10. *OPEN BEAK* gene regulates floral morphogenesis in rice

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To understand how floral organs are formed, a lot of studies have been done in *Arabidopsis thaliana*, which led to the establishment of a genetic model, ABC model. In this model, class A, B and C genes act in two adjacent whorls, whorl 1,2, whorl 2, 3 and whorl3, 4, respectively, and determine floral organ identity. Also in rice, a model plant of monocots, this model is suggested to be applicable except for carpel identity that is specified by *DL* gene. However, regulatory mechanism of flower formation in monocots is mostly unknown.

We isolated two recessive mutants affecting spikelet/flower morphogenesis from M2 populations mutagenized with N-methyl-N-nitrosourea. Since they were revealed to be allelic and their malformed lemma and palea resembled open beak, they were designated, *open beak-1* (*opb-1*; cv. Taichung 65 background) and *opb-2* (cv. Kinmaze background).

In the vegetative phase, about 15% of *opb-1* seedlings did not show a bending of second leaf blade at the lamina joint (Fig. 1A, B). In about 20% of *opb-1* seedlings, the third and/or fourth leaf blades were malformed (undulated) (Fig. 1B). Thus, *OPB* is required for correct development of second to forth leaves. In the late vegetative phase, however, *opb-1* plants became indistinguishable from wild-type plants.

As the mutant name suggested, conspicuous abnormalities were detected in spikelet/flower. Although the two mutants showed similar phenotypes, abnormality was more severe in *opb-1* than in *opb-2* (Fig. 1). The first two pairs of glumes, rudimentary glumes and empty glumes, were almost normal. Abnormalities were observed in lemma and subsequent organs. In both mutants, lemma and palea were suppressed in their lateral growth, thus unable to enclose internal floral organs (Fig. 1C, E, G). In addition, tip of the lemma curved toward the palea. Organization of floral organs was also abnormal. In wild-type flower, two lodicules, six stamens and one pistil are formed in whorl 2, 3, 4, respectively (Fig. 1D). In whorl 2 of *opb*s, lodicules were elongated as if they partially acquired glume identity. Often, the glume-like structure was also formed on the palea side of whorl 2. In *opb*s, a mosaic organ of lodicule and stamen was formed in whorl 2. In whorl 3, the number of normal stamens was reduced, and mosaic organs between stamen and pistil appeared (Table 1). The number of the mosaic organs was larger in *opb-1* than in *opb-2*. In whorl 4, two to five pistils were formed, suggesting a partial loss of determinacy of floral meristem. From these phenotypes, it is considered that identities of whorl 2 and 3 organs (lodicules and stamen) are impaired.

