Rice Science, 2009, 16(1): 75–78

DOI: 10.1016/S1672-6308(08)60060-8

Characterization and Genetic Analysis of a Novel Mutant *mst* of Rice Defective in Flower Development

LI Yun[1, 2], XU Pei-zhou[1, 3], ZHANG Hong-yu[1, 3], FU Shao-hong[2], YANG Jin[2], ZHANG Ru-quan[2], WU Xian-jun[1, 3]

(*[1]Rice Research Institute, Sichuan Agricultural University, Wenjiang 611130, China; [2]Crops Research Institute, Chengdu Academy of Agricultural and Forestry Sciences, Wenjiang 611130, China; [3]Key Laboratory of Southwest Crop Genetic Resources and Improvement, Ministry of Education, Sichuan Agricultural University, Ya'an 625014, China*)

Abstract: A spontaneous mutant with multiple stigmas (*mst*) was found in an indica rice line 466. The *mst* mutant exhibits normal at the vegetative development stage and produces normal inflorescence structures. The difference between the mutant and the wild type was observed when the stamen primordium began to develop. In the *mst* florets, palea and lemma opened, lodicules were homeotically transformed into palea/lemma-like structures, and stamens were homeotically transformed into carpel-like structures. It looked like multiple stigmas being full of the whole floret. The phenotypic changes of *mst* were very similar to that of B-like mutant *spw1*. Compared with other mutants with pistillate morphologies, the severe *mst* florets showed that the inner three floral organs were completely changed into palea/lemma-like structures. Moreover, the mutant was female sterile. Occasionally, with the changing environment, one or two stamens were fertile. Genetic analysis indicated that the mutant traits were controlled by a single recessive gene.

Key words: rice; flower development; multiple stigmas mutant; floral organ identity

With the development of molecular and genetic biotechnology, significant progress has been made to elucidate the genetic control of floral pattern in the model dicotyledon species *Antirrhinum* and *Arabidopsis*. Genetic analyses of homeotic mutants in the two model plants led to the 'ABC' model [1-3] of flower development extending to the 'ABCDE' model [4-6]. Related studies have extended to monocot plants. In fact, considerable efforts have been made for many years in rice and several rice flower mutants related to carpel development have been reported. Huang and Ma [7] reported two mutants *fon1* and *fon2*, which cause an increase in flower organ number especially in carpel number. In the *spw1* mutant flowers [8], lodicules and stamens are homeotically transformed into palea-like organs and carpels, respectively. *SPW1* gene has been cloned, which is the same gene as *OsMADS16*. Nagasawa et al [8] reported other four mutants *dl-1*, *dl-2*, *dl-sup1* and *dl-sup2*, which are abnormal in leaf development, also specified in carpel identity. The *dl-1* and *dl-2* mutants show no midrib in leaves, along with the enlarged carpel and increase stigma number; whereas in the *dl-sup1* and *dl-sup2* flower mutants, carpels are homeotically changed into stamens completely. The Japanese researchers reported two pistil mutants *mp1* and *mp2*, which have the similar abnormal morphology with increased pistils and two ovaries. The above results show that the discovery and identification of natural flower mutants is necessary in studying the floral development of rice. We found a natural mutant with multiple stigmas and homeotically lodicule-transformed palea/lemma-like organs and stamen-transformed carpels. We thus named it *mst* temporarily.

MATERIALS AND METHODS

Plant materials

A new mutant *mst* with multiple stigmas was found in a rice line 466 (*Oryza sativa* subsp. *indica*). The normal plants in this population, from which the mutant was derived, were used as a wild type control.

The mutant was crossed with the wild phenotype rice, 93-11(indica), Nipponbare (japonica), Shuhui 527 (indica) and more than 20 indica rice varieties, respectively. F_1 progenies of those crosses were all normal phenotypes. The parents, F_1 and F_2 were all planted in the field of the Rice Research Institute, Sichuan Agricultural University, Wenjiang, China.

Morphological analysis

For anatomical observation, floral structures of the mutant and the wild type plant were observed under a stereo microscopy before flowering. Meanwhile, 300 spikelets were investigated and some images of them were captured.

