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Mapping and allelic sequencing of a long sterile lemma trait in rice

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ABSTRACT: Some outer floral organs are unique in gramineous plants, like the sterile lemma and rudimentary glume in rice. However, their development mechanisms are still poorly understood. In this study, we used 4 mutants with long sterile lemma (LSL), named JF11, JF12, JF13 and JNY-7, to be crossed with Aijiaonante (AJNT) and Nipponbare (NIP), respectively. Genetic analysis indicated that LSL trait exhibited recessive heredity and was controlled by a common allele named *sl-1(t)*. Using the method of bulk segregant analysis and linkage analysis between SSR markers and LSL trait based on F_2 populations, the *sl-1(t)* gene was located at the interval between RM20903 and RM20948 on chromosome 7. The interval harbors a known *G1* gene, which regulates the sterile lemma trait. The findings of allelic sequencing showed an 11-base deletion in gene *G1* happened in the mutants of JF11, JF12 and JF13, which led to a frame-shifting mutation, whereas the mutant of JNY-7 had a base substitution that caused the change of the amino acid residue. Eight substitutions in the ORF and 10 in the upstream region from –1 to –824 were found between *Indica* and *Japonica* rice by DNA sequence analysis, but those polymorphisms have no effect on the gene function. In conclusion, we fine mapped the LSL gene, *sl-1(t)*, and found 2 kinds of mutant alleles conferring the gene function and the DNA polymorphisms of *G1* between *Indica* and *Japonica* rice.

Key words: *Oryza sativa* L., gene mapping, ALOG domain.

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INTRODUCTION

A flower is the vital reproductive organ determining quality of fruits and seed yiled in plants. Since the 1980s, the mechanism of floral organ development has become one of hot spots in developmental biology by means of the floral organ mutants of model plants. The ABC model of plant flower development was put forward to elaborate the molecular mechanism of floral organ identity in eudicots based on their previous findings (Carpenter and Coen 1990; Bowman et al. 1991; Coen and Meyerowitz 1991; Coen and Carpenter 1993). Then, *FBP7* and *FBP11* genes, which regulated the identity and development of an ovule in petunia, were cloned in 1995 (Angenent et al. 1995; Colombo et al. 1995) and designated to an additional class D MADS box gene. Furthermore, the *SEP1/2/3* genes were found as necessary for the development of petals, stamens, and carpels in *Arabidopsis* and were identified as a new class gene (E-class gene) of the floral quartet model (Pelaz et al. 2000; Honma and Goto 2001; Galimba et al. 2012). The findings of D-class and E-class enriched the ABC model and made the classical one extending to the ABCDE model.

Moreover, many researchers found that the genes among the Classes A, B, and C are, relatively, conserved between grasses and edicts based on comparison with the homology of the MADS-box genes and analysis of the transgenic plants (Schmidt et al. 1993; Chung et al. 1995; Kang et al. 1995; Mena et al. 1996; Ambrose et al. 2000). It is known that rice is a typical model plant of the grasses and the monocots in general and has 5 kinds of genes showing similar function like the ones in Classes A, B, C, D and E. So far, many genes related to floral organ development in rice were identified and cloned, with *OsMADS15*, *OsMADS14* and *OsMADS18* belonging to Class A (Kyozuka et al. 2000; Lim et al. 2000; Fornara et al. 2004), *OsMADS2*, *OsMADS4, OsMADS16*, and *OsMADS32*, to Class B (Chung et al. 1995; Kang et al. 1998; Moon et al. 1999; Kyozuka et al. 2000; Rensing et al. 2008; Wang et al. 2015), *OsMADS3*, *OsMADS58*, *RAG*, *DL*, *CPP1* and *DFO1*, to Class C (Coen and Carpenter 1993; Kang et al. 1995; Kang et al. 1998; Kyozuka et al. 2000; Yamaguchi et al. 2004; Yamaguchi et al. 2006; Li et al. 2011a; Yan et al. 2015; Zheng et al. 2015), *OsMADS13* and *OsMADS21*, to Class D (Lopez-Dee et al. 1999; Dreni et al. 2007; Li et al. 2011a), *OsMADS1*, *OsMADS5*, *OsMADS6*, *OsMADS7*, *OsMADS8*, *OsMADS34*, *MJ706*, and *EMF2B*, to Class E (Chung et al. 1994; Jeon et al. 2000; Agrawal et al. 2005; Kalika et al. 2005; Sun and Zhou 2008; Cui et al. 2010; Gao et al. 2010; Li et al. 2011b; Lin et al. 2013; Conrad et al. 2014). The function analysis of these genes is very helpful to elucidate the molecular regulation mechanism of rice inflorescence.

