SQUAMOSA Promoter Binding Protein-Like Transcription Factors: Important Breeding Research Targets for Improving Cereal Grain Yield

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Grain size and shape are prime targets for crop breeders, as they affect both yield and quality in rice. In addition, breeders target plant vegetative and reproductive shoot architecture, because these traits influence grain number, and thus yield potential. Accordingly, the genetic control of grain size and shape, and of shoot architecture, has been extensively investigated in recent years. In an important recent advance, Bin Han and colleagues have used genome-wide association analysis of grain size in a diverse collection of rice varieties of worldwide origin to identify a major quantitative trait locus *GLW7*, which encodes a *SQUAMOSA* promoter binding protein-like 13 (OsSPL13), a member of the plant specific SBP domain family of transcription factors (Si et al., 2016). *GLW7* positively regulates the size of rice grain cells, thus increasing grain length. *GLW7* also regulates rice reproductive shoot (panicle) architecture, and the combined effects of *GLW7* on panicle and grain structure increase yield (Si et al., 2016).

What are *SQUAMOSA* promoter binding protein-like (SPL) transcription factors?

The SPL proteins are relatively diverse in sequence and function, but all contain a highly conserved region of 76 amino acids known as the SBP domain. The first described SPL proteins were AmSBP1 and AmSBP2 of snapdragon (*Antirrhinum majus*), which bind to the promoter of the floral meristem identity gene *SQUAMOSA* (Klein et al., 1996). Subsequently, *SPL* genes have been found to regulate diverse plant biological functions, including vegetative-reproductive phase change and shoot architecture. In *Arabidopsis*, *SPL* genes have been particularly implicated in regulating the timing of the floral transition, via both DELLA-dependent and DELLA-independent pathways (Wang et al., 2009 and Yu et al., 2012). Of particular interest is the fact that many *SPL* genes encode mRNAs having sequence complementarity with an evolutionarily conserved miR156. These mRNAs are specifically cleaved following interaction with miR156, and the miR156-*SPL* regulatory module functions as a master regulator for the age-dependent flowering responses (Wang et al., 2009).

Rice SPLs: opposing roles in the regulation of vegetative and reproductive shoot architecture

In contrast to *Arabidopsis*, and although the rice (*Oryza sativa*) genome contains 18 *OsSPL* genes (Preston et al., 2013), none of these has up to now been reported to modulate rice vegetative-reproductive phase changes, whilst several of them appear to be concerned with the specification of plant vegetative or reproductive architecture, or of grain size or shape. For example, *OsSPL14* plays important roles in controlling ideal plant architecture, and has major consequent effects on grain productivity (Jiao et al., 2010 and Miura et al., 2010). Intriguingly, *OsSPL14* plays opposing roles in the regulation of tiller and panicle branching. Whilst up-regulation of *OsSPL14* expression leads to the production of fewer tillers with stronger, more robust culms, it conversely increases the number of panicle branches (Jiao et al., 2010 and Miura et al., 2010). Similarly, transgenic plant lines having elevated levels of *OsSPL13* expression have increased numbers of both primary and secondary panicle branches, whereas the numbers of panicle branches and grains per panicle are greatly reduced in RNAi lines having reduced levels of *OsSPL13* expression (Si et al., 2016). Other SPLs are also known to be involved in the regulation of inflorescence architecture, again in contrasting ways: whilst up-regulation of *OsSPL17* and *OsSPL7* both reduce tiller numbers, the down-regulation of *OsSPL17* or miR156-targeted *OsSPL* genes expression greatly reduces panicle branch numbers (Xie et al., 2006 and Wang et al., 2015a), except that overexpression of *OsSPL16* reduces the numbers of both tillers and panicle branches (Wang et al., 2012). Taken together, these observations suggest that multiple *OsSPL* genes play redundant functions in regulating the activities of vegetative (tiller-forming) and inflorescence (panicle branch-forming) meristems, and often in contrasting ways (Figure 1).