For observation with a scanning electron microscope (SEM), young panicles and flowers were fixed following the method of

Received: 2 July 2008; **Accepted:** 24 November 2008
Corresponding authors: WU Xian-jun (wuxjsau@126.com); ZHANG Ru-quan (ruquanzhang@163.com)

Li et al [9]. For light microscopic observations, rice flowers were fixed in 50% ethanol, 0.9 mol/L glacial acetic acid, and 3.7% formaldehyde at 4°C for 15 h. The specimens were stained with Alcian blue and dehydrated through a graded-ethanol series, infiltrated with xylene, and embedded in paraffin. Sections with the thickness of 12 μm were attached to gelatin-coated glass slides and observed under a light microscope (Olympus AX-80, Tokyo, Japan).

RESULTS

Morphological and anatomical structure of the mutant florets

No obvious difference was observed between the wild type and *mst* before heading. After heading, *mst* began to show abnormal floral organ structures. Compared with the wild type, the mutant florets showed opened lemma and palea and the purple stigma exposed from the first view. As we know, normal rice floret in the wild type consists of one lemma, one palea, two lodicules, six stamens and one pistil (Fig. 1-B). Florets of *mst* plant exhibited severe phenotypic changes, showing alterations in stamens, carpels and lodicules. Lodicules were homeotically transformed into palea/lemma-like structures (Fig. 1-C, E), while stamens were homeotically transformed into carpel-like structures and multiple stigmas were more obvious. Occasionally, one or two stamens were not changed and showed male fertility (Fig. 1-F, G). Moreover, the mutant florets would show more severe morphological changes under low temperature, for example, the inner three floral organs (lodicules, stamens and carpels) were completely changed into palea/lemma-like structures (Fig. 1- D).

The *mst* mutant was pollinated with more than 20 indica rice varieties, but no rice seed was obtained. The result indicates the presence of female sterility in the mutant. In order to investigate the histological variations of the florets, more than 50 mature *mst* and the wild type florets were embedded in paraffin and cross sectioned. These sections showed that stamens were homeotically changed into stigma-like and carpel-like structures (Fig. 2-E, F and G). Most of carpel-like tissues were non-functional and sterile, because the ovaries or the ovules were underdeveloped.

Floral organ development at the early stage

To study the abnormalities in *mst* florets in detail, we examined the phenotypes of the *mst* florets at the early developmental stage under a scanning electron microscope. No difference was observed between the wild type and the mutant before the lemma and palea primordia development. The *mst* florets were distinguishable from those of the wild type when lodicule primordia began to develop. In the whorl 2, lodicules could not be found, whereas ectopic palea- or lemma-like organs developed on the lateral side. In the whorl 3 (Fig. 2-D), either stamen primordia were rarely observed, or too many primordia were irregularly produced in the mutant florets (Fig. 2-B). In the whorl 4, three or more carpel primordia were formed. At last, the whole floret showed multiple stigmas enclosed by palea and lemma (Fig. 2-C).

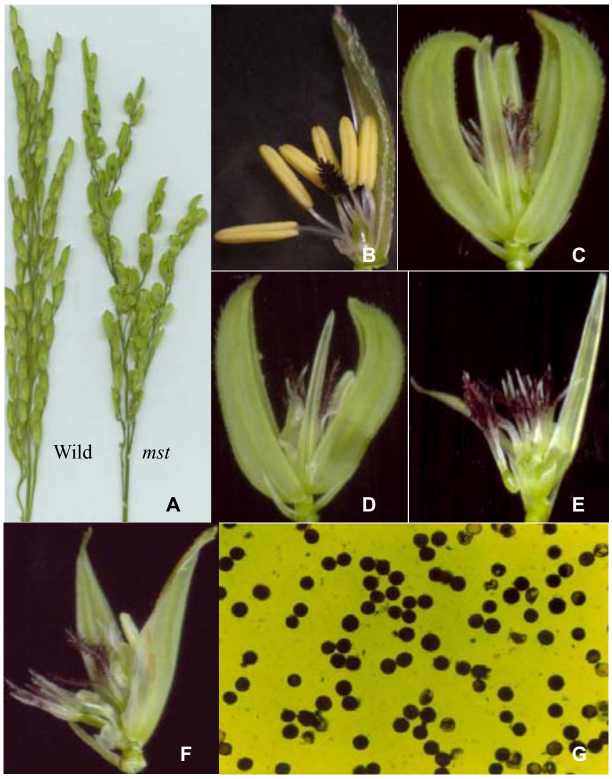

Fig. 1. Morphological and anatomical structure of the florets in *mst* mutants.