It was also known that the divergence of monocot and dicot happened about 200 million years ago (Wolfe et al. 1989). However, the morphological characteristics and development process of monocot flower are distinct from those of dicot flower. For example, the lemma, palea, and lodicule around the stamen and pistil in rice floret are obviously different from the calyx and petal in dicot flower; in addition, there is a pair of sterile lemmas and rudimentary glumes remained outside each rice floret, respectively (Yoshida and Nagato 2011), which seems an unique floral organ in grass species. In some studies, it was believed that rice lodicule is similar to the petal in dicot flower (Kang et al. 1998; Ambrose et al. 2000) and the lemma and the palea amount to the calyx (Prasad et al. 2001). Besides, the sterile lemmas might be derived from the lemma (Prasad et al. 2001; Yoshida et al. 2009). Nevertheless, many questions still remain to be further studied, such as "the lemma and the palea are the same organ?", "where do the sterile lemma and the rudimentary glume derive from?", and "how does every gene of floral organ work in perfect union?". In this study, we attempted to analyze the genetic characteristics of sterile lemma trait and cloned the target gene using 4 mutants with long sterile lemma in order to reveal the gene function and structure.

MATERIAL AND METHODS

The *Indica* long sterile lemma (LSL) mutants, JF11, JF12 and JF13, were selected from a mutant population that derived from rice mature pollens induced by ^{60}Co-γ ray irradiation with 30Gy in the Rice Genetics and Breeding Laboratory of Xiamen University, China (Figure 1a). The *Japonica* LSL mutant, JNY-7, was donated from China National Center for Crop Germplasm Preservation (Figure 1a). Aijiaonante (AJNT) and Nipponbare (NIP) are the germplasm materials preserved in our lab. F_1 and F_2 populations were constructed from the crossings between JF11 and AJNT (JF11/AJNT), NIP and JF11 (NIP/JF11), JNY-7 and AJNT

(JNY-7/AJNT), NIP and JNY-7 (NIP/JNY-7), JF11 and JNY-7 (JF11/JNY-7) and JF11 and JF13 (JF11/JF13), respectively.

Figure 1. Grain phenotypes of the parents and their F_1s. (a) The parent grains, from left to right, are: AJNT, JNY-7, JF13, JF12 and JF11. White arrows show sterile lemmas and the red ones show long sterile lemmas; (b) The grains, from left to right, are JNY-7, JF11, AJNT, F_1 from JNY-7/JF11 and F_1 from JNY-7/AJNT, respectively; (c) The grains, from left to right, are JF11, JF13, AJNT, F_1 from JF11/AJNT and F_1 from JF11/JF13, respectively; (d) The grains, from left to right, are NIP, JNY-7, and F_1 from NIP/JNY-7, respectively; (e) The grains, from left to right, are NIP, JF11 and F_1 from AJNT/JF11, respectively.

DNA extraction and linked SSR marker screening

DNA extraction referred to Cetyltrimethyl Ammonium Bromide (CTAB) extracting method (Wang and Fang 2003). The marker tightly linked to the target trait was followed the approach of Bulk Segregant Analysis (BSA) (Michelmore et al. 1991). In this study, DNA was extracted from individual F_2 plant. DNAs from 15 individuals with LSL trait were equally mixed and developed the mutant gene pool. Similarly, the wild type (WT) gene pool was developed as well. These 2 gene pools were subjected to screen the simple sequence repeats (SSR) markers. The polymorphic markers between the 2 gene pools might link tightly to the locus of LSL trait.

