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Fine Mapping of a *Retarded-Palea2 (REP2)* Gene on Chromosome 9 in Rice

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Abstract

Floral organ development influences plant reproduction and crop yield. However, mechanisms underlying the development of floral organs in specific group of species like grasses remain unclear. To understand how palea was formed, we identified a *retarded-palea2* (*rep2*) mutant, which showed that the palea was degenerate, and the lemma was crooked and just like sickle shaped. Genetic analysis confirmed that the *rep2* mutant phenotype was due to a single recessive gene mutation. F_2 population derived from the *rep2* mutant crossed with *Oryza sative* subsp. *Japonica* Taigeng16, was used for molecular mapping of the *REP2* gene. Using simple sequence repeats (SSR) and insertion-deletion (Indel) markers, the *REP2* gene was fine mapped into a 12.9 kb physical distance on chromosome 9, where two open reading frames were predicted. Sequence analysis indicated that a 10-bp-deletion was found in LOC_Os09g24480 between 8PW33 and the *rep2* mutant. The rice *RETARDED PALEA1 (REP1)* gene was in this locus. Thus, we suspected that a 10-bp deletion in the *rep2* mutant caused a frame shift and premature translational termination, and led to the functional alteration of the *REP2* gene.

Keywords: Rice (*Oryza sative L.* Subsp. *indica*); *Rep2* mutant; Molecular marker; Gene mapping; Floral development

Introduction

The flower is the reproductive organ of angiosperm plants, and its formation occurs through different steps. Firstly, the fate of the floral meristem is specified through the activity of floral meristem genes; secondly, the floral meristem is patterned into the whorls of organ primordia through the activity of floral organ identity genes; thirdly, the floral organ identity genes activate downstream effectors, which specify various tissues and cell types that constitute different floral-organ types [1]. Since some genes involved in flower development have been cloned from the model plants, rapid progress has been made in elucidating the molecular mechanisms regulating flowering [2,3]. Intensive molecular and genetic analyses in those species, notably *Arabidopsis thaliana*, and snapdragon (*Antirrhinum majus*), establish the ABC model [4,5]. According to this model, three classes of homeotic genes control the floral organ formation. A-class genes alone specify sepal formation, A- and B-class genes determine petal identity, B-class genes in combination with C-class genes together regulate stamen development, and C-class genes alone specify the innermost whorl, the carpel [6,7]. Although the floral model is proved by some studies in different plants [3,8-10] many questions remain unanswered. For example, the molecular mechanism underlying its floral organ development has not been fully investigated [11], how the target gene is identified by floral homeotic proteins, what is the target of floral homeotic proteins [12,13]. The grasses (Poaceae) is one of the largest monocot families with ~10 000 species, including many important cereal crops such as rice, maize, and barley [14,15]. Grass species have highly specialized flowers that differ from those of eudicots. A typical monocot, such as rice, a single spikelet consists of two pairs of sterile glumes (rudimentary glumes and empty glumes) and one floret, and a single floret is comprised of one lemma, one palea, two lodicules (the equivalent of petals), six stamens, and one pistil [16,17], but the surrounding structures, lodicules, lemma and palea, are unique to grasses [17,18]. In the eudicots, molecular genetic and morphological studies have revealed that lodicules and stamens are organs homologous to petals and stamens [2,19-21]. However, the origin and mechanism of lemma and palea development have long been controversial. Some researchers think of the lemma and palea as sepals, whereas others consider them as additional bracts because of their similar cellular patterns [19]. Hence, it is necessary to identify more mutants related to the development of lemmas and paleas and to isolate these relevant genes. However, to date, there are very few examples of molecular characterization of palea and lemma development in rice. In this study, we analyzed a rice mutant, *retarded-palea2* (*rep2*), which had defects in several floral organ identities, including the palea identity, and in floral meristem determinacy. We also reported the investigation and comparison of the morphological features of the floral organs between the *rep2* mutant and WT, genetic analysis of the mutant trait, and mapping of the gene for the *rep2* locus to a small physical region. We isolated the *REP2* gene by map-based cloning, and the *REP2* gene encoded a TCP family transcription factor.

