect the expression of *CPD* and *DWF4*, suggesting that AP2 and ARF2 act through distinct pathways, which is consistent with the reports that *AP2* influences development of maternal integuments, the zygotic embryo, and endosperm to repress seed size (Ohto et al., 2009) and *ARF2* represses seed size by regulating the development of integuments (Schruff et al., 2006).

BR and Other Signals Coregulate Seed Size

In addition to the SHB1-MINI3-IKU2 pathway, several other pathways affecting seed size were regulated by BR. *KLU* expresses in the inner integument of developing ovules and promotes cell proliferation in integuments to determine the growth potential of seed coat and seed. The *klu* mutant has smaller seeds, and by contrast, overexpression of the *KLU* gene results in significantly enlarged seeds (Adamski et al., 2009). The expression level of *KLU* was up-regulated by BR, and *KLU* was a direct target of BZR1. These results suggested that BR might positively regulate integument development through BZR1 activating *KLU*. *FIS2* also regulates seed development through the epigenetic regulation pathway of endosperm development (Pien and Grossniklaus, 2007). Our results confirmed that BR activated *FIS2* by BZR1 targeting *FIS2*. *RGE1* and *ANT*, two BZR1 target genes induced by BR, positively regulate embryo proliferation. BR might regulate seed development through regulation of embryo proliferation through BZR1 targeting and regulating *RGE1* and *ANT*.

Recent studies reported that BR and gibberellins regulate common cellular activities and developmental processes (Bai et al., 2012), and BZR1 interacts with phytochrome-interacting factor4 to control a core transcription network, which indicate that plant growth can be coregulated by the steroid and environmental signals (Oh et al., 2012). Our results showed that the important genes for cytokinin signaling, *AHK* and *CKI1* (Riefler et al., 2006; Deng et al., 2010), were the target genes of BZR1 and were regulated by BR (Supplemental Fig. S3). BR and cytokinin might cross talk to coregulate seed development, but the detailed mechanism needs further investigation.

BR Regulates Seed Shape through Unknown Maternal Tissue Factors after Fertilization

Our results showed that BR might have a major effect on seed shape. The ratio of length to width in wild-type seed ranged from 1.5 to 1.7, depending on growth conditions and metrical methods, which resembles the golden ratio of 1.61803399 (Cervantes et al., 2010), and this ratio was notably lower in *det2*, *dwf4*, or *bri1-116* (Fig. 2). Cross pollination of these mutants with wild-type pollen, restoring BR signaling in the embryo and endosperm, rescued seed size/mass to that of the wild type but did not rescue the seed shape. Before pollination, the ovule shape, including length, width, and area, of *det2* was not significantly different from the wild type (Supplemental Fig. S8), indicating the BR regulated seed shape after fertilization. Although the *SHB1-MINI3-IKU2* pathway, *AP2*, and *ARF2* mediated the BR control of seed weight and seed size, the BR regulation of seed shape was independent of these genes. Therefore, the mechanism for BR regulation of seed shape was different from that of seed size. BR synthesis and signaling in the maternal tissues, such as integument, determined the seed shape, whereas the BR produced in the embryo/endosperm had little effect on seed shape. Such distinct cell type-specific effects of BR suggested that BR did not transport over long distance from source cells (for our case, from zygote to integument cells) and thus can function as a local signal in morphogenesis, which was consistent with previous reports (Symons et al., 2008), and also in accord with the recent finding of a role of BR in regulating organ boundary formation (Bell et al., 2012; Gendron et al., 2012). Our study indicated that BR regulated distinct pathways in specific cell types to control the seed size and shape. Further study of these pathways at the molecular and developmental levels would be important for increasing grain yield.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The *det2*, *dwf4*, *bri1-116*, *bzr1-1D*, *shb1*, *mini3*, *iku2*, *ap2-5*, *ap2-6*, and *arf2-8* mutants and transgenic Arabidopsis (*Arabidopsis thaliana*) plants are with Col-0 background. *bri1-5* and *shb1-D* mutants are with Ws. Plants were grown at 23°C, and seeds were harvested when the plant was completely mature. At later developmental stages, the plants were also fitted with a plastic well to catch all seeds released by dehiscing siliques. Isolated siliques of 4 to 5 d after hand-pollination of Col-0 and *det2* were dipped with mock solution or 10 μM 24-epibrassinolide (BR) treatment and mock solution or 1 μM BRZ for 3 h as determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis. All siliques were cut in the same length of center joint position for easily absorbing the solutions.

