RESEARCH ARTICLE

Characterization and fine mapping of a female fertility associated gene *Ff1*(*t*) in rice

LEI ZHAO[1], SONG YAN[2], RENLIANG HUANG[2], SHAN ZHU[2], HONGLIANG XIONG[2], ZHIQIN PENG[2,3], QINGYOU ZHOU[4], YINGJIN HUANG[1,3]* and XIANHUA SHEN[2]*

[1]*Key Laboratory of Agriculture Responding to Climate Change, Jiangxi Agricultural University, Nanchang 30045, People's Republic of China*
[2]*Rice Research Institute, Jiangxi Academy of Agricultural Sciences, Rice National Engineering Laboratory (Nanchang); Jiangxi Provincial Key Laboratory for Physiology and Genetics of Rice, Nanchang 330200, People's Republic of China*
[3]*Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education, Jiangxi Agricultural University, Nanchang 330045, People's Republic of China*
[4]*Agriculture Bureau of Wuning Country 332300, Jiangxi, People's Republic of China*

Abstract

Female-sterile line can be used as a pollinator which has a great potential for hybrid seeds production. However, reports on female fertility are fewer than male fertility. Here, we characterized a recessive female fertility weakening mutant *ff1(t)* from rice. The spikelet fertility was seriously affected in the mutant. Reciprocal crosses and pollen vitality assay suggest that the decreased fertility was caused by the defective female gametophytes. Further investigation indicated that the mutant ovary development was inhibited before fertilization and failed swelling after flowering. Genetic analysis and fine mapping showed that the mutant was controlled by a single recessive gene, residing on a 16.8 kb region on the long arm of chromosome 1. The gene annotation indicated that there was only one putative gene encoding lysine decarboxylase-like protein in this region, which was allelic to *LOG*. Further, the sequence analysis was carried out and a substitution at the splice site of intron 2 / exon 3 was revealed in *ff1(t)* mutant, resulting in the change of reading frame. The finding of novel allele of *LOG* locus will facilitate the understanding of the mechanisms of female gametophyte development.

[Zhao L., Yan S., Huang R., Zhu S., Xiong H., Peng Z., Zhou Q., Huang Y. and Shen X. 2015 Characterization and fine mapping of a female fertility associated gene *Ff1*(*t*) in rice. *J. Genet.* **94**, 67–73]

Introduction

Fertility is an important agronomic trait which is closely related to yield in rice. In recent years, a large scale of rice male organs and male-sterile mutants have been reported and successfully applied to heterosis breedings (Ling 1996). However, little effort has been devoted to female sterility (Dou *et al.* 2009). Indeed, the female-sterile line, reflecting the evolution of a plant species from gynodioecy to dioecy can be used as a pollinator in hybrid seed production system in which both parents are sown in mixtures to generate hybrid seeds (Daskalov and Mihailov 1988). Moreover, the system can also ensure permanent flowering and abundant pollen quality that leads to increased yield of hybrid seeds without additional labour for removing male plants till maturity (Daskalov and Mihailov 1988).

Many female sterile materials have been found in various plants (Chen and Walsh 2009), which is mainly caused by the abnormal development of female gametophyte, including the development of ovule, formation of the embryo sac and growth of the embryo (Li *et al.* 2006). As of now, only a few genes such as *OsRPA1a*, *LONELY GUY* (*LOG*), *OsDEES1, OsAPC6, RAD51C* and *OsMSH5* have been reported to be involved in the development of female gametophyte or female fertility in rice. *OsRPA1a* gene plays an essential role in meiotic and somatic DNA repair. In *OsRPA1a* mutant, no embryo sac was formed in female meiocytes and abnormal chromosomal fragmentation occurred in male meiocytes after anaphase I, thereby leading to completely sterile female gametophytes and partially fertile male gametophytes (Chang *et al.* 2009). The *LOG* gene is required to maintain

*For correspondence. E-mail: Xianhua Shen, shen_xh20000913@126.com; Yingjin Huang, yihuang_cn@126.com.
Lei Zhao and Song Yan contributed equally to this work.

Keywords. rice; female fertility weakening; ovary development; molecular mapping.

meristem activity and its loss of function causes premature termination of the shoot meristem (Kurakawa *et al.* 2007). The ovule formation is abolished inside the normally developing carpel in *log-3*, which is regarded as a female-sterile mutant (Yamaki *et al.* 2011). *OsDEES1* is involved in the regulation of early embryo sac development. Knockdown of *OsDEES1* expression disturbed female gametophyte formation, resulting in a degenerated embryo sac and defective seed formation (Wang *et al.* 2012). Abnormal endosperm development caused high female sterility in *OsAPC6* mutant (Awasthi *et al.* 2012). *RAD51C* gene is required for the meiosis of both female and male gametocytes and DNA repair of somatic cells. Knockout of *RAD51C* resulted in both female and male sterility in rice (Kou *et al.* 2012). A similar role of *OsMSH5* was found by Luo *et al.* (2013).