Genotyping and mapping

The SSR markers linked tightly to LSL trait were used to genotype 700 individuals with LSL in the F_2 population derived from the JF13/AJNT crossing. Linkage analysis between genotype and phenotype was conducted by MAPMAKER/EXP 3.0 (Lander et al. 1987; Lincoln et al. 1992), and the linkage map was drawn by MapChart 2.2 (Voorrips 2002).

SSR markers and specific primers

Sequences of 522 SSR markers used in this research were downloaded from the GRAMENE website (http://www.gramene.org/microsat/ssr.html). According to the NIP sequence at the 2 flanks of the target locus, 72 SSR markers were designed by Preimer 5.0 and synthesized by Life Technologies Corporation.

A pair of primers, XMsl-7 (Forward primer: GAATGGAGGGTTGGGTCACT; XMsl-7, and Reverse primer: CGAAGCAACGGAACGAACAC), was designed and synthesized to amplify the Open Reading Frame (ORF), its upstream and downstream sequences of *G1*.

RESULTS AND DISCUSSION

All F_1 individuals generated from the crossings JF11/AJNT, NIP/JF11, JNY-7/AJNT and NIP/JNY-7 showed the phenotype of wild type (Figures 1b,c,d,e), whereas the F_1 plants from the crosses JNY-7/ JF11 and JF11/JF13 showed the phenotype of long sterile lemma (Figures 1b,c). In the F_2 population from the crossing of JF11/AJNT, there are 4,741 wild type individuals and 1,565 mutant individuals, respectively, presenting 3:1 segregation ratio (WT/LSL) ($\chi^2 = 0.102$; $\chi^2_{0.05} = 3.84$). This indicated that the LSL trait of JF11, JF13, and JNY-7 should be controlled by a recessive locus named *sl-1(t)*.

A set of 137 SSR markers with polymorphism between JF11 and AJNT was used to test the genotype of the DNA pools

of wild type and mutant. Two markers with polymorphism on the short arm of chromosome 7, RM51 and RM295, were further applied to validate the genotype of 200 F_2 LSL individuals. Linkage analysis between molecular marker and phenotype trait showed that the locus of *sl-1(t)* was mapped on the inner side of RM51 and RM295, and the genetic distances from the target locus to RM51 and RM295 were 13.5 and 12.7 cM, respectively. In addition, another 6 SSR markers on chromosome 7, RM20828, RM20852, RM20903, RM20948, RM21004, and RM21035, were used to fine map the *sl-1(t)*, through genotyping 700 mutants of F_2 plants. The *sl-1(t)* was narrowed in the interval between RM20903 and RM20948, and the genetic distances between *sl-1(t)* and the 2 markers were 3.8 and 4.9 cM, respectively (Figure 2).

The *G1* gene (LOC_Os07g04670) with a 831-base length ORF was deemed to confer the sterile lemma trait (Yoshida et al. 2009). In the present research, *sl-1(t)* was located in the interval between RM20903 and RM20948, which harbored *G1*. So we speculated that the *SL-1(t)* might be allelic to *G1*.

