SPL transcription factors prevent floral reversion in rice

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1 Plants show an incredible diversity of forms, dimensions, architectures, structures, and reproductive
2 behaviors. Yet, they can be geometrically described as composed of simple building units called
3 phytomers, which are produced by the shoot apical meristem (SAM). A phytomer is made of a node
4 bearing a leaf and an axillary meristem, and of an internode connecting the node to the following
5 phytomer. Piling of phytomers creates the plant body, similarly to a LEGO® construction made of
6 simple bricks. Modifications of the phytomeric unit creates diversity. The leaf can change in size
7 and shape; the axillary meristem can be dormant or active, determinate or indeterminate; the
8 internode can elongate considerably, or be compressed to the extent it might look invisible.

9 During vegetative growth, the phytomer produces leaves and meristems that give rise to lateral
10 branches. However, upon perception of favorable environmental conditions, the SAM is
11 reprogrammed to reproductive growth, and is converted to an inflorescence meristem that starts
12 developing flowers. In several plant species, the reproductive switch is coordinated by the florigens,
13 small soluble proteins belonging to the Phosphatidyl Ethanolamine Binding Protein family.
14 Florigens are expressed in companion cells of the phloem and are translocated to the SAM through
15 the sieve elements. Upon entering cells of the SAM, they form higher-order complexes which
16 include transcription factors of the bZIP family, directing transcriptional activities that initiate the
17 reproductive transition. This moment is key for every annual species, including rice and
18 Arabidopsis, because the reproductive switch is irreversible. Once committed to flowering, the
19 SAM suppresses the vegetative program, to establish the reproductive one.

20 In rice, reproductive phytomers repress leaf (also called bract) development, while allowing
21 outgrowth of axillary meristems and thus branching. This pattern results in the formation of a
22 determinate inflorescence (the panicle), bearing primary and secondary branches and harboring
23 spikelets which contain the flowers.
24 The study of rice inflorescence architecture has received substantial interest, given its prominent
25 role in determining yield. A major regulator of inflorescence development is encoded by the
26 *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14* (*SPL14*) gene, also known as *IDEAL*
27 *PLANT ARCHITECTURE 1* (*IPA1*)/*WEALTHY FARMER'S PANICLE* (*WFP*), hereafter *SPL14*.

28 Modulation of its expression can increase panicle branching and yield, while reducing vegetative

29 branching, limiting tiller number. Thus, the gene has opposite roles during the vegetative and

30 reproductive phase. *SPL14* expression is reduced by the microRNAs *miR156/miR529* and mutations

31 disrupting their binding site result in a more active *SPL14* allele increasing panicle branching

32 (Miura et al., 2010).

33 *SPL14* belongs to a large family of transcription factors, some of which, including *SPL17*, are

34 redundant with *SPL14* in regulation of panicle branching (Jiao et al., 2010; Wang et al., 2015). The

35 full extent of redundancy has likely not been appreciated yet, but a study from Wang et al.,

36 addressing this problem, is now revealing novel features of the SPLs-*miR156/529* regulatory

37 module (Wang et al., 2021).

SPLs are required to suppress the vegetative program during rice flowering

39 After the reproductive transition, panicle development is characterized by bract suppression and

40 branches are formed with spiral phyllotaxy. The triple mutant *spl7 spl14 spl17* displays a striking

41 phenotype with fully developed leaves replacing the bracts at the base of the panicle and leaf

42 sheaths forming in the more distal positions. Basal primary branches are converted to vegetative

43 shoots in the axil of proximal bracts and are inserted on the main rachis with spiral phyllotaxy, like

44 reproductive branches, but forming leaves with an alternate phyllotaxy.

45 These floral reversion phenotypes indicate that transition to flowering occurs normally in the

46 mutant, but the reproductive program cannot be correctly established or maintained, leading to

47 persistent vegetative development of panicle meristems. Overexpression of *miR156/529* leads to

48 similar, albeit milder, phenotypes. Thus, the SPLs-*miR156/529* module maintains the reproductive

49 program after transition to flowering.