A, Spikelets of *mst* (right) and wild type (left); B, The wild type floret with normal floret organs (parts of the lemma and palea are deleted); C and E, The mutant florets with lodicules and stamens homeotically transformed into palea/lemma-like and carpel-like structures, respectively; D, Extra palea/lemma-like structures where the stamens developed; F, One stamen was not changed in *mst* floret; G, I_2-KI staining of the pollen of *mst* mutant.

Genetic analysis

When the *mst* mutant was crossed with the wild type rice varieties, 93-11, Nipponbare and Shuhui 527, respectively, all the F_1 hybrids showed wild-type phenotypes, and all the F_2 populations showed the typical Mendelian segregation (3:1), indicating that the mutant phenotypes resulted from a single recessive gene mutation (Table 1). In this study, the newly found floral identity mutant with multiple stigmas is referred as *mst* temporarily.

DISCUSSION

A lot of progress has been made in the studies on flower development of rice. Many flower mutants have been reported, such as B-like mutant *spw1* [8], E-like mutants *lhs1* [10], *lh* [11]

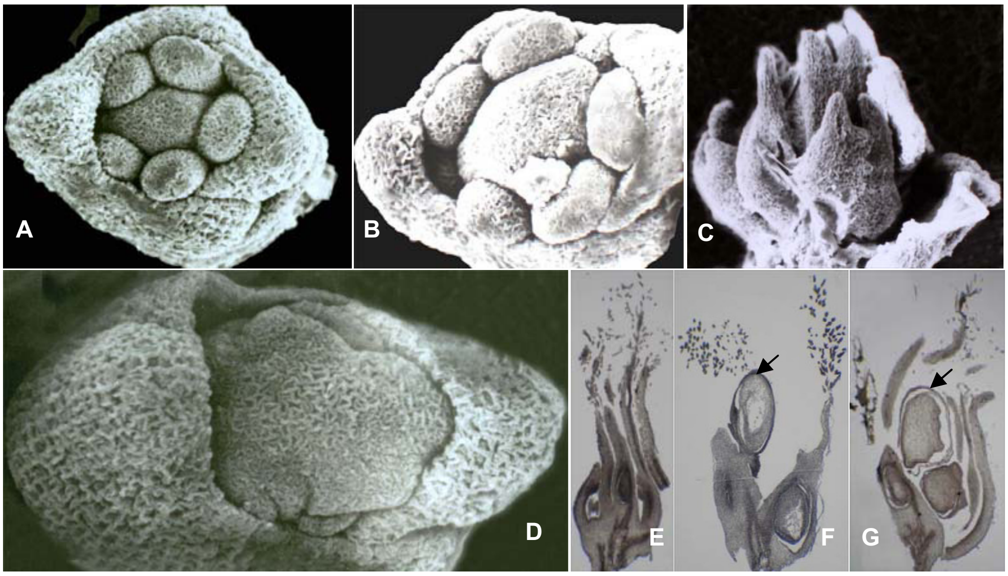

Fig. 2. Scanning electron micrographs of wild type and *mst* florets at the early developmental stage and histological analysis of *mst* florets.
A, Wild type floret at the early developmental stage; B to D, The *mst* florets at the early developmental stage; B, Seven organ primordia are formed at the positions where stamen primordia are formed in the wild type; C, The mutant floret produces a big global carpelloid structure and tips with many stigma papillae at the later stage; D, Emergence of ectopic palea/lemma-like organ primordia in the *mst* mutant; E to G, Multi-ovules in the fused ectopic carpelloids; F and G, Bulged tissue: incomplete transformation structure (arrowed); F, Three sac structures with a completely developed one in the middle.

Table 1. Segregation proportion of F_2 population in each cross.

Cross	Number of total plants	Number of normal plants	Number of mutant plants	Normal Mendelian segregation	χ^2
Wild type×*mst*	423	319	104	3:1	0.039
93-11×*mst*	361	271	90	3:1	0.001
Nipponbare×*mst*	387	293	94	3:1	0.104
Shuhui 527×*mst*	296	227	69	3:1	0.450

and *lrs* [12], flower number mutants *fon1*, *fon2*, *fon3* [13], *fon4* [14] and *fon*(t) [9] , as well as multiple carpel mutants *mp1* and *mp2* [15]. Among these mutants, the B-like mutant *spw1* was the most intensively studied. In the flower of the *spw1* mutant, the odicules and stamens are homeotically transformed into palea/lemma-like organs and carpel-like structure, respectively. We have noted that most of the *mst* florets showed the similar phenotypes with *spw1*. However, under the given environment, some of the *mst* florets showed severe phenotypes that the inner three floral organs were completely transformed into palea/lemma-like structures. The special character is very similar with the mutation for *OsMADS1* gene [17]. However, the *mst* mutant was complete female sterility and partial male sterility. Thus, the *mst* mutant is a novel floral organ identity mutant in rice different from other reported floral mutants.