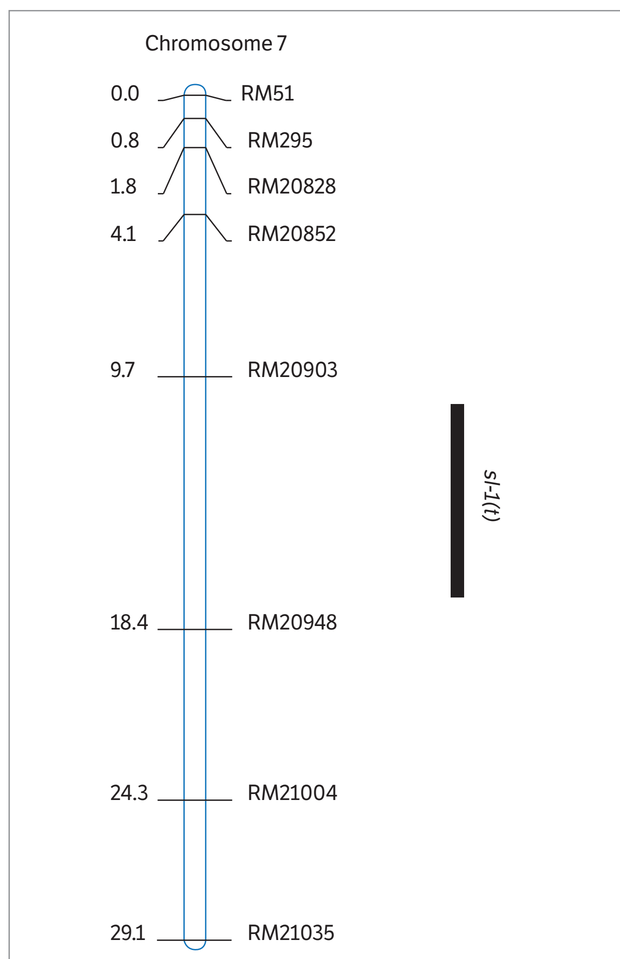

Figure 2. The location of *sl-1(t)* in the molecular linkage map on the short arm of chromosome 7. The bar is *sl-1(t)*, and the numbers on the left of the linkage map represent genetic distance (cM).

The PCR product with 1,873 bases included an 831-base ORF, an 874-base upstream sequence, and a 168-base downstream sequence of *G1*. Allelic sequencing showed that the homologous *G1* sequences of JF11, JF12 and JF13 mutants had a same deletion with 11 bases (GACGGCGCCGC) in the exon (Figure 3a), which directly led to the event of frame-shifting mutation, while JNY-7 had a base substitution which made an arginine convert into a histidine at the site of the 117th amino residue (Figures 3b,c). The results indicated that the mutation of *G1* should be able to generate the phenotype of long sterile lemma of the 4 mutants in this study.

Rice sterile lemma was a characteristic floral organ that was considered to be a vestigial floret in rice (Yoshida et al. 2009). Some genes related to the sterile lemma have been mapped or cloned in rice. *G1* was cloned by the map-based cloning method (Yoshida et al. 2009). *Osleg* was mapped in a 207-kb region on the short arm of chromosome 7, which was close to *G1* (Chen et al. 2010). In the present study, it was believed that *sl-1(t)* gene should be allelic to *G1* based on the locus location and allelic sequencing. It was very obvious that *G1* would be a key gene for further research on rice sterile lemma.

G1 has only 1 exon with 831 bp, which codes a ALOG domain, a distinct N-terminal DNA-binding domain with an additional zinc ribbon (Figure 4) (Yoshida et al. 2009; Iyer and Aravind 2012). The g1-4 mutant has a 68-base deletion in the downstream of ALOG domain; g1-2 and g1-3 have a same base transversion in the zinc ribbon insert region and a transition in the third helix of the ALOG domain, respectively (Yoshida et al. 2009). In this research, the mutant locus, an 11-base deletion near to the mutant site of g1-4, happened downstream the ALOG domain (Figure 4). In the *G1* gene of JNY-7, a base transition happened at the base No. 344 in the third helix of the ALOG domain (Figure 4). In the ORF and the upstream from –1 to –824 base of *G1*, there were 8 and 10 base substitutions between *Indica* and *Japonica* rice, respectively, and 2 of the 8 mutants located at the ORF caused the changes of the amino acid sequence. The aminoacid No. 133, V, becomes G, and the No. 190, M, becomes R (Figure 4). These 2 amino acids are located at the ALOG domain, but do not impact on the gene function.