In addition, the ABCDE model of plant flower morphological formation proposed that class D genes specify, ovule identity. Two D-class genes, *OsMADS13* and *OsMADS21*, have been reported in rice. While *OsMADS13* controls the ovule identity, *OsMADS21* has lost its ability to determine ovule identity (Dreni *et al.* 2007). Moreover, the E-class gene *OsMADS6* defines carpel/ovule development by interacting with *OsMADS13* (Li *et al.* 2011). It is shown that the mechanism of female gametophyte development is very complex. These above findings present valuable information on the development of female reproductive organ and provide a clue for developing female sterile materials. Nevertheless, it is still a long way to apply female sterility to rice breeding. The main issue is that the female sterile materials reported cannot be directly utilized unless the fertility is regulated by day length or environmental temperature changes like the photoperiod-thermo-sensitive genic male-sterile lines in rice. Thus, it is important to create more female fertility defective mutants, and subsequently characterize and clone these genes responsible for the female organ development in rice.

In this study, we identified a rice female fertility weakening mutant derived from spontaneous mutation. The mutant showed ultra-low seed setting percentage (<1%). Reciprocal crosses and pollen vitality assay experiment suggested that the defective female gametophyte caused female sterility. The genetic analysis showed that the mutant was controlled by a recessive gene, which was temporarily named *ff1(t)*. Here, we report the fine mapping of the *Ff1(t)* gene through map-based cloning strategy.

Materials and methods

Rice materials

The female fertility weakening mutant *ff1(t)* was identified from the natural variation materials by screening the high backcross generations between an *indica* cultivar rice Jin23B (recurrent parent, maintainer line, *Oryza sativa* ssp. *indica*) and Dongxiang wild rice (*O. rufipogon* Griff.). Reciprocal crosses were made between *ffw1(t)* and two *indica* cultivars, Jin23B and 93-11, respectively in summer, 2012, Nanchang, China. F_1 generation was planted in Hainan province of China, and all the seeds were harvested and planted in Nanchang the following year.

Morphology analysis

To eliminate the influence of photoperiod and temperature, sowing was conducted in stages and seasons for the experiment. Morphologic characters of the mutant, the wild type and F_1 generation were observed during the whole growing period. Plant height, panicle length, total grain number per plant, and seed-setting rate were investigated in *ff1(t)* and its wild-type plant (Jin23B) after maturity. The pollen fertility was observed under optical microscope through the 1% I_2–KI staining method. Flower organ investigation and photography were carried out with the Motic BA210 (Maike Aodi Ltd, Xiamen, China) series biological microscope and anatomical lens.

Coarse mapping of ffw1(t)

The mutant was crossed with Sasanishiki (*japonica*) to generate F_2 population for molecular mapping. The mapping population was composed of 46 female fertility weakening plants from the F_2 population. A total of 165 simple sequence repeat (SSR) markers and 135 insertion–deletion (InDel) markers evenly distributed on the 12 rice chromosomes were used to determine the approximate map position of the *ff1(t)* locus on rice chromosome. The genetic linkage map between the *ff1(t)* locus and molecular markers was constructed using Map-Maker 3.0 (Cambridge, USA) on the basis of the results of SSR/InDel marker analysis. The polymerase chain reaction (PCR) programme included 4 min at 94°C, followed by 32 cycles at 94°C for 20, 56°C for 20 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. PCR products were separated on 4% agarose gel or 6% polyacrylamide gel stained with Gel Red or silver, respectively.

Fine mapping of ff1(t)

Based on the results of primary mapping, BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search the diversities of the nucleotide sequences between Nipponbare and 93-11. InDel markers were then developed using the Primer Premier 5.0 software (table 1). The recombinant plants detected by coarse mapping markers (Os1_94.5 and Os1_95.7) were used for gene fine mapping with the In/del markers. The target region was finally narrowed based on the number of recombinant plants.

Sequence analysis

Candidate gene annotation was obtained from the rice GRAMENE database (http://www.gramene.org/) and the Rice Annotation Project Database (RAP-DB) (http://rapdb.dna.affrc.go.jp/cgi-bin/gbrowse/IRGSP40/). In the target region, the entire 16.8 kb genomic DNA of *ff1(t)* mutant and its corresponding wild-type plant were amplified. The

Table 1. Primers used in this study.