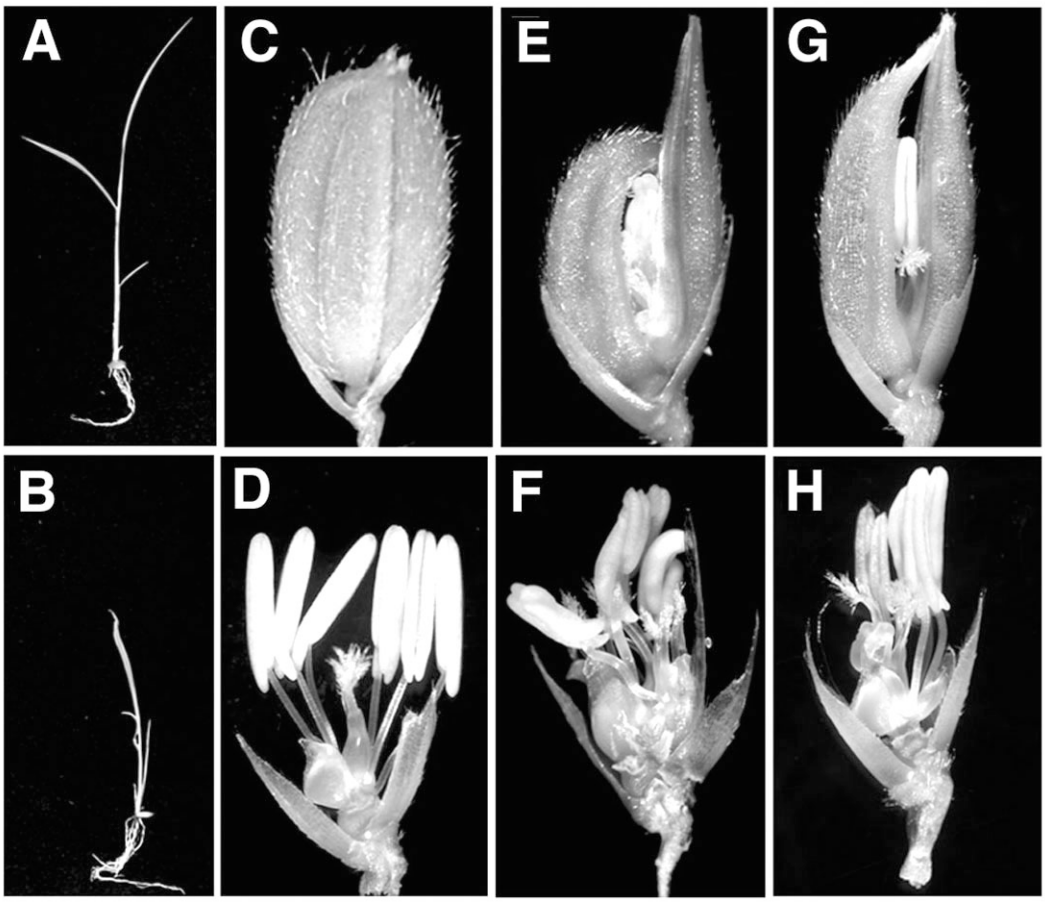

Fig. 1. Phenotypes of *opb*. A: Two-week-old wild-type seedling. B: Two-week-old *opb-1* seedling. C: Mature wild-type spikelet. D: Mature wild-type flower. E: Mature *opb-1* spikelet. F: Mature *opb-1* flower. G: Mature *opb-2* spikelet. H: Mature *opb-2* flower.

The above results indicate that *opb* mutation affects the identities of whorl 2 and 3 organs and meristem determinacy. Thus, it is probable that *OPB* regulate class B gene expression. Then, we examined the expression of *SUPERWOMAN1* (*SPW*1) gene (class B gene) that specifies whorl 2 and 3 organs (Nagasawa *et al*. 2003), and *DROOPING LEAF* (*DL*) gene that is involved in carpel identity and meristem determinacy (Nagasawa et al. 2003, Yamaguchi et al. 2004). In *opb*s, *SPW1* was expressed in narrower domains of both whorl 2 (lodicule) and 3 (stamen) organs than in wild type (Fig. 2A, B), suggesting that *OPB* promotes *SPW1* expression in whorl 2 and 3. On the contrary, *DL* was expressed in wider region of *opb* flower than in wild-type flower. (Fig. 2C, D). Thus, *OPB* would be required for correct expression of these two genes. Alternatively, it may function to limit the expression domains of floral organ identity genes and to establish organ boundaries.

Table 1 Number of floral organs (mean ± s.d.) in wild type and *opb*

Genotype	Lodicule	Staminoid loducule	Stamen	Pistiloid stamen	Pistil	Total
Wild type	2.0±0	0	6.0±0	0	1.0±0	9.0±0
opb-1	2.2±0.6*	0.8±0.8	2.7±0.8	3.7±1.1	2.3±1.6	11.7±1.8
opb-2	2.0±0	0.6±1.1	3.5±1.4	2.4±1.5	1.8±0.4	10.3±1.0

* Most are elongated.

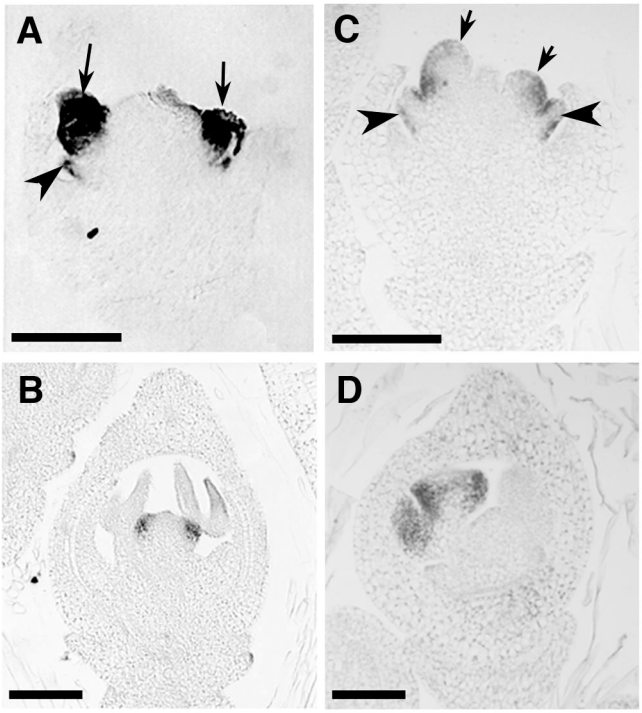

Fig. 2. Expression pattern of two floral organ identity genes, *SPW1* and *DL*. A: Expression of *SPW1* in lodicule and stamen primordia of wild-type flower. B: Expression of *DL* in carpel primordium of wild-type flower. C: Expression of *SPW1* in lodicule and stamen primordia of *opb-1* flower. D: Expression of *DL* in carpel primordia of *opb-1* flower. Arrows indicate lodicules. Arrowheads indicate stamens. Scale bars, 100 μm.

References

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