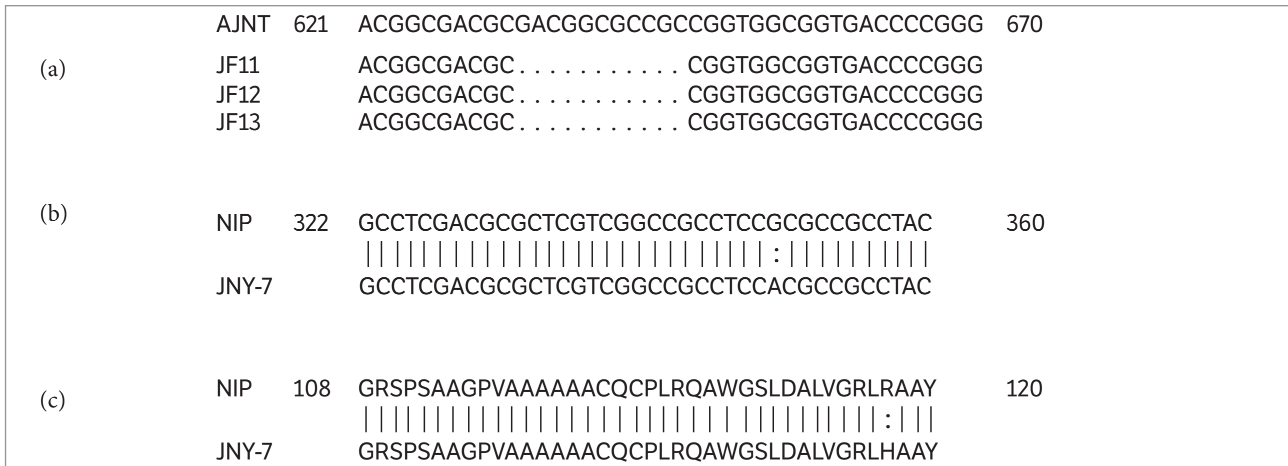

Figure 3. DNA or amino sequence alignment of *G1* in the research materials. (a) DNA sequence alignment of *G1* between AJNT and the 3 mutants. Each dot (.) means a 1-base deletion; 621 and 670 mean the base position in gene sequence; (b) DNA sequence alignment between NIP and JNY-7; 322 and 360 mean the base position in gene sequence; "|" shows the same base between NIP and JNY-7; ":" shows the different base between the 2 materials. The base, G, in NIP, was turned to A in JNY-7; (c) Amino sequence alignment of *G1* between NIP and JNY-7; 108 and 120 mean the amino position in protein sequence; "|" shows the same amino between NIP and JNY-7; ":" shows the different base between the 2 materials. One base mutation led to 1 amino change, from H to R.