Materials and Methods

Plant materials

The *Indica* rice 8PW33 and *Japonica* Taigeng16 were kept in the Rice Research Institute, Fujian Academy of Agricultural Sciences, China. The *retarded-palea2* mutant with the background of *Indica* cultivar 8PW33, was obtained through a screen of a M_2 population treated by ^{60}Co γ-ray, and was designated as *rep2*. About 300 plants in M_1 population and 3000 plants in M_2 population were grown at Fuzhou Experimental Station in Fujian Academy of Agricultural Sciences in April 2006 and in April 2007, respectively. The *Indica* cultivar, 8PW33, was derived from a progeny of a cross between Minghui 86 and Dongnanhui 307, which was designated as WT in this paper. In April 2011, the *rep2* mutant was crossed with WT, 9311 and Taigeng16 at Sanya Experimental Station in Hainan Province. The F_1 seeds, WT and the *rep2* mutant were sown at Fuzhou Experimental Station in Fujian Province in June 2011 and all F_2 seeds were harvested in October 2011. The F_2 seeds, *rep2* and 8PW33 were planted at Sanya Experimental

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Station in Hainan Province in April 2012. Plant height, panicle number per plant, spikelet number per panicle, flag leaf length, flag leaf width and setting percentage were measured at maturity in October 2011. The segregation ratios of mutants versus WT were investigated at flowering stage. All plants were grown according to standard commercial practices, with spacing of 13.3 cm between plants within each row and 26.4 cm between rows, and field management essentially followed normal agricultural practices.

Morphological and pollen fertility observation

In September 2011, 30 florets of mutant were selected randomly and the components of each floret were separated by forceps, and the types of florets were investigated. Images of florets were recorded by the Olympus digital anatomy microscope SZ61-SET. Estimation of pollen fertility was based on the I_2-KI stain method [22]. Pollen was placed on slides with 1% I_2-KI solution, and nipped into pieces using forceps to make the pollen grains spill out. The pollen fertility was examined under an optical microscope according to the morphology and staining gradation. The pistil fertility was investigated by the seed-setting rate of self-crossing and hybridization.

Construction of mapping population

The mapping population was constructed by crossing the *rep2* mutant (*Indica*) with Taigeng16 (*Japonica*), and the F_2 mapping population was generated from the self-cross of F_1 population. Totally, 1808 mutant plants in F_2 population were selected for fine mapping.

Microsatellite analysis

SSR (simple sequence repeats) primers were synthesized referring to the published rice database (http://www.Gramene.org/microsat/ssr.htm1). Indel (insertion-deletion) markers were designed according to the sequences comparison results of the genome sequences of *Japonica* (cv. Nipponpare) [23] with *Indica* (cv. 93-11) manually [23]. The BAC clone's sequences of *Japonica* and *Indica* were aligned, and primers were designed using Primer premier 5.0 based on the polymorphism region between the two rice subspecies and the polymorphic markers were used for gene mapping.

PCR amplification and marker detection

Plant DNA was extracted from the frozen leaves of rice plants using the CTAB method [24] with minor modifications. For PCR amplification of markers, each 20 μL reaction mixture contained 50 ng DNA, 5 μmol of each primer, 10× PCR buffer (100 mM Tris (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, 2 μg gelatin), 250 μM of each dNTP and 0.5 U of *Taq* polymerase. Amplification was performed with the following program: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, with a final extension of 5 min at 72°C. Amplified PCR products were resolved by electrophoresis in 3% agarose gels with ethidium bromide staining or 8% polyacrylamide denaturing gels with silver-staining for SSR markers [25].

Bulked segregant analysis

Bulked segregant analysis was used to search for markers linked to the target gene. A mutant DNA pool was constructed with the DNA extracted from leaves of 15 mutants randomly selected from the F_2 population. SSR markers distributed in the rice genome were used to detect linkage, with DNAs extracted from the *rep2* mutant and Taigeng16 used as a control. The band type of the markers linking with the mutant gene was the same as that of the *rep2* mutant.

