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Spotlight

Crop Seed Size: BR Matters

Sufficient food supply is critically important to humans, and food security will be particularly challenging in view of the ever-growing world population, decreasing arable cropland, and global warming. To meet the challenges, most crop geneticists think it will be necessary to enrich the cultivated gene pool for breeding programs, and thus equally important to increase our understanding of the underlying genetic and molecular mechanism for grain yield. However, crop yield is a complex trait that is controlled simultaneously by multiple genes (i.e., quantitative trait loci [QTLs]) and heavily influenced by the surrounding environment; hence, to dissect such a complex trait into component contributory traits is necessary. Seed size (weight) is an essential yield component trait, and the past two decades have witnessed the identification of several hundred grain shape QTLs in rice; however, the genetic and molecular regulatory network of these QTLs remains largely unknown. Increasing lines of experimental evidence suggested that the plant hormone brassinosteroids (BRs) play a key role in seed size (weight) and hence crop yield regulation. Here, I briefly discuss the recent progress on how BR biosynthesis and signaling pathway have an impact on the important agronomic trait in crops.

WHAT ON EARTH IS RICE *GW5* GENE AND ITS FUNCTION?

A major *QTL for grain width on chromosome 5* (*GW5*, also called *qSW5*, or *GSE5*; hereafter called *GW5*) in rice was fine mapped by independent research groups and reported to be closely associated with a 1212-bp DNA deletion fragment that caused decreased *GW5* expression and increased grain width (Shomura et al., 2008; Weng et al., 2008; Duan et al., 2017). Most importantly, significant human selection has been taking place at the *GW5* deletion during rice domestication, likely due to artificial selection for breeding high-yield varieties (Shomura et al., 2008). More interestingly, Duan et al. (2017) found that, in addition to the aforementioned 1212-bp deletion that is present mainly in *japonica* varieties, a 950-bp DNA deletion is also closely linked to grain width alteration that prevails in *indica* varieties; also, the three major haplotypes of *GW5* identified in cultivated rice could have originated from different wild accessions. An interesting question remains, therefore, as to why the rice subspecies *indica* and *japonica* use different *GW5* haplotypes to enlarge grain size.

Transgenic plants with loss of function of the candidate gene *LOC_Os05g09520* by a CRISPR/Cas9 strategy produced a wider grain, while upregulated expression level of the gene bore a narrower grain (Figure 1; Duan et al., 2017; Liu et al., 2017), suggesting that *GW5* exerts a negative effect on grain width. Sequence analysis indicated that the *GW5* is a domestication-related gene encoding a protein with two IQ calmodulin-binding motifs (Duan et al., 2017; Liu et al., 2017). GW5 could physically interact with the rice calmodulin OsCaM1-1 (Duan et al., 2017). In previous studies, the *GW5* gene has been described to encode an unknown protein that interacts with ubiquitin (i.e., so-called ubiquitin-related protein) (Shomura et al., 2008; Weng et al., 2008); however, Liu et al. and Duan et al. have independently confirmed that its neighboring gene turned out be the viable candidate, which actually encodes a calmodulin-binding protein. However, it remains unclear whether the calcium signaling pathway is indeed involved in GW5 regulation of grain size and how it works.

Furthermore, using a carboxyl-terminal fragment of GW5 as the bait to screen a yeast two-hybrid library, GSK2 was identified as a viable interacting partner of GW5, which was confirmed by BiFC and pull-down assays (Liu et al., 2017). It is known that GSK2 is a rice ortholog of the *Arabidopsis* GSK3/SHAGGY-like kinase BIN2 and functions as a negative regulator of BR signaling in rice. Consistently, in the lamina joint inclination assays, transgenic rice plants overexpressing *GW5* in the genetic background of *GSK2* RNAi transgene greatly enhanced the phenotypes, which exhibited hypersensitivity to exogenously applied brassinolides (Liu et al., 2017). In addition, a series of biochemical analyses indicated that GW5 could repress the kinase activity of GSK2 toward OsBZR1 and DLT, resulting in accumulation of their unphosphorylated forms and altered BR signaling (Liu et al., 2017). Therefore, as proposed by the authors, GW5 might be a positive regulator of the BR signaling pathway in regulating grain width and weight in rice.

