

Functional Dissection of Class C Genes in Rice

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The classic genetic ABC model of floral organ identity control was established nearly 30 years ago (Coen and Meyerowitz, 1991). The model explains the process by which four types of floral organs are produced from undifferentiated floral meristems through the action of three classes of transcription factors. The class C MADS-domain transcription factors specify carpel identity and control floral meristem determinacy, while combinatorial action of class B and C transcription factors specifies stamen identity. Originally, the ABC model was derived from genetic studies conducted in *Arabidopsis thaliana* and *Antirrhinum majus*, but it was subsequently shown to hold true for other flowering species, including members of the grass family (Ciaffi et al., 2011; Guo et al., 2015).

In this issue, Sugiyama et al. (2019) re-address the function of class C genes in rice (*Oryza sativa*) and provide evidence to suggest that, in contrast to *Arabidopsis*, rice class C genes are not key regulators of carpel specification (Sugiyama et al., 2019). They did this primarily by identifying and analyzing novel loss-of-function mutants of *OsMADS58* by TILLING and CRISPR/Cas9 approaches (Sugiyama et al., 2019). Previous genetic analyses of *OsMADS58* had used RNAi or unstable transposon-insertion lines to disrupt the locus (Dreni et al., 2011; Yamaguchi et al., 2006). These RNAi lines had shown morphological defects that were not observed in the *osmads58* mutants, suggesting that RNAi approaches might have affected additional genes. Sugiyama and colleagues further created and examined the loss-of-function double mutant *osmads3 osmads58* (Sugiyama et al., 2019). The *osmads3* loss-of-function mutant was previously described as forming chimeric organs consisting

of lodicules and stamens in whorl 3, caused by the partial loss of stamen identity (Yamaguchi et al., 2006). This prior finding implicated OsMADS3 involvement alone in stamen specification. However, the loss-of-function double mutant *osmads3 osmads58* failed to form stamens altogether, suggesting that both OsMADS3 and OsMADS58 redundantly specify stamen identity (Sugiyama et al. 2019). In addition, the double mutant had indeterminate meristems with lodicules in whorl 3, and green organs produced in whorl 4 that maintained *DROOPING LEAF (DL)* expression as well as D-class *OsMADS13* expression. Sugiyama et al. (2019) claim that these organs are carpel-like, while Dreni et al. (2011) previously interpreted them as perianth lemma/palea-like organs. Thus two different theories currently exist based on the different alleles studied and/or to diverse interpretations of the data; Dreni et al (2011) proposed that OsMADS3 and OsMADS58 redundantly regulate carpel identity as a conserved function of class C proteins, and implicated DL as a fourth-whorl marker without an identity function due to its inability to specify carpel identity in the absence of *OsMADS3* and *OsMADS58*. By contrast, Sugiyama et al. (2019) now propose that *OsMADS3* and *OsMADS58* are dispensable for carpel specification, and that DL is the key carpel identity determinant (Fig. 1).

DL is the rice ortholog of Arabidopsis *CRABS CLAW*, which encodes a YABBY-type transcription factor (Yamaguchi et al., 2004). In *dl* mutant flowers, the carpel is replaced by multiple stamens (Nagasawa et al., 2003). This indicates that DL is a key player for fourth-whorl carpel identity, by repressing the B-function genes and/or by inducing carpel identity genes. If the sole purpose of DL is to repress the class B genes, the fourth whorl carpels should be restored when *dl* is combined with a class B mutant. Mutations in rice *OsMADS16/SUPERWOMANI (SPW1)*, an *APETALA3* homolog, transforms lodicules and stamens into palea-like organs and an indefinite number of carpels, respectively (Nagasawa et al., 2003). Here, the *dl spw1* double mutant was reported to show palea/leaf-like organs in whorl 2 and multiple unidentifiable organs in whorls 3 and 4, despite still expressing *OsMADS3* (Sugiyama et al., 2019). This result strongly suggests that DL induces carpel identity and that OsMADS3 alone is not sufficient to induce carpel identity without DL. DL has evolved a grass-specific function in carpel development via the acquisition of a novel expression pattern and protein function

(Nagasawa et al., 2003). Thus, in rice neo-functionalization of DL appears to have happened, which was subsequently followed by the reverse-diversification and non-functionalization of class C proteins in carpel identity control. Recently, Arabidopsis CRC was shown to function in the control of auxin homeostasis (Yamaguchi et al., 2017; Yamaguchi et al., 2018). It would be interesting to determine whether a similar form of regulation occurs in rice.