Molecular mapping of the *REP2* gene

The band patterns of the mutant (*rep2 rep2*) and Taigeng16 (*REP2 REP2*) were recorded as 1 and 3, respectively, whereas 2 was used to denote the heterozygote (*REP2 rep2*). In this study, linkage analysis between the *rep2* locus and the SSR markers was conducted using MAPMAKER version 3.0 software [26] and map distances were estimated with MapDraw V2 [27]. At the same time, the linkage map was basically the same as reported [28].

Bioinformatics analysis

Candidate genes were predicted according to the available sequence annotation databases (http://rice.plantbiology.msu.edu/; http://www.tigr.org/). DNA and amino acid sequences were used for a complete alignment using Clustal X version 1.81.

Results

Main agronomic characteristics of *rep2*

To elucidate the genes that control the development of rice flowers, we screen for comparisons of phenotypes between the *rep2* mutant and WT. The results showed that the *rep2* mutant showed some special traits (Table 1). For example, the *rep2* mutant is taller with wider flag leaf, higher with plant height and more spikelets than WT, which shows difference at 0.05 probable levels or significant difference at 0.01 probable levels. Especially, we observe that the seed setting rate of the *rep2* mutant (48.4%) is lower than that of WT (92.6%) (Table 1), which shows significant difference at 0.01 probable levels.

Phenotype investigation of the *rep2* mutant

WT and the *rep2* mutant show indistinguishable phenotypes in their vegetative stage; however, their spikelets are different from booting stage to maturity (Figure 1a-1d). A wild type rice floret consists of a palea, a lemma, two lodicules, six stamens and a pistil with two stigmas, and these organs are all normal. In *rep2* floret, the palea is degenerate, and the lemma is crooked, which is just like sickle shaped. Close examination of the base of the spikelet revealed that the degenerate palea in *rep2* mutation was formed at the booting stage (Figure 1a). In addition, no abnormality was detected in other spikelet organs such as the pistil and stamens, suggesting that the *rep2* mutation specifically affects the palea development. The *rep2* mutant flowers were male fertile and female fertile. Microscopy analysis indicated that 95.75% of pollen grains of the mutated plants are normal (Figure 1g). When artificially pollinated with the WT pollens, about 300 germinative seeds were yielded from 400 flowers of 3 panicles on one plant, suggesting that the *rep2* mutant was basically female fertile.

Genetic analysis of the gene for the *rep2* trait

To determine whether *rep2* was controlled by a single gene or multiple genes, the *rep2* mutant was crossed with 9311and WT. All F_1 hybrids showed normal phenotypes, and all F_2 populations showed normal Mendel's segregation (Table 2). Segregation of WT and the mutant type plants fitted a 3:1 segregation ratio in the two F_2 populations (χ^2=0.330~0.688, P>0.05) (Table 2). So these results indicated that the mutant phenotype was controlled by a single recessive gene.

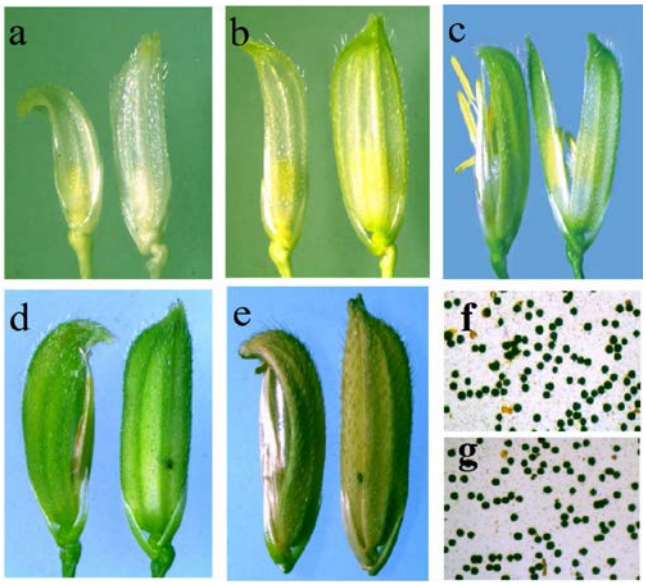
Preliminary molecular mapping of the *REP2* gene

