

# Brassinosteroid functions in *Arabidopsis* seed development

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**Abbreviations:** BR, brassinosteroid; *SHB1*, *SHORT HYPOCOTYL UNDER BLUE1*; *IKU1*, *HAIKU1*; *MINI3*, *MINISEED3*; *IKU2*, *HAIKU2*; *AP2*, *APETALA2*; *AGL*, *AGAMOUS-LIKE*; *ARF2*, *AUXIN RESPONSE FACTOR2*; *TTG2*, *TRANSPARENT TESTA GLABRA2*; *TTI6*, *TRANSPARENT TESTA 16*; *FIS2*, *FERTILIZATION INDEPENDENT SEED 2*; *FIE*, *FERTILIZATION INDEPENDENT ENDOSPERM*; *MEA*, *MEDEA*; *MSI1*, *MULTICOPY SUPPRESSOR OF IRA*; *SWN*, *SWINGER*; *MET1*, *METHYL TRANSFERASE*; *ANT*, *AINTEGUMENTA*; *RGE1*, *RETARDED GROWTH OF EMBRYO1*; *SUP*, *SUPERMAN*; *INO*, *INNER NO OUTER*; *SIN*, *SHORT INTEGUMENTS*; *BEL1*, *BELL*; *AG*, *AGAMOUS*; *HLL*, *HUELLENLOS*; *AP2*, *APETALA2*; *dwf*, *dwarf*; *shk1-D*, *shrink1-dominant*; *brd2*, *brassinosteroid-deficient dwarf2*; *BRI1*, *BRASSINOSTEROID INSENSITIVE1*; *AHK*, *ARABIDOPSIS HISTIDINE KINAS*; *CKI1*, *CYTOKININ INDEPENDENT 1*; *ats*, *aberrant testa shape*; QTLs, quantitative trait loci

Seed development of flowering plant is a complicated process controlled by a signal network. Double fertilization generates 2 zygotic products (embryo and endosperm). Embryo gives rise to a daughter plant while endosperm provides nutrients for embryo during embryogenesis and germination. Seed coat differentiates from maternally derived integument and encloses embryo and endosperm. Seed size/mass and number comprise final seed yield, and seed shape also contributes to seed development and weight. Seed size is coordinated by communication among endosperm, embryo, and integument. Seed number determination is more complex to investigate and shows differences between monocot and eudicot. Total seed number depends on siliqua number and seed number per siliqua in *Arabidopsis*. Seed comes from fertilized ovule, hence the ovule number per flower determines the maximal seed number per siliqua. Early studies reported that engineering BR levels increased the yield of ovule and seed; however the molecular mechanism of BR regulation in seed development still remained unclear. Our recent studies demonstrated that BR regulated seed size, shape, and number by transcriptionally modulating specific seed developmental pathways. This review summarizes roles of BR in *Arabidopsis* seed development and gives clues for future application of BR in agricultural production.

## Introduction

The molecular mechanisms of seed development are extensively studied, and many genes involved have been already identified. *SHB1*,<sup>1</sup> *IKU1*,<sup>2</sup> *MINI3*,<sup>3</sup> *IKU2*,<sup>3</sup> *AP2*,<sup>4-6</sup> and MADS-box

transcription factors *AGL61*,<sup>7,8</sup> *AGL62*,<sup>9</sup> and *AGL80*<sup>10</sup> mainly regulate endosperm development to affect seed size/weight. *ARF2*,<sup>11</sup> *TTG2*,<sup>12</sup> and *TTI6*<sup>13</sup> regulate integument development to affect seed development. *FIS2*, *FIE*, *MEA*, *MSI1*, *SWN*,<sup>14</sup> and *MET1*<sup>15</sup> influence seed development by epigenetic regulation of endosperm development and paternal imprinting. *ANT*<sup>16</sup> and *RGE1*<sup>17</sup> are involved in regulating embryo proliferation and affect seed development. Hormones play crucial roles in seed development,<sup>18</sup> including auxins, cytokinins, gibberellins, and BR. Also many genes that take part in ovule development and regulate the seed number have been identified in *Arabidopsis*, such as *SUP*,<sup>19</sup> *INO*,<sup>20</sup> *SINI*,<sup>21</sup> *SIN2*,<sup>22</sup> *TSO1*,<sup>23</sup> *BEL1*,<sup>21,24</sup> *AG*,<sup>25</sup> *HLL*,<sup>26</sup> *ANT*,<sup>26</sup> and *AP2*.<sup>24</sup>

Steroid hormone BR is required in seed development. Deficiency in BR synthesis and signal transduction pathway leads to severe phenotypes in reproductive development. Many excellent articles and reviews covered BR synthesis, signal transduction, and related plant growth and development. Here we briefly summarize our current understanding of BR functions and regulation mechanisms in *Arabidopsis* seed development.