Marker	Physical position (bp)	Forward primers (5′–3′)	Reverse primers (5′–3′)
RM3598	7,348,862–7,348,967	CGACTTCTCCTCCATTTTCG	CAAATTCACGCAGTGACCAC
RM8133	9,390,211–9,390,430	AAAACTGACTGTTTGTTTAAATGAAAT	GTTACTGCTGTAATGTGAATTGCT
STS1-10	12,281,142–12,281,353	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
STS1-11	14,796,344–14,796,517	ATGCTCTGTTTGGCTTATTTCACG	ATACAGTTCGTCCGCTACAGGC
Os1_87.4	22,117,547–22,117,709	AGATCCATGTAATCATTCGG	ATGTGCTAGTCCATCACTCC
Os1_94.5	22,764,784–22,764,929	AAAGAACTAGCGAGTCAACG	TCACATTTTGACTCTATGTTG
Os1_B131	22,931,856–22,932,054	TCCAAATACAAAAAGAGCGCA	TCGGAGCGTCAAAAGGTAG
Os1_C7.0	22,940,535–22,940,725	CACGCTTCTAAGATGGATGA	AGGTCGCAAAAGTATCATGT
Os1_C21	22,954,511–22,954,729	GTGAAAACCTATCACAAAGTCA	ATTTGGACGTGCCGTT
Os1_C23	22,957,270–22,957,484	GGATAGGCGCATTAGATTCA	CTGTTTTTAATGTGGCCGTG
Os1_C37	22,971,143–22,971,322	GGTATATCAGTTCCGTCGTC	GGACAAATTAACCTGCCAAG
Os1_22.98	22,997,756–22,997,951	AAATCTCTCCCACCACCA	GATTGGTTCCTTGGCTTC
Os1_95.7	23,106,078–23,106,232	TGATGAACAGCACAGAAAGA	ACACACCGAGAAACTGAAAT
Os1_23.5	23,183,407–23,183,549	TTTTGGGTAAGTTCACTCGT	ATGAGAGCATTTGGATGAAT
RM11307	24,257,472–24,257,619	AAAGCTCTGCAATCTTCTCTCC	GAATACGACATCAGAACAGTGC
RM7124	24,389,784–24,389,950	ATCGGTTAAGCTGAAACCCC	AACCGTGAACACACACATGC
STS1-15	25,121,260–25,121,446	CATCTCAAAGTCGTGAGTGCAAC	ATACGTGACCCCTCCGAGCT
Os01g40630sq-1		GGCACGAGAAGAAGGTATCG	ATCTCTCTCCTCCAATCGCT
Os01g40630sq-2		GCAATGCTGTTGGATAGG	TGCATGCTTGAATTGTTG

PCR products were subjected for direct sequencing, and the sequence-alignment was performed using the software Vector NTI 10.0 (Invitrogen, California, USA).

Results

The performance of ff1(t) mutant

The *ff1(t)* mutant showed more tillers, fewer grain numbers, especially lower seed sets (0.8±0.1%) compared with the wild-type Jin23B (figure 1, a & b; table 2). In addition, about one quarter of the spikelets per panicle of the mutant were defective as paleas were degenerated (figure 1c–e). One to two stamens were lost in the defective florets (figure 1f). Majority of spikelets were empty and stayed green until maturity in *ff1(t)* mutant (figure 1, a&b). To determine the cause of ultra-low seed setting rate of *ff1(t)* and whether the male or female reproductive organs had functional defects in the mutant plants, we checked the mature pollen fertility by 1% I_2–KI staining method. The microscopy assay showed that there was no difference between the mutant and wild-type. The pollen grains shape, staining fraction and