At first, the polymorphisms between the *rep2* mutant and Taigeng16 were examined with 324 pairs of SSR primers from the RM series, of which 206 pairs exhibited polymorphism. Using BSA (bulked segregant analysis) method analysis, these 206 primer-pairs, the *rep2*

Main traits	WT	The *rep2* mutant	Difference
Plant height (cm)	99.3	119.6	-20.3**
Productive panicles per plant	6.8	7.3	-0.5
Flag leaf length (cm)	55.0	58.4	-3.4
Flag leaf width (cm)	2.00	2.80	-0.8*
Spikelets per panicle	152.3	255.3	-102.0**
Seed setting rate (%)	92.6	48.4	44.2**

* Difference between *REP2* and WT at P<0.05; **difference between *rep2* and WT at P<0.01.
Data are derived from the trial performed at Fuzhou experimental station in October 2011.

Table 1: Comparison of main agronomic traits between the *rep2* mutant and WT.



(a) Compare of booting stage; (b) Compare of heading stage; (c) Compare of flowering stage; (d) Compare of filling stage; (e) Compare of the mature stage; (f) Pollen grains of the *rep2*mutant; (g) Pollen grains of WT.

Figure 1: Phenotypes of WT (image on the right of each panel) and the *rep2* mutant (left)

Crosses	F_1 phenotype	F_2 population			χ^2 (3:1)	P
		Normal plants	Palea degradation	Total plants		
rep2/WT	Normal type	156	46	202	0.330*	0.5-0.75
rep2/9311	Normal type	135	55	190	0.688*	0.25-0.5

*Denote the segregation ratio of normal plants to mutant plants complied with 3:1 at 0.05 significant probability level.

Table 2: Segregations of F_2 populations crossed by the *rep2* mutant.

mutant, Taigeng16, and 15 mutant plants from the F_2 population were then used for linkage analysis between markers and the mutant gene. The result suggested that SSR marker RM3796, RM6051, RM6854and RM566 were linked to *rep2* locus, and the polymorphic linkage-markers RM3796, RM6051, RM6854and RM566 were used to survey 189 F_2 plants derived from *rep2* mutant × Taigeng16. Based on the segregation data and linkage map analyzed with MAPMAKER version 3.0 and Map Draw V2.1 software, the *rep2* locus was located between markers RM6051and RM6854 on chromosome 9, at a respective distance of 3.6 cM and1.2 cM (Figure 2a), Then, ten pairs of new primers in the proximal region of the *rep2* locus were used for mapping. Among them, four primers, RM24282, RM24301, RM24323, and RM24334 showed polymorphism between the *rep2* mutant and Taigeng16 (Table 3). Then the *REP2* gene was preliminarily mapped between molecular markers RM24301 and RM24323 in the terminal region of chromosome 9, at a respective distance of 0.7 cM and 0.4 cM (Figure 2b).

Fine mapping of the *REP2* gene

To map the gene to a smaller region, 1808 mutant individuals were identified from the F_2 population derived from *rep2* × Taigeng16. A higher precision map was constructed using published markers in the region between RM24301 and RM24323 (Figure 2c, Table 3). Seven polymorphic InDels were selected from 16 new InDels (Table 3). The Indel markers were designed from the publicly available rice genome sequences, and the likelihood of detecting polymorphism between the *rep2* mutant and Taigeng16 was predicted by comparing sequences from *Nipponbare* (http://rgp.dna.affrc.go.jp/) and the *Indica* cultivar 93-11 (http://rice.genomics.org.cn/). All recombinants were genotyped using seven polymorphic markers within the above interval. Recombinant screening with nine markers (RM24301, Indel-9-1, Indel-9-3, Indel-9-4, Indel-9-8, Indel-9-12, Indel-9-13, Indel-9-16 and RM24323), which were more internal to the *rep2* locus. The *REP2* gene was precisely defined in an 80.0 kb region by Indel-9-4 and Indel-9-12 (Figure 2c). To delimit the gene to a smaller region, new polymorphic molecular markers were developed, and three polymorphic InDels were selected from 8 new InDels (Table 3). Recombinant screening with six other markers (Indel-9-4, Indel-9-17, Indel-9-8, Indel-9-19, Indel-9-21 and Indel-9-12), which were more internal to the *rep2* locus, detected two, one, zero, two, two and six recombinants, respectively (Figure 2d). Thus, the *REP2* gene was precisely defined in a 12.9 kb region by Indel-9-17 and Indel-9-19.