Rice SPLs: differing roles in the regulation of grain size and shape

The Si et al. (2016) paper shows that, in addition to regulating panicle architecture, *OsSPL13* also promotes increases in the length and thickness of the rice grain, but does not regulate grain width (Figure 1). Analysis at the cellular level (cell number and cell size) of the spikelet hull revealed that *OsSPL13* regulates grain shape via control of the mechanisms determining cell size, and predominantly regulates organ development by regulating the size that individual cells achieve during growth, rather than the number of cells comprising an organ (Si et al., 2016). They also show that OsSPL13 binds to the promoter of a gene called *SMALL AND ROUND SEED 5* (*SRS5*), and likely acts as a positive regulator of *SRS5* transcription. *SRS5* mRNA encodes an ∝-tubulin subunit component of the microtubule cell growth machinery, thus providing a link between *OsSPL13* and cell growth regulation.

In contrast, yet another rice SPL-encoding gene, *OsSPL16*, the gene underlying the *Grain-width 8* (*GW8*) QTL, is a positive regulator of cell proliferation and grain filling (Wang et al., 2012). High-level expression of *OsSPL16* results in enhanced grain width and yield: cell division is promoted, cell numbers are increased, and grains become wider (Figure 1). OsSPL16 binds to the promoter of gene *Os07g0603300* (the gene underlying another major rice grain shape QTL, *GW7*), and represses its expression (Wang et al., 2015b). *Os07g0603300* encodes a TON1 RECRUIT MOTIF (TRM)-containing protein that likely recruits the TON1 protein to cortical microtubule arrays, thus influencing cell division patterns (Wang et al., 2015b and Wang et al., 2015c). *GW7* is a major quantitative trait locus for rice grain quality because it confers the much prized slender grain phenotype. The formation of slender grain is thus thought to be caused by an *Os07g0603300*-dependent alteration in the properties of the microtubules of the preprophase band of dividing cells that favours longitudinal over transverse organ growth, an alteration that is itself inhibited by OsSPL16-mediated transcriptional repression of *Os07g0603300* (Wang et al., 2015b). Thus, different *OsSPL* genes affect grain size and shape in different ways, either by regulating cell elongation (*OsSPL13*), or by regulating the frequency or orientation of cell division events during cell proliferation (*OsSPL16*).

Cereal SPLs: a paradigm of evolutionary gene neo-functionalization and breeding or engineering targets for improving yield potential

It has been suggested that the ancestral SPL function was to regulate vegetative and floral transitions, this function has subsequently been partitioned through differential sub-functionalization following both gene duplication and speciation, and that neo-functionalization (e.g., in glume architecture) and parallel recruitment (e.g., in branching) occurred more recently in angiosperm evolution (Preston et al., 2013). Whilst the liguleless gene (*OsLG1*) provides an important example of how the capturing of variation in SPL function was crucial to the origin of a closed panicle trait during rice domestication (Ishi et al., 2013), the various rice examples discussed here provide powerful examples of how relatively rapid gene evolution, perhaps driven by post-domestication human selection, can contribute to the fine-tuning that has contributed to further improvements in crop performance. It is striking that so many of the shoot branching and grain size/shape traits identified in rice QTL analyses are conferred by variant *OsSPL* genes, rather than by variance in genes belonging to other gene families.

How was this rapid evolution of SPL function achieved? Changes in time, place or extent of *SPL* gene expression (e.g. 11 *OsSPL* genes were putative targets of miR156), alterations in down-stream target gene specificity, or modulation of biochemical function of individual SPL proteins are all possible routes to functional variation. However, it is perhaps noteworthy that in most of the QTL examples discussed above, variation in the expression pattern of specific *OsSPL* genes confers trait variation, suggesting that this might be the majority mechanism driving the genetic variation selected during rice breeding programmes. Irrespective of the mechanisms via which SPL functions evolved to regulate rice vegetative and reproductive shoot architecture and grain size/shape, it is clear that discovering and exploiting further useful genetic variation affecting SPL function is now a major goal for breeders seeking to improve grain yield, not only in rice, but also in closely related cereal crops such as wheat, barley and maize.