Based on the above results, it could be concluded that *mst* might be a functional mutant aroused by class B-like gene. But some special florets indicated that they might also belong to E-like mutation. Can severe B-like mutation induce E-like mutation? The results remain unknown. The identification of *mst* mutant may provide insight into the floral structure of rice and other monocots.

The primary genetic analysis showed that the *mst* was controlled by a single gene. It is possible for us to clone this gene in the near future. Further molecular and biochemical studies will shed light on the mechanisms how *mst* regulates these important developmental processes in monocot plants.

ACKNOWLEDGEMENTS

This work was supported by the Program for Changjiang Scholars and Innovative Research Team in University of China (Grant No. IRTO453).

REFERENCES

1 Carpenter R, Coen E S. Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. *Genes Dev*, 1990, **4**: 1483–1493.

2 Schwarz-Sommer Z, Huijser P, Nacken W, Seadler H, Sommer H. Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science*, 1990, **250**: 931–936.

3 Bowman J L, Smyth D R, Meyerowitz E M. Genetic interactions

among floral homeotic genes of *Arabidopsis*. *Development*, 1991, **112**: 1–20.

4 Colombo L, Franken J, Koetje E, van Went J, Dons H J, Angenent G C, van Tunen A J. The petunia MADS-box gene *FBP11* determines ovule identity. *Plant Cell*, 1995, **7**(11): 1859–1868.

5 Pelaz S, Ditta G S, Baumann E, Wisman E, Yanofsky M F. B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature*, 2000, **405**(6783): 200–203.

6 Pelaz S, Gustafson-Brown C, Kohalmi S E, Crosby W L, Yanofsky M F. *APETALA1* and *SEPALLATA3* interact to promote flower development. *Plant J*, 2001, **26**(4): 385–394.

7 Huang H, Ma H. *FON1*, an *Arabidopsis* gene that terminates floral meristem activity and controls flower organ number. *Plant Cell*, 1997, **9**(2): 115–134.

8 Nagasawa N, Miyoshi M, Sano Y, Satoh H, Hirano H, Sakai H, Nagato Y. *SUPERWOMAN1* and *DROOPING LEAF* genes control floral organ identity in rice. *Development*, 2003, **130**(4): 705–718.

9 Li Y, Xu P Z, Zhang H Y, Peng H, Zhang Q F, Wang X D, Wu X J. Characterization and identification of a novel mutant *fon*(t) on floral organ number and floral organ identity in rice. *J Genet & Gen*, 2007, **34**(8): 730–737.

10 Jeon J S, Jang S, Lee S, Nam J, Kim C, Lee S H, Chung Y Y, Kim S R. *Leafy hull sterile1* is a homeotic mutation in a rice MADS box gene affecting rice flower development. *Plant Cell*, 2000, **12**(6): 871–884.

11 Khush G S, Librojo M. Naked seed rice (*nsr*) is allelic to *op* and *lhs*. *Rice Genet Newsl*, 1996, **13**: 102–105.

12 Wu X J, Wang B, Han Z P, Xie Z H, Mou C H, Wang X D. Identification of the mutant of long rice spikelet. *Sci Agric Sin*, 2004, **37**(3): 453–455. (in Chinese with English abstract)

13 Jiang L, Qian Q, Mao L, Zhou Q Y, Zhai W X. Characterization of the rice floral organ number mutant *fon3*. *J Integr Plant Biol*, 2005, **47**(1): 100–106. (in Chinese with English abstract)

14 Chu H, Qian Q, Liang W, Yin C, Tan H, Yao X, Yuan Z, Yang J, Huang H, Luo D, Ma H, Zhang D. The *FLORAL ORGAN NUMBER4* gene encoding a putative ortholog of *Arabidopsis* CLAVATA3 regulates apical meristem size in rice. *Plant Physiol*, 2006, **142**: 1039–1052.

15 http://www.gramene.org/db/mutant/search_mutant?id=GR:0060532.