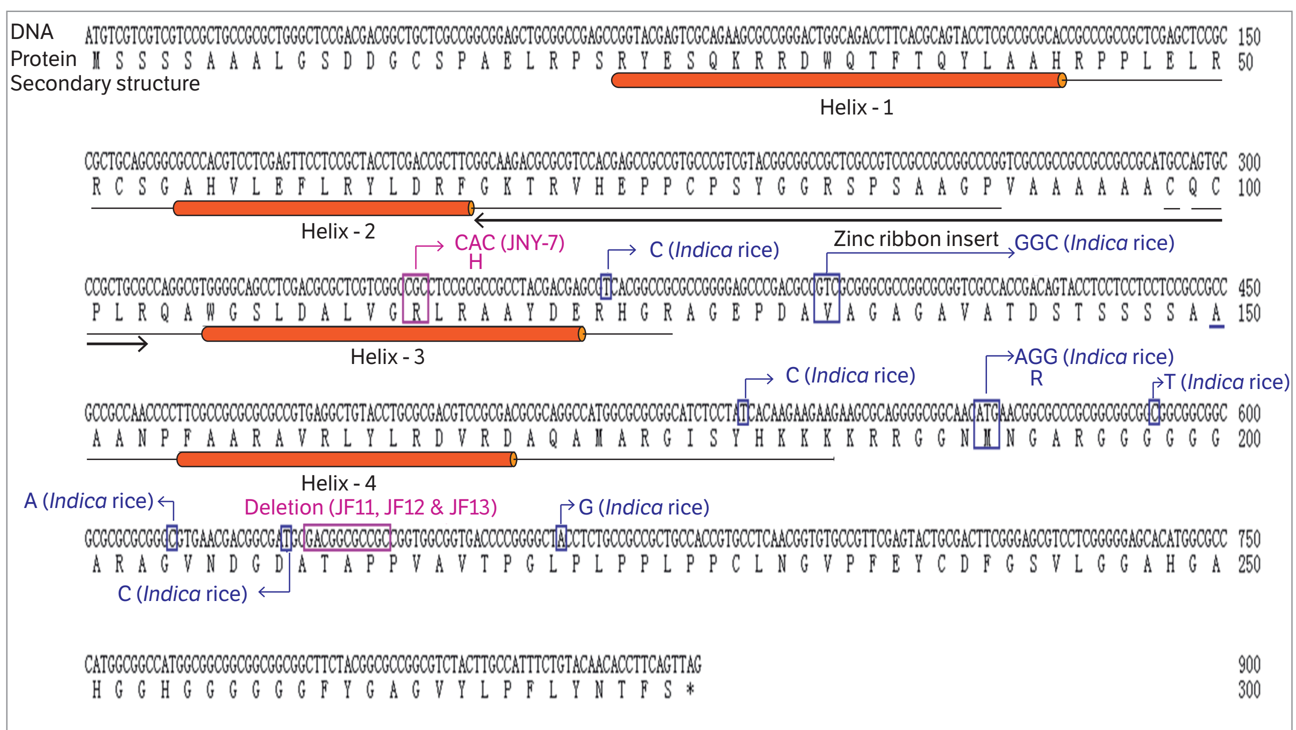

Figure 4. The CDS, protein, and ALOG domain of *G1*. Pink words represent the mutant loci of 4 mutants in the present study. Blue words mean the different loci between *Indica* and *Japonica* rice. Black lines and orange cylinders show the secondary structure of ALOG domain (Iyer and Aravind 2012).

It is known that ALOG domain is far different from the MADS domain tightly linked with the development of the floral organ in eudicots and monocots, which suggested that the molecular mechanism of rice sterile lemma identity is different from that of other floral organs, particularly in eudicots. However, in the transgenic rice plants with *Oryza sativa MADS box gene 1* (*OsMADS1*) and the rice *actin1* promoter, the 2 sterile lemmas elongated and developed palea-like and lemma-like glumes (Jeon et al. 2000), which seemed →

that the sterile lemma identity should also be related to the MADS gene family.

How is the rice sterile lemma regulated? Is there a real link between ALOG domain and MADS domain? How do the 2 domains work together? Now all of these questions are little known to us. The research on gene regulation and signal path should be further conducted in the future. The present study might provide some good mutant materials and theoretical basis for further studying the key domain, gene regulation, and signal path related to rice sterile lemma.

CONCLUSION

Rice sterile lemma is a unique outer floral organ in gramineous plants. Long sterile lemma is a recessive trait and regulated by a key gene, *G1*. The mutant genes, *sl-1(t)*, in JF11, JF12, JF13 and JNY-7 are alleles of *G1*. JF11, JF12 and JF13 have an 11-base deletion, which gives rise to a frame-shifting mutation. JNY-7 has a base substitution which triggers a missense mutation.

G1 codes an ALOG domain, which is a distinct N-terminal DNA-binding domain with an additional zinc ribbon. In this study, we found 2 mutations. One locates the downstream of the ALOG domain, and the other, in the third helix of the ALOG domain. These 2 mutations both affect the gene function. Also, 8 and 10 base substitutions happen in the ORF and the upstream from −1 to −824 base of *G1* between *Indica* and *Japonica* rice, respectively, and only 2 of them in the ORF lead to the change of amino acid sequence. Although these 2 polymorphisms are located at the ALOG domain, there is no impact on the gene function, which shows these polymorphism loci are not the key conservative ones for supporting the structure and function of ALOG domain.

In a word, this study makes the researcher to further understand the structure and function of the *G1* gene and provides 2 available mutants for exploring the development mechanism of rice floral organ.

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