Figure 1. Diverse and contrasting roles of *OsSPL* genes in regulating rice vegetative and reproductive shoot architecture, and grain size and shape. The *OsSPL* genes act as a negative regulator of the activity of the vegetative shoot meristems. The up-regulation of the *OsSPL* genes is associated with the reduction of tiller numbers, such as *OsSPL7*, *OsSPL14*, *OsSPL16* and *OsSPL17*. In contrast, the elevated expression of the *OsSPL* genes usually promotes panicle branching and improves grain yield, such as *OsSPL13*, *OsSPL14* and *OsSPL17*. Specifically, overexpression of *OsSPL16* inhibits both activities of vegetative (tiller-forming) and inflorescence (panicle branch-forming) meristems. Both *OsSPL13* and *OsSPL16* positively regulate grain weight, but they affect rice grain size and shape in different ways. *OsSPL13* promotes increases in grain length by regulating cell elongation, but does not regulate grain width. Conversely, the up-regulation of *OsSPL16* is corrected with the formation of short and wide grain by regulating the frequency or orientation of cell proliferation.

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REFERENCES

Ishii, T., Numaguchi, K., Miura, K., Yoshida, K., Thanh, P.T., Htun, T.M., Yamasaki, M., Komeda, N., Matsumoto, T., Terauchi, R., et al. (2013). *OsLG1* regulates a closed panicle trait in domesticated rice. Nat. Genet. 45(4): 462-465.

Klein, J., Saedler, H., and Huijser, P. (1996). A new family of DNA binding proteins includes putative transcriptional regulators of the Antirrhinum majus floral meristem identity gene SQUAMOSA. Mol. Gen. Genet. 250(1):7-16.

Jiao, Y., Wang, Y., Xue, D., Wang, J., Yan, M., Liu, G., Dong, G., Zeng, D., Lu, Z., Zhu, X. et al. (2010). Regulation of *OsSPL14* by OsmiR156 defines ideal plant architecture in rice. Nat. Genet. 42(6): 541-544.

Miura, K., Ikeda, M., Matsubara, A., Song, X.J., Ito, M., Asano, K., Matsuoka, M., Kitano, H., and Ashikari, M. (2010). *OsSPL14* promotes panicle branching and higher grain productivity in rice. Nat. Genet. 42(6): 545-549.

Preston, J.C., and Hileman, L.C. (2013). Functional evolution in the plant *SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE* (*SPL*) gene family. Front. Plant Sci. 4(80):1-13.

Si, L., Chen, J., Huang, X., Gong, H., Luo, J., Hou, Q., Zhou, T., Lu, T., Zhu, J., Shangguan, Y., et al. (2016). *OsSPL13* controls grain size in cultivated rice. Nat. Genet. 48(4): 447-456.

Wang, J.W., Czech, B., and Weigel, D. (2009). miR156-regulated *SPL* transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. Cell. 138(4):738-749.

Wang, L., Sun, S., Jin, J., Fu, D., Yang, X., Weng, X., Xu, C., Li, X., Xiao, J., and Zhang, Q. (2015a). Coordinated regulation of vegetative and reproductive branching in rice. Proc. Natl. Acad. Sci. U S A. 112(50):15504-15509.