Candidate genes in the 12.9 kb region

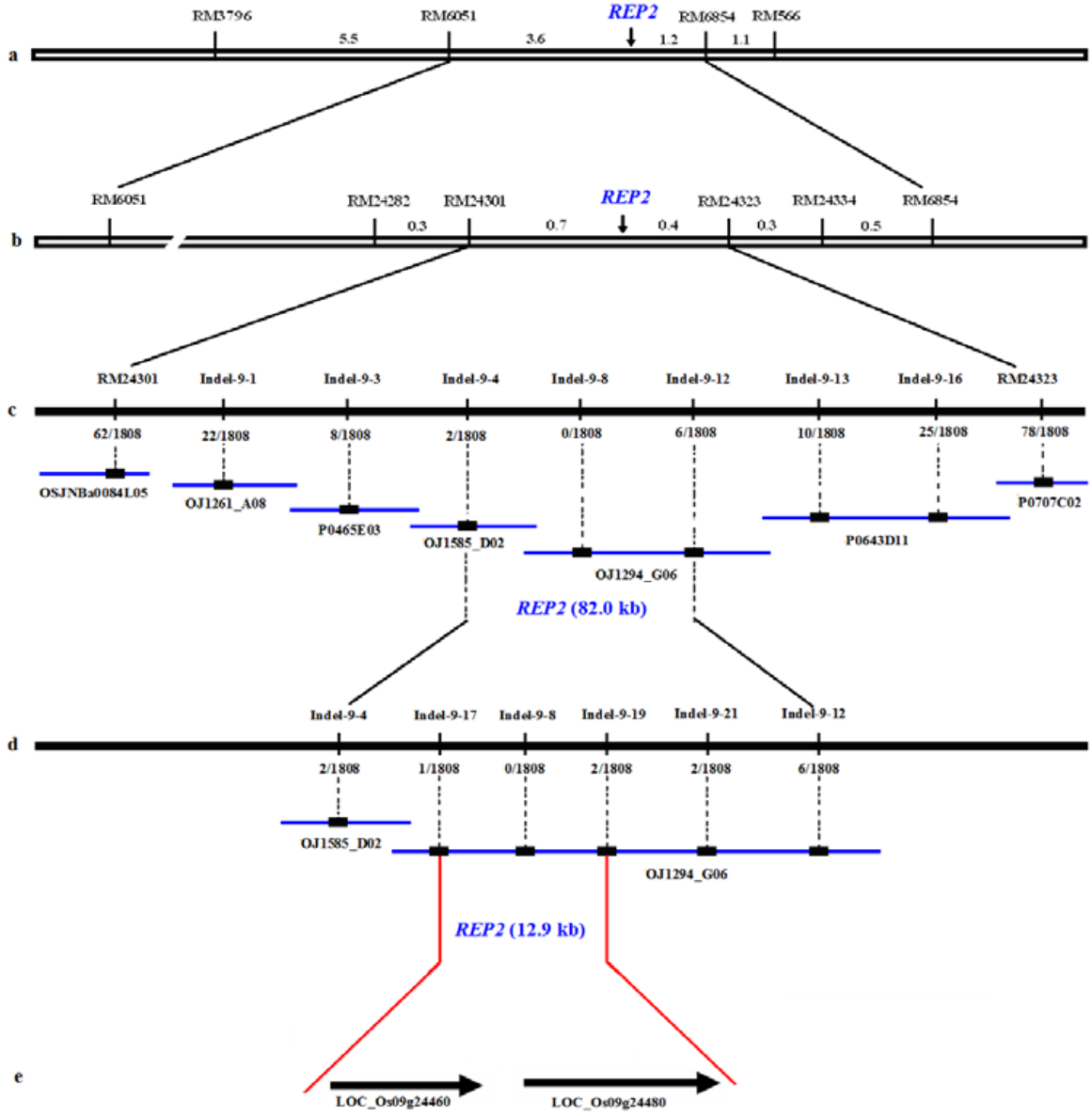
There are two annotated genes (LOC_Os09g24460 and LOC_Os09g24480) in the 12.9 kb region, according to the available sequence annotation databases (http://ricegaas.dna. affrc.go.jp/; http://www.tigr.org/). The two annotated genes have a corresponding full-length cDNA. LOC_Os09g24460 is an expressed protein, and LOC_Os09g24480 encodes a TCP family transcription factor.