THE IMPACT OF BRs ON SEED-SIZE-RELATED TRAITS

BRs are a class of growth-promoting steroidal hormones that were initially isolated from rapeseed (*Brassica napus*) pollen, which are crucial for normal growth and development, such as plant height, leaf angle, panicle architecture, and seed size (Wu et al., 2016). To date, several rice BR biosynthesis mutants that bear seeds of reduced length have been functionally characterized, such as *d11* (*dwarf11*/*cpb1*), *d2* (*dwarf2*/*smg11*), *brd1* (*BR-deficient dwarf1*), and *brd2* (*BR-deficient dwarf2*) (Figure 1; Hong et al., 2002, 2005; Fang et al., 2016; Wu et al., 2016). Similarly, genes affecting BR perception and signaling have also been shown to control grain size in rice plants; for example, the rice *d61* mutant produced shorter grain and was less sensitive to exogenous BR, compared with the wild-type, most likely caused by loss of function of the rice ortholog of the BR receptor BRI1 in *Arabidopsis* (Yamamuro et al., 2000). This is also exemplified by the *OsmiR397-OsLAC* module, wherein the laccase-like protein OsLAC was targeted by OsmiR397; transgenic plants overexpressing *OsmiR397* bore enlarged grain and were much more sensitive to exogenously applied BR

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*Xian-Jun Song**

Institute of Botany, Chinese Academy of Sciences, No.20 Nanxincun, Xiangshan, Beijing 100093, China

***Correspondence: Xian-Jun Song (songxj@ibcas.ac.cn)**

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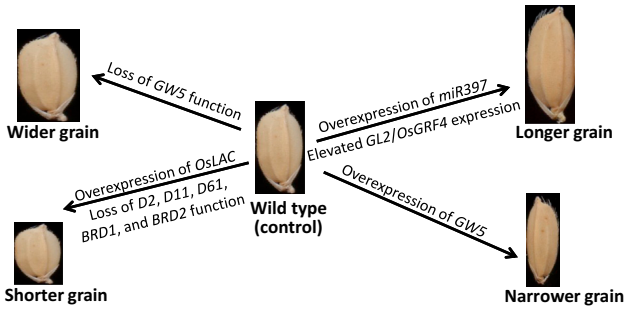

Figure 1. A Summary Diagram of how the BR-Related Genes Act Individually to Change Seed Size in Rice Plants Described in this Paper.

treatment, while those plants overexpressing *OsLAC* reduced grain size and were almost insensitive to the same treatment (Figure 1; Zhang et al., 2013). Furthermore, a grain-length QTL *GS2/GL2* was recently shown to encode a plant-specific transcription factor *OsGRF4* (growth regulating factor 4) that targeted by miR396; perturbation of the binding site of *OsGRF4* by miR396 caused elevated *OsGRF4* expression, and consequently upregulated the expression of the BR-induced genes (Hu et al., 2015; Che et al., 2016). Moreover, GSK2 was found to interact with and repress the transcription activation activity of OsGRF4 (Che et al., 2016). In addition, a major QTL for soybean seed weight encoding a putative phosphatase 2C (PP2C) was very recently characterized, and similar to the scenario for rice GW5, PP2C from the wild allele could interact with and enhance the accumulation of dephosphorylated GmBZR1 (soybean BZR1 ortholog) (Lu et al., 2017). It is of interest to note that the outer epidermal cell size in spikelet hulls of BR synthesis mutants *d2* or *d11* were smaller, while those of overexpression of either the *D2* or *D11* gene became significantly larger than the wild-type controls in rice plants (Fang et al., 2016; Wu et al., 2016), suggesting that BR might control grain size via altering cell expansion in spikelet hulls. Interestingly, GW5 (or other factors such as miR397) regulates grain size by restricting an increase in cell number (Duan et al., 2017; Liu et al., 2017). To better understand the mechanism underlying BR regulation of seed size, it would be worth investigating how GW5 works in the BR signaling pathway. Despite the progress, the role of BRs in regulating seed size needs to be further dissected, and the genetic regulatory networks among these seed-size regulators also remain to be determined.

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