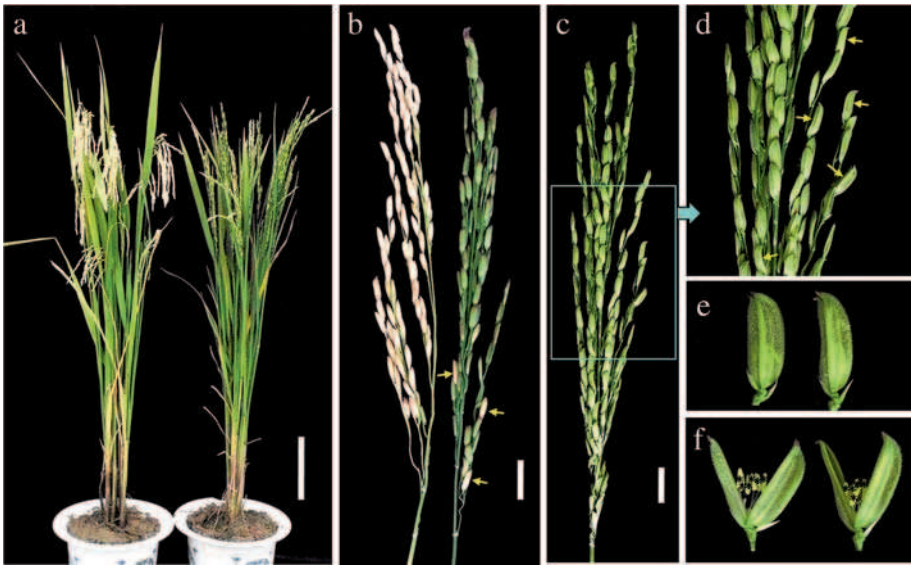

Figure 1. Phenotypes of wild-type jin23B and *ff1(t)* mutant. (a) Wild-type and *ff1(t)* mutant plants. Bar = 10 cm. (b) Panicles of wild type and *ff1(t)* mutant at the mature stage. The arrows indicate normal seeds. Bar = 2 cm. (c) Panicle of *ff1(t)* mutant at the flowering stage. Bar = 2 cm. (d) Magnified image of the rectangle in (c). The arrows indicate spikelets with degenerated paleas. (e) Spikelet of wild type (left) and spikelet with a degenerated palea from *ff1(t)* mutant (right). (f) Spikelet of wild type and spikelet with a degenerated palea from *ff1(t)* mutant. Asterisk in (f) indicates the stamen.

Table 2. Comparison of the main agronomic characters between *ff1(t)* and wild-type Jin23B.

Materials	Plant height (cm)	Tiller number per plant	Panicle length (cm)	Total grains per panicle	Seed setting rate (%)
Mutant (*ff1(t)*)	82.4±3.5	18.2±2.1**	20.2±1.5	78.8±5.0**	0.8±0.1**
Wild type (Jin23B)	83±4.2	12.3±3.2	21.3±2.1	115.4±4.5	78.5±2.4

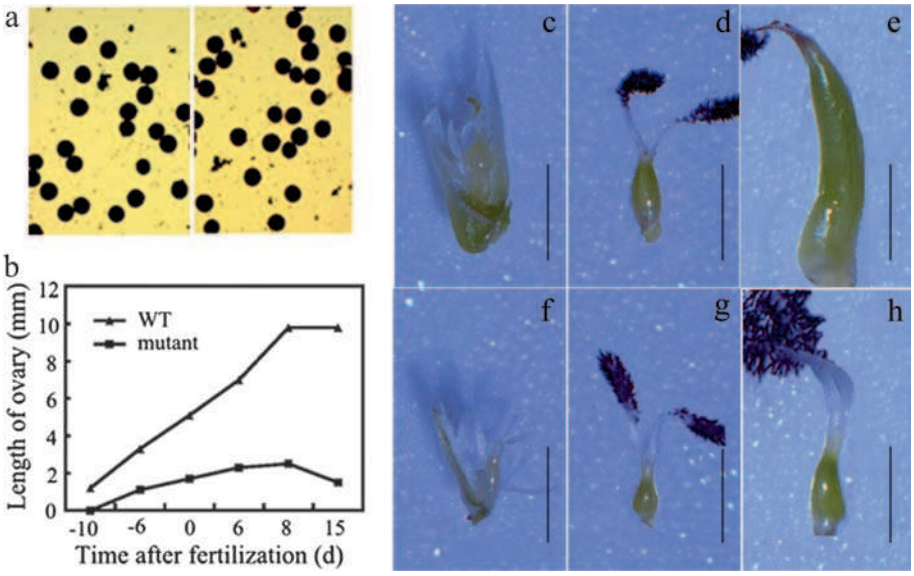

Figure 2. Investigation of the pollen fertility and floral organ. (a) Pollen grain stainability of the wild type (left) and *ff1(t)* mutant (right). (b) Characterization of the ovary swelling rate of wild type and *ff1(t)* mutant. The time course of ovary length is shown. The data are presented as mean ±SD ($n > 10$). (c–h) Ovary development comparision between wild type (above) and *ff1(t)* mutant (below) at the booting stage (c and f), heading stage (d and g) and filling stage (e and h); scale bars, 2 mm.

intensity of *ff1(t)* were normal (figure 2a). Subsequently, we performed artificial pollination experiments between *ff1(t)* mutant and wild-type plants, Jin23B and 93-11. The reciprocal crosses showed that seed sets were very low while using the *ff1(t)* mutant as female parent. In contrast, the seed sets were normal when wild-types as female parents pollinated with the mutant pollens were used. It is indicated that the female gametophyte defect was the main cause for fertility weakening which led to low-seed sets in the *ff1(t)* mutant.