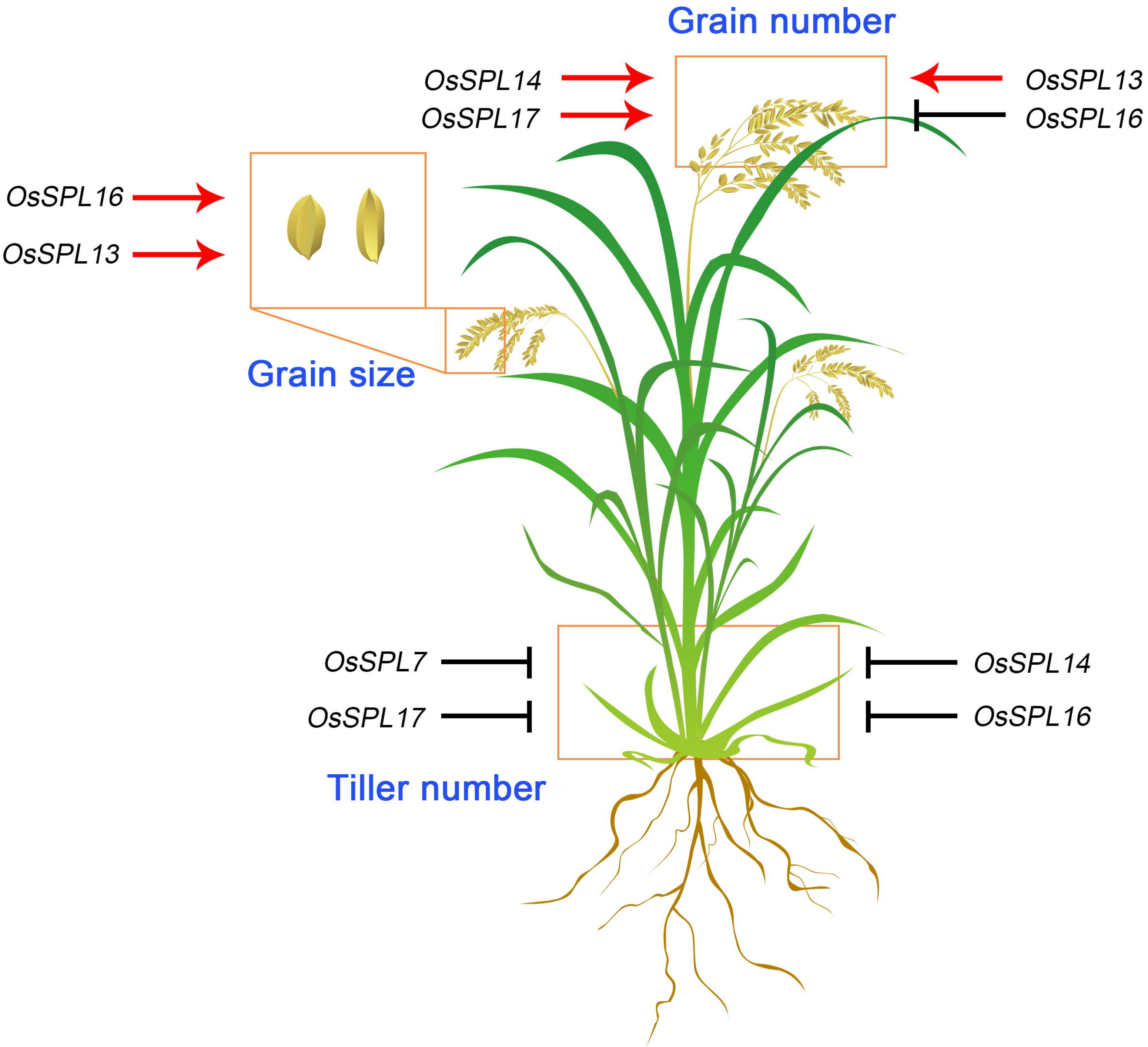
Wang, S., Wu, K., Yuan, Q., Liu, X., Liu, Z., Lin, X., Zeng, R., Zhu, H., Dong, G., and Qian, Q. (2012). Control of grain size, shape and quality by *OsSPL16* in rice. Nat. Genet. 44(8): 950-954.

Wang, S., Li, S., Liu, Q., Wu, K., Zhang, J., Wang, S., Wang, Y., Chen, X., Zhang, Y., and Gao, C., et al. (2015b). The *OsSPL16-GW7* regulatory module determines grain shape and simultaneously improves rice yield and grain quality. Nat. Genet. 47(8): 949-954.

Wang, Y., Xiong, G., Hu, J., Jiang, L., Yu, H., Xu, J., Fang, Y., Zeng, L., Xu, E., Xu, J., et al. (2015c). Copy number variation at the *GL7* locus contributes to grain size diversity in rice. Nat. Genet. 47(8): 944-948.

Xie, K., Wu, C., and Xiong, L. (2006). Genomic organization, differential expression, and interaction of SQUAMOSA promoter-binding-like transcription factors and microRNA156 in rice. Plant Physiol. 142(1):280-293.

Yu, S., Galvão, V.C., Zhang, Y.C., Horrer, D., Zhang, T.Q., Hao, Y.H., Feng, Y.Q., Wang, S., Schmid, M., and Wang, J.W. (2012). Gibberellin regulates the *Arabidopsis* floral transition through miR156-targeted *SQUAMOSA* promoter binding-like transcription factors. Plant Cell. 24(8): 3320-3332.


Grain number
OsSPL14
OsSPL17
OsSPL13
OsSPL16
OsSPL16
OsSPL13
Grain size
OsSPL7
OsSPL17
OsSPL14
OsSPL16
Tiller number