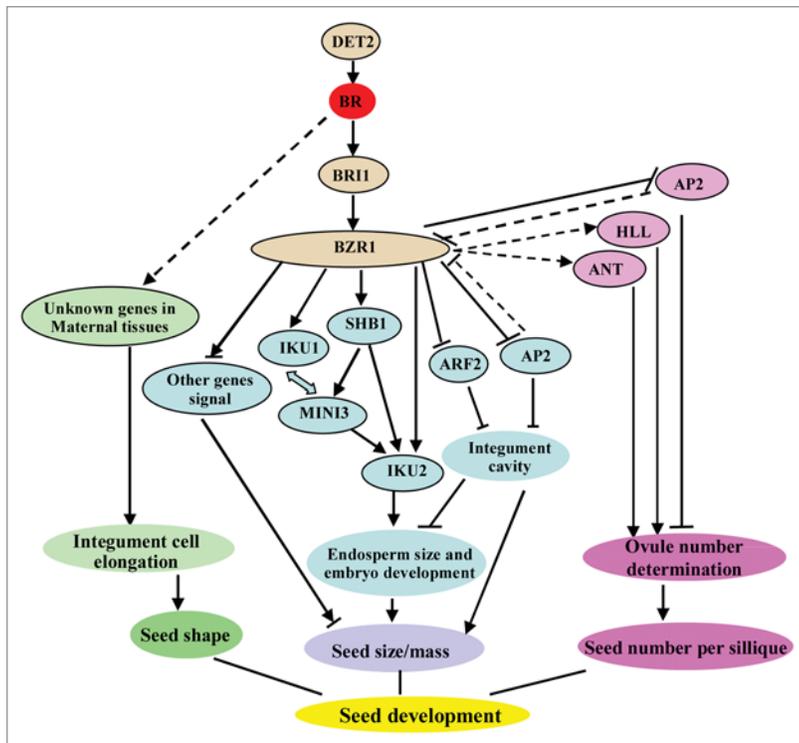
## BR Plays Key Roles in Seed Size/Mass Regulation

The functions of BR in seed development have been demonstrated by studies of BR deficient and insensitive mutants of *Arabidopsis*, *Oryza sativa*, *Pisum sativum*, and *Vicia faba*. *Arabidopsis* BR deficient mutant *dwf5* produces small seeds.<sup>27</sup> Another *Arabidopsis* dwarf mutant *shk1-D* (overexpression of a P450 monooxygenase family gene *CYP72C1*<sup>28</sup>) has lower endogenous BR level and produces general short organs and small seeds. Rice BR deficient mutant *brd2* exhibits shortened and smaller

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**Figure 1.** BR regulation model of seed development in *Arabidopsis*. 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 *MINI3* and *IKU2*, or alternatively through BZR1 binding to the promoter of *IKU2* and regulates *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*. BZR1, activated by BR signal, stimulates the ovule development-related genes like *HLL*, *ANT*, and *AP2* indirectly or directly and regulate ovule number. Besides, *AP2* and BZR1 possibly antagonize each other to participate in the ovule number determination process. BR affects the seed shape through regulating related genes in maternal tissues like integument (modified after Jiang et al.<sup>35</sup> and Huang et al.<sup>50</sup>).

grains.<sup>29</sup> A cytochrome P450 gene *DWF11* of rice is implicated in BR biosynthesis, and the *dwf11* mutant also bears reduced seeds.<sup>30</sup> Rice dwarf mutant *d61* produces small grain size, which is caused by loss of function of a rice *AtBRI1* ortholog, *OsBRI1*.<sup>31</sup> Contrarily, overexpression of BR-biosynthetic gene increases rice seed filling and yield.<sup>32</sup> BR-deficient *Vicia faba* mutant also produces small seeds,<sup>33</sup> and dwarf mutant *lk*, a severe BR deficient mutant of pea, has smaller seeds.<sup>34</sup> These facts indicate that BR is required for normal seed development and seed size/mass determination.

So far, the molecular mechanisms of BR involved in seed development remain unclear. Our recent studies introduce advanced understanding of BR regulation in seed size/mass.<sup>35</sup> First, we found the mature dry seeds of the BR-deficient mutant *det2* and the BR insensitive mutant *bri1-5* (a weak allele of *bri1* mutant) were smaller than corresponding wild type seeds. Furthermore, the exogenous BR partially rescued seed size and weight of *det2*, confirming BR positive regulation of seed size/mass. Detailed studies showed that the reduced seed size of *det2* was possibly caused by reduced seed cavity and endosperm volume, delayed

embryo development, decreased embryo cell size, number, and integument cell length. Our results are in accordance with the theory that final seed size depended on interactions among zygotic embryo and endosperm, maternally derived seed coat, and mother plant.<sup>11</sup> We further analyzed the effects of BR synthesis and signaling in specific tissues on seed size determination, and deduced that BR produced by the embryo and endosperm contributed to the increased seed size.