We also observed the development process of ovary to investigate the female abnormalities of *ff1(t)* spikelets. The ovary of *ff1(t)* developed slowly than that of the wild type before and after fertilization (figure 2b). At the booting stage, the ovary was clearly visible in the wild type, whereas the mutant ovary was too small to be seen (figure 2, c&f). At the heading stage, the ovaries of both the mutant and wild type were swollen. However, the size of ovary was smaller in mutant (figure 2, d&g). After fertilization, the wild-type ovary developed rapidly and showed whole endosperm appearance within five days, while the morphological change of *ff1(t)* was not obvious (figure 2, e&h). Moreover, the ovary of *ff1(t)* failed to swell and degenerated gradually at the filling stage (figure 2b). These results suggested that some physiological defects might exist in the female gametophyte which inhibited the development of *ff1(t)* ovary, and subsequently resulted in significant decrease of female fertility.

Genetic analysis of ff1(t)

To study the inheritance of phenotypic trait in *ff1(t)*, crosses were made between *ff1(t)* and the two wild types (Jin23B and 93-11), respectively. All F_1 plants from the cross displayed wild-type phenotypes. In F_2 populations, the phenotypes of all progenies from each population were investigated (table 3). The mutant phenotype was identified by two characteristics, abnormal spikelet with degenerated paleas and ultra-low seed sets. Table 3 shows that the segregation of

Table 3. Genetic analysis of *ff1(t)* in F_2 populations.

F_2 population	Population	Normal plants	*ff1(t)* plants	χ^2 (3:1)
93-11/*ff1(t)*	1200	922	278	2.1511
Jin23B/*ff1(t)*	470	366	104	2.07

$P<0.05$.

Figure 3. Plants segregated from the F_2 population of *ff1(t)*/Sasanishiki. Wild type (left), mutant type (right).

normal and mutant plants in the two F_2 population was in accordance with the expected 3:1 ratio. These results suggest that *ff1(t)* is controlled by a single recessive nuclear gene.

In F_2 progeny of 93-11/*ff1(t)* and Sasanishiki/*ff1(t)*, the phenotypes of the recessive individuals were found to be more severe than that of *ffw1(t)* mutant and exhibited degenerated paleas in almost all spikelets (figure 3), implying that the extent of genetic background difference between parents could affect the *ff1(t)* phenotype.

Coarse mapping of ff1(t)

Forty six mutant plants from F_2 population of Sasanishiki/*ff1(t)* were used for coarse mapping *ff1(t)*. A total of 165 SSR markers and 135 InDel markers were employed to detect the polymorphism between the two parents, of which, 138 markers were polymorphic. Subsequently, the polymorphic markers were further used to determine the approximate map position of the *ff1(t)* locus on rice chromosome. Finally, two markers RM3598 and RM1297, located on chromosome 1, were associated with the tagged traits. To confirm this, four polymorphic markers, STS1-11, Os1_94.5, Os1_95.7 and RM11307, between RM3598 and RM1297, were used for linkage analysis. The results showed that there were 15 single recombinations in STS1-11, five single recombinations in