50 Some features of the *spl7 spl14 spl17* mutant are reminiscent of the *osmads14, 15, 18, 34* quadruple

51 mutant, including replacement of reproductive branches with vegetative shoots and correct

52 establishment of spiral phyllotaxy upon reproductive transition (Kobayashi et al., 2012). *OsMADS*

53 genes are transcriptionally induced at the apical meristem upon arrival of florigenic signals from

54 leaves (Figure 1D). These observations lead to the possibility of a crosstalk between the florigen-

55 *OsMADS* and *SPLs-miR156/529* modules. In Arabidopsis, SPL3, 4 and 5 can interact with FD, a

56 bZIP transcription factor mediating florigen binding to the DNA. The interaction facilitates FD

57 binding to target gene promoters, including those of MADS box genes required for flower

58 development (Jung et al., 2016). If rice shared a similar mechanism, SPLs could interact with OsFD

59 proteins, to coordinate flowering induction, driven by environmental cues, with its maintenance

60 determined by an endogenous program (Cerise et al., 2021).

Expression data indicate that SPLs and their antagonizing miRs display a complementary pattern in the reproductive stage, consistent with their function (Figure 1A, B). This pattern, and the analysis of SPL loss-of-function mutants, gain-of-function and overexpressors show that dosage effect is crucial to establish panicle architecture. An excess of SPLs rapidly stops branching by differentiating terminal spikelets, while loss-of-function mutants reduce branching similarly to what observed in *miR156* overexpressors (Wang et al., 2015; Yang et al., 2019). Only a right dosage that bypasses *miR156* control is optimal to increase branching. Thus, engineering the system for increasing yield will likely require fine tuning of expression levels, e.g. by exploiting editing to increase cis-regulatory variation (Rodríguez-Leal et al., 2017).

Downstream of SPLs into the regulatory networks maintaining reproductive fate

In a further effort, Wang and colleagues set out to define the gene regulatory network associated to SPLs activity. Using a comprehensive set of experiments, revealing expression, protein-protein interaction, and DNA-interaction patterns, they identified the NECK LEAF1 (NL1) protein as interactor of SPLs. *NL1* encodes a GATA transcription factor, whose expression is also directly activated by the SPL14 protein.

NL1 activity limits the expansion of the vegetative phase into the reproductive one and, when mutated, gives rise to vegetative shoots in the panicle, similarly to *SPL* mutants. Genetic analyses corroborated these findings, indicating that *NL1* is epistatic to *miR156* and *SPL14/17*. *NL1* expression localizes to the adaxial side of bract primordia, overlapping with *SPL* transcripts (Figure 1C). Most notably, NL1 can interact with all SPL proteins. Thus, the SPLs-NL1 module is a central coordinator of downstream events that prevent floral reversion.

Consistent with this observation, transcriptional profiling and chromatin accessibility analysis in the *spl7 spl14 spl17* and *nl1* mutants, identified a large overlap of differentially regulated genes, that globally indicate how the vegetative program persists also during panicle development in the mutants. Among common targets, the *PLASTOCHRON1* (*PLA1*) gene was identified as transcriptionally activated, as well as directly bound, by SPL14 and NL1 proteins (Wang et al., 2009). The resulting SPLs-NL1-PLA1 cascade is now empowered with a terminal element. Its molecular function, as well as the spatial expression profiles of the genes involved is evidencing interesting features of the system.

Transcription of *SPLs*, *NL1* and *PLA1* overlaps in developing bract primordia at the base of the panicle and in incipient bract primordia on the reproductive branches, consistent with these genes being organized in a common regulatory module (Figure 1C). From these very localized sites, the module can suppress bract development in a cell-autonomous manner. However, maintenance of the

reproductive fate of the developing axillary meristems might require non-cell autonomous activity. SPLs and NL1 are unlikely to act non-cell autonomously, but *PLA1* encodes a cytochrome P450 monooxygenase, whose enzymatic activity could be necessary to produce a non-cell autonomous signal preventing phase reversion (Miyoshi et al., 2004). The nature of such signal remains unclear, but its identification would contribute to understanding how inflorescence shape is determined, and possibly use it to increase crop yield.

Figure 1. Spatial expression patterns of marker genes during the late primary branch initiation stage.

(**A**) Description of morphological domains in the panicle.

(**B**) Expression patterns of *SPL7*, *14* and *17* and of *miR156* and *529* transcripts. These two gene families display a complementary pattern.

(**C**) Expression patterns of *PLA1* and *NL1* transcripts, which overlap with expression of *SPLs*.

(**D**) Expression patterns of Hd3a and OsMADS15 proteins and of *OsMADS14* and *34* transcripts.

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