Measurement of Seed Weight, Seed Length, Seed Width, and Seed Area

For seed weight, plants were grown concurrently under identical conditions and seeds were harvested when the plant was mature. One thousand seeds per genotype were dried at 24°C for 7 d and weighed. Data are presented as means ± SD from at least three independent experiments. For seed length and width, dried seeds were photographed using a Zeiss Axio Imager A1 microscope and then measured by ImageJ software. The measured seed length and width were used to calculate the ratio of length to width. ImageJ software was used to calculate the seed area. Young seeds at early-heart or middle-heart stage were cleared in Hoyer's solution overnight and photographed using a Leica differential interference contrast microscope, followed by using ImageJ software to calculate the seed dimensions.

Developmental Alteration Analysis

To examine the developmental alterations of *det2*, *ap2-6*, and *ap2-6 det2* mutants, flowers of Col-0 and *det2*, *ap2-6*, and *ap2-6 det2* mutants were hand pollinated, and the developing seeds were harvested at different days after pollination. The siliques were dissected under an Olympus SZX9 microscope to isolate the young seeds, and then these young seeds were cleared in Hoyer's solution overnight and photographed using a Leica differential interference contrast microscope. To investigate the endosperm development, young seeds were fixed in formaldehyde-acetic acid solution overnight at 4°C and dehydrated through an ethanol series (50% (v/v), 70% (v/v), 80% (v/v), 90% (v/v), and 100% (v/v), each for 30 min). The samples were then embedded in LR White (Sigma-Aldrich). Sections were stained with 0.1% (w/v) toluidine blue O in distilled water. Stained sections were photographed using a Zeiss Axio Imager A1 microscope.

Scanning Electron Microscopy Analysis

Mature dried seeds were imbibed for 1 h and dissected under an Olympus SZX9 microscope to isolate the mature embryos. The embryos were incubated for 12 h in buffer that contains 50 mM sodium phosphate (pH 7.0), 10 mM EDTA, 1% Triton X-100, and 1% dimethyl sulfoxide at 37°C and fixed for 24 h in formaldehyde-acetic acid (10% formalin, 5% acetic acid, 45% ethanol, and 0.01% Triton X-100). After fixation, the samples were dehydrated through an ethanol series (70%, 85%, 95%, and 100%, each for 30 min) and then dehydrated through an ethanol and isopentyl acetate series (75% ethanol and 25% isopentyl acetate, 50% ethanol and 50% isopentyl acetate, 25% ethanol and 75% isopentyl acetate, and 100% isopentyl acetate, each for 30 min). The embryos were then dried using a Hitachi Critical Point Dryer. The individual embryos were mounted on scanning electron microscopy stubs, sputter coated with platinum using the Hitachi Ion Sputter, and examined under a Hitachi S-4800 scanning electron microscope. Around 50 embryos were photographed, and the cell length and width were determined using ImageJ software. Data are presented as the average of three independent experiments.

Total RNA Isolation and Quantitative RT-PCR Analysis

Total RNA was extracted from siliques 4 to 5 d after hand pollination using the TRIzol RNA extraction kit (Invitrogen). The first-strand complementary DNA was synthesized by using moloney murine leukemia virus reverse transcriptase (Promega) and used as templates for RT-PCR analysis. Quantitative RT-PCR analyses were carried out on Mx3000P (Stratagene) by using the SYBR Green reagent (Toyobo) according to the manufacturer's instructions. The primer sequences for quantitative RT-PCR were listed in Supplemental Table S2. Data were calculated from three biological replicates, and each biological replicate was examined in triplicate.