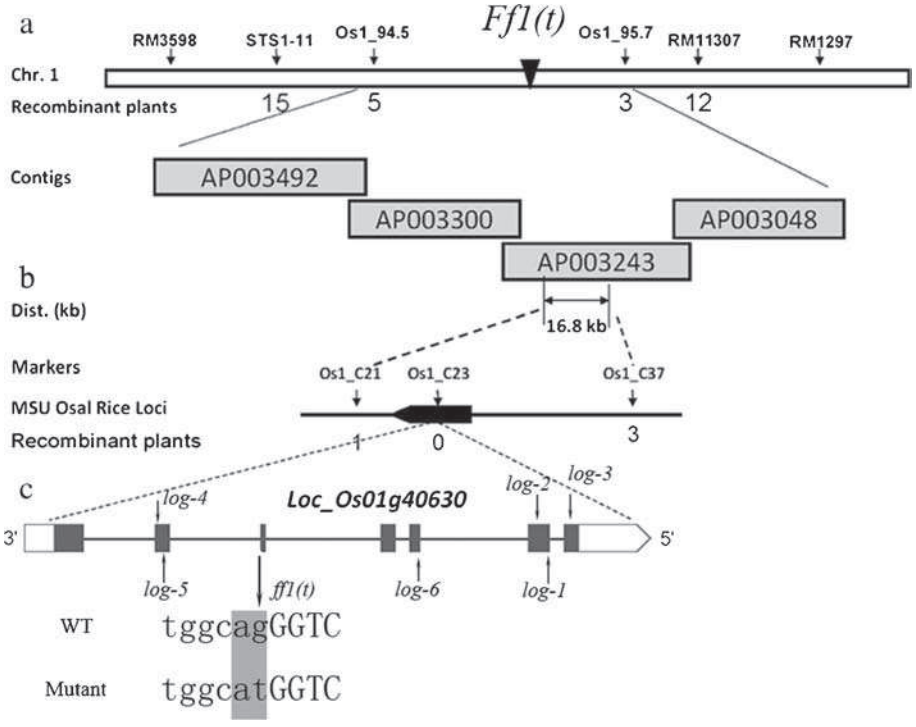

Figure 4. Positional cloning of *Ff1(t)*. (a) Coarse mapping of the *Ff1(t)* locus. (b) Fine mapping of the *Ff1(t)* locus. The region of the *Ff1(t)* locus was narrowed within the 16.8 kb region on chromosome 1 (chr. 1), which contained only one predicted gene. (c) Exon/intron structure of *ff1(t)*. The *ff1(t)* gene contains seven exons (black box) and six introns. A base substitution occurs (ag→at) at the splice site of intron 2 / exon 3. Mutation sites of six mutant alleles of the *LOG* gene are shown in the figure.

Os1_94.5, three single recombinations in Os1_95.7 and 12 single recombinations in RM11307. Hence, the *Ff1(t)* gene was preliminarily mapped between Os1_94.5 and Os1_95.7 with the genetic distance of 5.4 cM and 3.3 cM, respectively, on the long arm of chromosome 1 (figure 4a).

Fine mapping of ff1(t)

To further determine the locus of *ff1(t)* gene on chromosome 1, 30 more InDel markers between Os1_94.5 and Os1_95.7 were developed, of which, 12 were polymorphic between the two parents. Linkage analysis using 929 recessive individuals from F_2 (Sasanishiki/*ff1(t)*) indicated that Os1_C23 was cosegregated with *ff1(t)*, while the markers Os1_C21 and Os1_C37 were found to be tightly linked to *ff1(t)*, only 1 and 3 recombinants were found. Therefore, *ff1(t)* was narrowed down to a 16.8 kb genomic region between the InDel markers Os1_C21 and Os1_C37 on AP003243. The physical map encompassing *Ff1(t)* locus was then constructed (figure 4b).

The ff1(t) gene encoding lysine decarboxylase-like protein

Within the 16.8 kb region, there is only one annotated gene (*LOC_Oso1g40630*) with seven exons and six introns, a 729 bp coding sequence, encoding 242 amino acids. The gene encodes a lysine decarboxylase-like protein according to the gene annotation information provided by the Rice Genome Annotation Project (figure 4c). Further, we sequenced the gene in *ff1(t)* mutant and wild type. The results showed that a substitution of G (wild type) with T at the splice site of intron 2 / exon 3 occurred in *ff1(t)* (figure 4c), thus, this mutation was predicted to alter the reading frame resulting in delayed termination.

Discussion

The female fertility weakening or sterility can be caused by various defects in female organs which resulted in decreased rice yield. In this study, we characterized a female fertility weakening mutant *ff1(t)* which caused an ultra-low seed set. The reciprocal crosses and pollen vitality assay showed that the poor fertility of the mutant was caused by defective female organs. Further investigation revealed that the cause for the sterility of *ff1(t)* lay in the defect of ovary expansion. In rice, pollination occurs at the same time as flower opening and double fertilization takes place several hours after pollination (Satoh and Omura 1979) followed by rapid cell division and morphological enlarging in 1–3 days after fertilization (Itoh *et al.* 2005). However, these changes were hardly observed in *ff1(t)* mutant. In addition, *ff1(t)* showed reduced grain numbers and increased tillers, and some florets with degenerated paleas compared to the wild type, Jin23B. This implies that the *Ff1(t)* gene has pleiotropic effects on agronomic traits.

Molecular mapping showed that *ff1(t)* was located at 16.8 kb region on the long arm of chromosome 1. Within the restricted region, there was only one annotated gene, *LOC_Oso1g40630*, encoding a lysine decarboxylase-like (LDC-like) protein. LDC is the key enzyme functioning in the formation of cadaverine by the decarboxylation of lysine. Moreover, cadaverine, putrescine and other aliphatic polyamines are involved in a number of growth and developmental processes (Bagni and Tassoni 2001). Recently, a rice lysine decarboxylase-like 1 (*OsLDC-like 1*) gene, a homologous gene of *LOC_Oso1g40630* was identified by the analysis of T-DNA insertion mutants under H_2O_2 induced oxidative stress. Mutation of *OsLDC-like 1* was shown conferring an oxidative stress-tolerant phenotype. However, *OsLDC-like 1* did not exhibit lysine decarboxylase activity (Jang *et al*. 2012). Another LDC-like gene, *MC126*, isolated from tobacco, worked as a putative catalyst in pyrimidine alkaloid biosynthesis, including synthesis of nicotine, nornicotine, anabasine, and anatabine (Häkkinen *et al.* 2007). In addition, LOG, annotated as LDC, was verified to be required to maintain meristem activity. Also, LOG did not exhibit lysine decarboxylase activity (Kurakawa *et al.* 2007).