What's the major function of OsMADS3 and OsMADS58 in *whorl4*? They are clearly important players for shutting off floral meristem activity together with SPW1 and DL (Sugiyama et al., 2019). The double mutant of the class C genes shows strong indeterminate growth with continuous expression of the class I KNOX gene, *OSHI*. At the same time, the carpel-like organs of *osmads3 osmad58* also ectopically express *OsMADS15*, a lemma/palea-specific gene. Therefore, the authors concluded that OsMADS3 and OsMADS58 might function to repress A-class genes.

In the strong *dl* mutants, many ectopic stamens are formed in the region where the gynoecium would normally be produced. The *dl spw1* shows enhanced strong indeterminacy with persistent *OSHI* expression in the center of developing flowers (Sugiyama et al., 2019). It would be interesting to determine how OsMADS3, OsMADS58, SPW1 and DL synergistically function to achieve floral meristem termination.

Thus, Sugiyama et al. (2019) concluded that OsMADS3 and OsMADS58 are not essential for carpel identity control (Fig. 1). Could it then be concluded that rice class C proteins are not involved in carpel specification? As the authors suggest, it might be possible that in rice the genes necessary for carpel identity and morphogenesis can be co-regulated by OsMADS3, OsMADS58 and DL. In Arabidopsis, it has been shown that class C and D proteins redundantly confer carpel and ovule identity (Pinyopich et al., 2003). AGAMOUS (AG)-lineage genes *SHATTERPROOF1* (*SHP1*) and *SHP2* partially complement the loss of carpel identity and produce carpel-like organs in the absence of the Arabidopsis C-class *AG* function in a certain genetic background (i.e. *ag apetala2*). AG controls reproductive organ identity and morphogenesis by controlling the expression of multiple genes throughout reproductive development (Ito et al., 2007;

Guo et al., 2015). *DL* and *OsMADS13* are expressed in the carpel-like organs of *osmads58 osmads3* (Sugiyama et al., 2019). This indicates that in rice *DL* and *OsMADS13* might control carpel identity and morphogenesis redundantly with *OsMADS3* and *OsMADS58*. Careful analysis of the organs produced in the triple loss-of-function mutants of *osmads3 osmads58 dl* and *osmads3 osmads58 osmads13* should reveal whether *DL* and D-class *OsMADS13* confer carpel specification in *osmads3 osmads58*. The organs produced in *dl spw1* have neither stamen nor carpel identities even though *OsMADS3* expression is intact. Again, this does not mean that *OsMADS3* is not involved in (stamen and) carpel identity specification, but that not all of the genes necessary for carpel specification are induced in *dl spw1* mutants. Thus while *DL* activity is essential for the activation of carpel specification genes, *OsMADS3* alone may not be sufficient to induce these carpel identity genes. With regards to the molecular mechanisms involved, one possible scenario is that genes acting downstream of *DL* could be co-regulated by class C genes, including *OsMADS3*. Once the genome-wide targets of *OsMADS3*, *OsMADS58*, and *DL* are known, and the protein complex sufficient to induce carpel identity genes identified, the evolutionary aspects of C class genes will become much clearer.

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Figure legend

Fig 1. Essential genes for C-class activity in rice. As a class C gene, *OsMADS58* functions partially redundantly with *OsMADS3* in floral meristem (FM) determinacy, repression of A class genes and stamen identity control. The defects in FM determinacy

are clearly observable only when both *OsMADS3* and *OsMADS58* are mutated. *DL*, *DROPPING LEAF*; gl, glume; le, lemma; lo, lodicule; pa, palea.

- FM determinacy – *DL*, *OsMADS3* & *OsMADS58*
- Repression of A class genes - *OsMADS3*
- Stamen identity - *OsMADS3*
- Carpel identity - *DL*

**Essential genes
for rice C-class activity**

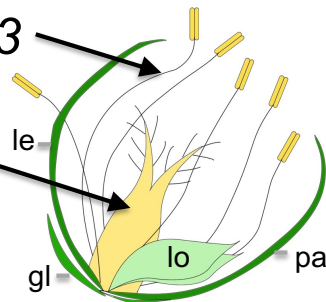


Fig 1. Essential genes for C-class activity in rice

As a class C gene, *OsMADS58* functions partially redundantly with *OsMADS3* in floral meristem (FM) determinacy, repression of A class genes and stamen identity control. The defects in FM determinacy is clearly observable only when both *OsMADS3* and *OsMADS58* are mutated. *DL*, *DROPPING LEAF*; gl, glume; le, lemma; lo, lodicule; pa, palea;