Sequence analyses of the *REP2* gene

To investigate which gene was responsible for the mutation phenotype, sequencing of two genes in WT and the *rep2* mutant revealed that a 10-bp-deletion was found in LOC_Os09g24480 between WT and the *rep2* mutant (Figure 3), while no difference in the LOC_Os09g24460 between WT and the *rep2* mutant was observed. Thus, we concluded that the LOC_Os09g24480 locus corresponded to *REP2*. Interestingly, the *RETARDED PALEA1* (*REP1*) gene, encoding TCP gene family members in defining the diversification of floral morphology [33], was in this locus. The phenotype characters of the *rep2* were very similar to that of *rep1* (Figure 1a-1e). According to the phenotypic resemblance, mapping and sequencing analysis, we suspected that *rep2* was probably allelic to *rep1*. The analysis of the ORF region showed that the *REP2* gene (LOC_Os09g24480) had corresponding full length cDNAs of 777bp (Figure 4). In *rep2*, a 10-bp deletion was found in LOC_Os09g24480, causing a frame shift and premature translational termination (Figure 4). Thus, mutation as such would be expected to significantly alter the functions of the protein.

Homology analyses of *REP2* genes from other species

To gain insight into the function of *REP2*, we generated homology analyses between rice and *Arabidopsis* (Figure 5) according to available sequence annotation databases (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/). In *Arabidopsis*, an ortholog of *REP2*, AT3G18550,



(a) Primary mapping of the *REP2* gene. The gene was mapped to the region between markers RM6051 and RM6854; (b) Further mapping of the *REP2* gene. The gene was mapped to the region between markers RM24301 and RM24323; (c) Fine mapping of the *REP2* gene. The *REP2* gene was precisely defined in an 80.0 kb region by Indel-9-4 and Indel-9-12, and the recombinants number between markers and target gene was indicated under the linkage map; **(**d) High resolution mapping of *REP2*. The *REP2* gene was finally localized to a 12.9 kb region in the BAC clone OJ1294_G06, and the recombinants number between markers and target gene was indicated under the linkage map; (e) Candidate genes in the 12.9 kb target region.

Figure 2: Genetic and physical maps of the *REP2* gene.

which belonged to the TCP family, was identified to encode TCP family transcription factor. As shown in Figure 5, *REP2* and AT3G18550 had high homology. Interestingly, a 10-bp deletion was found in the *rep2* mutant (Figure 4, 5), which caused a frame shift and premature translational termination, and destroyed the conservative region, leading to a functional alteration of the *REP2* gene. Thus, we suspected that the phenotype of the *rep2 mutant* would be caused by the functional alteration of the TCP structural protein.