Rice ovary consists of carpel and its enclosed ovule (Itoh *et al.* 2005). In the development progress of normal rice florets, carpel is primarily initiated by the floral meristem (FM). With the rapid enlarging of carpel, palea side of the FM was exhausted and leads to ovule primordium enclosed (Yamaki *et al.* 2011). The weak mutant *log-3* showed female sterile phenotype. Histological analysis demonstrated that the *log-3* volume of the floral meristem dome was normally maintained before carpel protrusion, but was decreased by the smaller number of cells. Thus the ovule founder region did not differentiate any organs, although the carpel developed normally in *log-3* (Yamaki *et al.* 2011). In this study, the ovary of *ff1(t)* was also found to be defective. It is suggested that *Ff1(t)*/*LOG* was necessary for ovary development especially for the ovule formation.

The molecular analysis showed that a base substitution occurred at the splice site of intron 2 / exon 3 of *ff1(t)*, which was different from the *log* mutations, indicating that *ff1(t)* is a novel allelic variant of *LOG* gene (figure 4c). It was demonstrated that cytokinins (CKs) play a vital function in controlling meristem activity, and fine-tuning of concentrations and the spatial distribution of bioactive CKs by *LOG* enzyme will lead to different phenotypes (Kurakawa *et al.* 2007). This findings may explain the phenotype differences between *ff1(t)* and *log* mutants. For example, the flowers of *log* mutants often consisted of only one stamen but no pistil (Kurakawa *et al.* 2007), whereas the *ff1(t)* exibited a moderate phenotype of floret in that most of the florets had six stamens, and all pistils had normal appearances showing a potential application in breeding.

Female sterile materials could be used as restorer lines and has a great potential to break the yield bottleneck of hybrid seeds production (Daskalov and Mihailov 1988). However, little progress has been made since the system was

proposed, the lack of usable female sterile resources is one of the main reasons. Although a few female fertility associated materials have been reported, most of them have negative effects which largely limited their application, such as partially female fertile (Awasthi *et al.* 2012) or male sterile (Chang *et al.* 2009; Kou *et al.* 2012; Luo *et al.* 2013). In addition, the seed production of female sterile restorer lines is another crucial bottleneck which restricts the application of this system. To solve this problem, it is necessary to explore or create more germplasm resourses that show female fertility conversion under permissive conditions similar to photoperiod-thermo-sensitive genic male-sterile lines. Moreover, the female sterile restorer lines could be reproduced by means of the vegetative propagation technique via tissue culture, which relies on the technical progress and cost reduction.

In conclusion, we have characterized a rice female fertility weakening mutant and fine mapped the controlling gene *ff1(t)*, allelic to *LOG*. It plays an important role in the development of female gametophyte. Loss of *ff1(t)* function could cause female sterility. Further study is necessary to understand the regulation network of *ff1(t)*/*LOG* underlying the female gametophyte development.

Acknowledgements

This study was financially supported by the Key Program for Science and Technology of Jiangxi Province, China (grant no. 20133ACF60001).

References

Awasthi A., Paul P., Kumar S., Verma S. K., Prasad R. and Dhaliwal H. S. 2012 Abnormal endosperm development causes female sterility in rice insertional mutant *OsAPC6*. *Plant Sci.* **183**, 167–174.

Bagni N. and Tassoni A. 2001 Biosynthesis, oxidation and conjugation of aliphatic polyamines inhigher plants. *Amino Acids* **20**, 301–317.

Chang Y., Gong L., Yuan W., Li X., Chen G., Zhang Q. and Wu C. 2009 Replication protein A (*RPA1a*) is required for meiotic and somatic DNA repair but is dispensable for DNA replication and homologous recombination in rice. *Plant Physiol.* **151**, 2162–2173.

Chen J. and Walsh B. 2009 Method for the mapping of a female partial-sterile locus on a molecular marker linkage map. *Theor. Appl. Genet.* **119**, 1085–1091.

Daskalov S. and Mihailov L. 1988 A new method for hybrid seed production based on cytoplasmic male sterility combined with a lethal gene and a female sterile pollinator in *Capsicum annuum* L. *Theor. Appl. Genet.* **76**, 530–532.