*SHB1*, *IKU1*, *MINI3*, and *IKU2* are reported to be positive regulators of seed size and act in the same pathway.<sup>1-3,36</sup> We demonstrated they were positively regulated by BR and *SHB1*. *IKU1* and *IKU2* were found as direct targets of BZR1,<sup>35</sup> confirming ChIP results of Sun et al.<sup>37</sup> Unlike *MINI3*, its interacting protein *IKU1*<sup>2</sup> is a direct target of BZR1. Genetic experiments showed these genes acted downstream of *DET2* and *BZR1* in seed size regulation. We proposed the model that BR regulated embryo and endosperm development to determine seed size through binding to *SHB1* promoter region by BZR1, consequently regulated the expression of *MINI3* and *IKU2*,<sup>1</sup> or through BZR1 binding to *IKU1*, which interacted with *MINI3*<sup>2</sup> to regulate the activity of *MINI3* and the expression of *IKU2*, or alternatively through BZR1 binding to *IKU2* and regulated the transcription of *IKU2* directly (Fig. 1). *FIS2*, *ANT*, and *RGE1* were detected to be direct targets of BZR1 and positively regulated by BR. We proposed that BR mediated the development of embryo and endosperm through regulating the expression of these BZR1 target genes to affect seed development (Fig. 1).

Our studies illustrated that BR was implicated in the regulation of integument development as well. The first evidence observed was the significant decrease of integument cell length in *det2*, indicating that BR positively regulated integument cell elongation. The second evidence was that *ARF2* gene mutation (*arf2*) resulted in dramatically increased seed size/mass due to the extra cell division in the integuments.<sup>11</sup> *ARF2*, a direct target gene of BZR1, was negatively regulated by BR. Genetic evidences demonstrated *ARF2* acted downstream of *BZR1* and BR signal in regulating seed size. These results rose up a hypothesis that BR might regulate the seed size/mass also through BZR1 binding and repressing *ARF2* to positively regulate the integument development (Fig. 1). Because cytochrome P450 *CYP78A5* (*KLU*) was reported to act as a maternal regulator of seed size by stimulating the integument cell proliferation,<sup>38</sup> and we demonstrated *KLU* was a direct target gene of BZR1, we gained another evidence supporting the hypothesis that BR regulated the integument development to influence seed development through BZR1 (Fig. 1). Besides, *AP2* acts as an important negative regulator of seed development by inhibiting integument growth (and embryo/endosperm development) to reduce the seed size.<sup>4</sup> *AP2* also affects seed size/mass by coordinating the endosperm/embryo growth.<sup>4-6</sup> Our results showed *AP2*

was a target of BZR1, too, and negatively regulated by BR, and *AP2* acted downstream of BZR1 and BR signal in regulating seed size (Fig. 1). Above all, we demonstrated that BR regulated seed size by expanding seed cavity and endosperm volume, promoting embryo development, and increasing embryo cell size, number, and integument cell length through transcriptionally modulating specific seed developmental pathways (Fig. 1).

### BR Functions in Seed Shape Determination

Shape is an important seed trait. To date, there are a few reports mentioning seed shape regulation. *Arabidopsis* seed shape mutant *ats* is isolated from an ethyl methanesulfonate-treated population. Its length/width ratio is significantly lower than wild type. *ATS* functions in integument development and directly affects seed shape.<sup>39</sup> A series of rice QTLs for grain weight are identified. Among them, *GW3*, *GW6*, and *GS3* correlate with grain shape.<sup>40,41</sup> MADS genes also contribute to seed shape. *Osmads6-1* seeds seem to be more roundish than elliptical.<sup>42</sup> However, the underlying mechanisms of seed shape determination are mostly unknown.

BR is involved in seed shape determination. *dwf5* and *shk1-D* produce aberrantly shaped *Arabidopsis* seeds.<sup>27,28</sup> *dwarf1* and *dwarf11* mutants bear small, round rice seeds that are shorter length but equal width to wild type.<sup>30,43</sup> Rice *brd2* mutant also exhibits shortened grains.<sup>29</sup> *lk* mutant produces irregularly shaped pea seeds.<sup>34</sup> Although BR has major effect on seed shape and BR effect cannot be recovered by other signals, the detailed mechanism of BR regulating seed shape is still unclear.