Reciprocal Crosses and Double Mutant Analysis

The reciprocal crosses between Col-0 and *det2*, *dwf4*, and *bri1-116* were performed with flowers at identical positions (11th to 14th flowers) on secondary inflorescences. A series of double mutants of *det2* with mutants showing large-seed-size *ap2-5*, *ap2-6*, and *arf2-8* were generated, and the mutant of *bri1-5* with the mutant showing large-seed-size *shb1-D* was also generated. A succession of double mutants of *bzr1-1D* with mutants showing small-seed-size *shb1*, *mini3*, and *iku2* and with mutants showing large-seed-size *ap2-5* and *ap2-6* were generated. The transfer DNA-specific primer for *shb1*, *mini3*, and *iku2* is T-DNA-1 and for *arf2-8* is T-DNA-2. For *shb1-D* genotyping, the transfer DNA-specific primer is T-DNA-3. For *ap2-5* genotyping, the PCR fragment from the wild type but not from *ap2-5* can be cut by *Sac*II. For *ap2-6* genotyping, the PCR fragment from the wild type but not from *ap2-6* can be cut by *Pst*I. For *det2* genotyping, *Mnl*I cuts the PCR fragment amplified from the wild type but not det2. For *bri1-5* genotyping, *Pst*I cuts the PCR fragment amplified from the wild type but not bri1-5. For *bzr1-1D* genotyping, the PCR fragment from the wild type but not from bzr1-1D can be cut by *Cfr*10I. The primer sequences for mutants screening were listed in Supplemental Table S3.

ChIP Analysis

For ChIP experiments, Arabidopsis wild-type and *pBZR1::BZR1-cyan fluorescent protein* (*CFP*) transgenic Arabidopsis plants were grown at 23°C under 16-h-light and 8-h-dark conditions. Siliques at 4 to 5 d were cross linked for 15 min in 1% formaldehyde by vacuum filtration. ChIP analysis was performed by using an affinity-purified anti-GFP polyclonal antibody, and quantitative RT-PCR was performed using SYBR Green reagent (Toyobo). Results were presented as the ratio of the amount of DNA immunoprecipitated from BZR1-CFP samples to that of the wild-type samples. The *PROTEIN PHOSPHATASE2A* (*PP2A*) and *CO-FACTOR FOR NITRATE, REDUCTASE AND XANTHINE DEHYDROGENASE5* (*CNX5*) genes were used as the negative control, and the *DWF4* gene was used as the positive control. The ChIP experiments were performed three times, from which the means and SDs were calculated. The primer sequences for ChIP-qPCR were listed in Supplemental Table S4.

Sequence data from this article can be found in the Arabidopsis Genome Initiative database under accession numbers *UBIQUITIN10* (At4G05320), *DET2* (At2G38050), *BRI1* (At4G39400), *DWF4* (At3G50660), *BZR1* (At1G75080), *SHB1* (At4G25350), *IKU1* (At2g35230), *MINI3* (At1G55600), *IKU2* (At3G19700), *AP2* (At4G36920), *ARF2* (At5G62000), *KLU* (At1G13710), *HSF15* (At4G18870), *FIS2* (At2G35670), *ANT* (At4G37750), *RGE1* (At1G49770), *AHK1* (At2G17820), *AHK3* (At1G27320), *AHK4* (At2G01830), and *CKI1* (At2G47430).

Supplemental Data

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

Supplemental Figure S1. Integument cell size and endosperm cellularization of Col, *det2*, *ap2-6*, and *ap2-6 det2* mutant plants.

Supplemental Figure S2. BR regulates seed size and shape.

Supplemental Figure S3. BR regulates the expression levels of seed size-related genes through BZR1's targeting their promoters.

Supplemental Figure S4. Overexpression *MINI3* in Col does not result in the changed seed size.

Supplemental Figure S5. Seed phenotypes of *det2* mutants are rescued by *AP2* mutation.

Supplemental Figure S6. Seed development in Col, *det2*, *ap2-6*, and *ap2-6 det2* mutant plants.

Supplemental Figure S7. Expression levels of *DWF4* and *CPD* in single and double mutants.

Supplemental Figure S8. The *det2* ovule before pollination is similar to that of Col.

Supplemental Table S1. Overexpression of *MINI3* in Col resulted in heavier seeds and unaltered seed size.

Supplemental Table S2. The primer sequences for qRT-PCR analysis.

Supplemental Table S3. The primer sequences for mutants screening.

Supplemental Table S4. The primer sequences for ChIP-qPCR analysis.

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