Dou B., Hou B., Xu H., Lou X., Chi X., Yang J., Wang F., Ni Z. and Sun Q. 2009 Efficient mapping of a female sterile gene in wheat (*Triticum aestivum* L.) *Genet. Res.* **91**, 337–343.

Dreni L., Jacchia S., Fornara F., Fornari M., Ouwerkerk P. B., An G., Colombo L. and Kater M. M. 2007 The D-lineage MADS-box gene *OsMADS13* controls ovule identity in rice. *Plant J.* **52**, 690–699.

Gressel J. and Al-Ahmad H. 2005 Assessing and managing biological risks of plants used for bioremediation, including risks of transgene flow. *Z. Naturforsch C.* **60**, 154–165.

Häkkinen S. T., Tilleman S., Świątek A., De S. V., Rischer H., Vanhoutte I., Van-Onckelen H., Hilson P., Inzé D. and Oksman-Caldentey K. M. 2007 Functional characterisation of genes involved in pyridine alkaloid biosynthesis in tobacco. *Phytochemistry* **68**, 2773–2785.

Itoh J., Nonomura K., Ikeda K., Yamaki S., Inukai Y., Yamagishi H., Kitano H. and Nagato Y. 2005 Rice plant development: from zygote to spikelet. *Plant Cell Physiol.* **46**, 23–47.

Jang S. J., Wi S. J., Choi Y. J., An G. and Park K. Y. 2012 Increased polyamine biosynthesis enhances stress tolerance by preventing the accumulation of reactive oxygen species: T-DNA mutational analysis of Oryza sativa lysine decarboxylase-like protein 1. *Mol. Cells* **34**, 251–262.

Kou Y. J., Chang Y. X., Li X. H., Xiao J. H. and Wang S. H. 2012 The rice *RAD51C* gene is required for the meiosis of both female and male gametocytes and the DNA repair of somatic cells. *J. Exp. Bot.* **63**, 5323–5335.

Kurakawa T., Ueda N., Maekawa M., Kobayashi K., Kojima M., Nagato Y., Sakakibara H. and Kyozuka J. 2007 Direct control of shoot meristem activity by a cytokinin-activating enzyme. *Nature* **445**, 652–655.

Li H., Liang W., Hu Y., Zhu L., Yin C., Xu J., Dreni L., Kater M. M. and Zhang D. 2011 Rice MADS6 interacts with the floral homeotic genes *SUPERWOMAN1*, *MADS3*, *MADS58*, *MADS13*, and *DROOPING LEAF* in specifying floral organ identities and meristem fate. *Plant Cell* **23**, 2536–2552.

Ling D. H. 1996 In vitro production ofmale sterile rice plants. In *Somaclonal variation in crop improvement II* pp. 20–45. Springer Publishers, Guangzhou, China.

Li S. C., Yang L., Deng Q. M., Wang S. Q., Wu F. Q. and Li P. 2006 Phenotypic characterization of a female sterile mutant in rice. *J. Integr. Plant Biol.* **48**, 307–314.

Luo Q., Tang D., Wang M., Luo W., Zhang L., Qin B. *et al.* 2013 The role of OsMSH5 in crossover formation during rice meiosis. *Mol. Plant.* **6**, 729–742.

Satoh H. and Omura T. 1979 Induction of mutation by the treatment of fertilized egg cell with N-methyl-N-nitrosourea in rice [chicken]. *J. Fac. Agr., Kyushu Univ.* **24**, 165–174.

Velkov V. V., Medvinsky A. B., Sokolov M. S. and Marchenko A. I. 2005 Will transgenic plants adversely affect the environment? *J. Biosci.* **30**, 515–548.

Wang N., Huang H. J., Ren S. T., Li J. J., Sun Y., Sun D. Y. and Zhang S. Q. 2012 The rice wall-associated receptor-like kinase gene *OsDEES1* plays a role in female gametophyte development. *Plant Physiol.* **160**, 696–707.

Yadav S. R., Khanday I., Majhi B. B., Veluthambi K. and Vijayraghavan U. 2011 Auxin-responsive OsMGH3, a common downstream target of OsMADS1 and OsMADS6, controls rice floret fertility. *Plant Cell Physiol.* **52**, 2123–2135.

Yamaki S., Nagato Y., Kurata N. and Nonomura K. 2011 Ovule is a lateral organ finally differentiated from the terminating floral meristem in rice. *Dev. Biol.* **351**, 208–216.

Received 30 June 2014, in revised form 13 October 2014; accepted 17 October 2014
Unedited version published online: 20 October 2014
Final version published online: 12 March 2015