Our recent studies made progress in explaining the mechanism of seed shape regulation by BR. We found that the length to width ratio of seeds produced by BR-deficient and insensitive mutants was notably lower than of wild type. The ratio of wild type *Arabidopsis* seeds resembles the Golden Ratio of 1.61803399.<sup>44</sup> Owing to the fact seeds come from fertilized ovules, we further analyzed ovule shape before pollination and found ovule shape of BR-deficient and insensitive mutants was normal. Thus we concluded BR regulated seed shape after fertilization. Furthermore, our results suggested that BR synthesis and signaling in the maternal tissues integument determined the seed shape (Fig. 1). The reciprocal crosses demonstrated that seeds shape was kept roundish when maternal tissue contained less BR or abnormal BR signal, even if BR level and signal have been rescued in embryo/endosperm and seed size/mass already restored. These results suggested that BR level in zygote determined the seed size/mass and had little effect on seed shape, which was consistent with the opinion that BR could not be transported through tissue boundary.<sup>45,46</sup> Although *SHB1-MINI3-IKU2* pathway, *AP2*, and *ARF2* mediate the BR regulation of seed size/mass, the BR functions in seed shape determination is independent on these genes. Herein, the evidences we got suggested that mechanism of BR regulating seed shape was different from that for seed size. Furthermore, our studies indicated BR regulated distinct pathways in specific cell types to control seed size and shape. However, the detailed mechanism and unknown concerned genes of BR regulating seed shape (maybe a good model for studying BR transport), are worth further investigation in the future (Fig. 1).

### BR is Involved in Seed Number Determination

There are several reports about seed number determination in flowering plants; however there is a significant lack of deeper insight into the mechanism of this regulation. First study describes the ectopic overexpression of a BR-biosynthetic gene *DWF4* in *Arabidopsis* transgenic plants results in increased seed yield due to a greater total seed number.<sup>47</sup> Constitutive expression of *ZmDWF4* also increases the seed numbers.<sup>48</sup> Enhancement of BR biosynthesis in transgenic rice enhances the grain yield due to more tillers and seeds and higher seed weight.<sup>32</sup> These studies suggest that BR causes an increase of branches and siliqua number that leads to increased seed yield.

Due to the fact that many factors contribute to total seed number, we first focused on seed number per siliqua in *Arabidopsis*. The ovule number determines the maximal number of seeds in *Arabidopsis*. Many genes have been identified in each phase of ovule development. *INO* and *SUP* promote and suppress integument, and also affect the asymmetric form of integuments.<sup>19,20</sup> *SIN1* and *SIN2* are required for cell division during integument development.<sup>21,22</sup> *TSO1*, which encodes a nuclear protein, is required for the orientation of cell elongation and cytokinesis in integument development.<sup>23,49</sup> *BEL1* and *AG* regulate integument identity.<sup>21,24,25</sup> *HLL* and *ANT* both promote ovule primordial growth.<sup>26</sup> Interestingly, *AP2* also affects early ovule formation.<sup>24</sup>

Our recent studies showed the ovule or seed number per siliqua of *det2* and *bri1-5* were significantly decreased, whereas *bzr1-ID* contained obvious increased ovule and seed number.<sup>50</sup> Thus we demonstrated that BR positively regulated ovule and seed number determination, and BR functioned in ovule number determination through BZR1 regulating *HLL*, *ANT*, and *AP2* transcription directly or indirectly (Fig. 1).<sup>50</sup> *AP2* did not act downstream of BZR1 in ovule number determination, but functioned antagonistically with BZR1, which seemed to be different to seed size/mass regulation. The research of *Arabidopsis* seed number determination would provide opportunities for further use in yield enhancement of crops like *Brassica napus* and *Glycine max*.

Apart of ovule number, *Arabidopsis* seed number per siliqua determination is also affected by paternal tissues, fertilization, seed development, and other factors. These processes and their regulation will be worth further investigation. Total amount of seeds depends on seed number per siliqua and siliqua number, which reflects the meristem differentiation and maintenance from early stages of reproductive development. Although rice seed number determination differs from *Arabidopsis*, the signals in reproductive meristems required for seed number determination process in both monocot and eudicot may be conserved. How BR functions in reproductive meristem and affects flower, fruit, and seed number is an attractive field in future research.

### Conclusions and Perspectives

The economic potentiality of BR in agricultural production was recognized as early as the 1980s, and the ability of exogenous BR to increase yield was shown in a variety of plant species. Combining previous reports and our results, we conclude that

BR has crucial function in seed development, including seed size/mass, shape, and number determination. The regulation mechanisms of BR in these processes are independent and different from each other.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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