Discussion

The *rep2* mutant exhibits unique qualities

Facilitated by the recent developments in genome sequencing, molecular markers and bioinformatics, an impressive number of the floral organ identity genes are fine mapped and cloned in the past 20 years, and it is more and more important to identify mutants related to the development of lemmas and paleas in cloning the floral organ genes. In rice, several lemma and/or palea defective mutants have been reported; for example, *leafy lemma* and *calcaroides* in barley [29], *lhs1* [30], *dh1* [31], *sl1* [32], *rep1* [33], *mof1* [34], *dep* [35], *pal1* [36], *tob1* [37], *bls1*[2] and *slp1* [38] in rice. In the present study, we have characterized and identified the *rep2* mutant. The *REP2* gene was located on chromosome 9, and sequence analysis showed that a 10-bp deletion was found in LOC_Os09g24480 (the *rep1* locus) among *rep2* and WT. Meanwhile, the *rep2* mutant and the *rep1* mutant possessed

Marker name	Marker type	Sequence of forward primer	Sequence of reverse primer	Locations
RM5657	SSR	5'-TATGTGCATTTGTAAGGTGA-3'	5'-GCTTTAGATTATTGAGCGAG-3'	OJ1261_A08
RM24240	SSR	5'-ATGCAACCTCCTCCATCATAAGG-3'	5'-TGCTGCTCCTACCTCACTCACC-3'	B1040D06
RM24260	SSR	5'-ACTAAAGGTCCCTAGATGA-3'	5'-TAAAGATGTTGGCTATGTC-3'	P0435D08
RM24275	SSR	5'-TATAGCAAGAGCCATAGC-3'	5'-CTACCAACCCAGATGAAC-3'	P0650H04
RM24282	SSR	5'-TTGTGGTTATTTGGCTGTC-3'	5'-CGAACTGTTAAACGATGTG-3'	OSJNBb0014M19
RM24301	SSR	5'-GAGCTGGATGTCCTCGAACG-3'	5'-GACCACCTCTCCAAGCTCACC-3'	OSJNBa0084L05
Indel-9-1	Indel	5'-TATGTGCATTTGTAAGGTGA-3'	5'-GCTTTAGATTATTGAGCGAG-3'	OJ1261_A08
Indel-9-3	Indel	5'-TCTTGCATTGACACCTCTTGAGC-3'	5'-AGTCCCAACAACTGGAAGAGAGG-3'	P0465E03
Indel-9-4	Indel	5'-TGACGTGTCTAGGTCCATAATGC-3'	5'-TTTCCTGTTCCGTTTGTCAGG-3'	OJ1585_D02
Indel-9-17	Indel	5'-GCCACGGCCAGTTCAACTCC-3'	5'-CCGTCCGGATCTTGCTGTGC-3'	OJ1294_G06
Indel-9-8	Indel	5'-GAACAGAGGAGGAGATCGAGAGG-3'	5'-CTTCTTGGGAGATGCAGAAATGG-3'	OJ1294_G06
Indel-9-19	Indel	5'-ATCACCCGCCCATTATGCTACCC-3'	5'-GATGTGGTCACCCTGACATGTGG-3'	OJ1294_G06
Indel-9-23	Indel	5'-GAACACGAGCGTCTTCTTCACC-3'	5'-GTTGGCTTTGATCGATGTGTCG-3'	OJ1294_G06
Indel-9-12	Indel	5'-CGATGTGTCGTCGTCGTC-3'	5'-AGCTCCTCGTGCAGAAGAAG-3'	OJ1294_G06
Indel-9-13	Indel	5'-ACCCAACTACGATCAGCTCG-3'	5'-CTCCAGGAACACGCTCTTTC-3'	P0643D11
Indel-9-16	Indel	5'-TCCACTTCATCTTCTCAACC-3'	5'-CGGAGTAGATCAGTAGGATCG-3'	P0643D11
RM24323	SSR	5'-GTATATATCCGTGCGAATCACTCTCC-3'	5'-AACACAGCTCACGCCAGTTCC-3'	P0707C02
RM24334	SSR	5'-GAACGGTTTGAGGAAGAAGAAGACG-3'	5'-ATCCATCCACGACACACCATCC-3'	P0668D04

Table 3: In Del and SSR molecular marker used for fine mapping of the *REP2* gene.

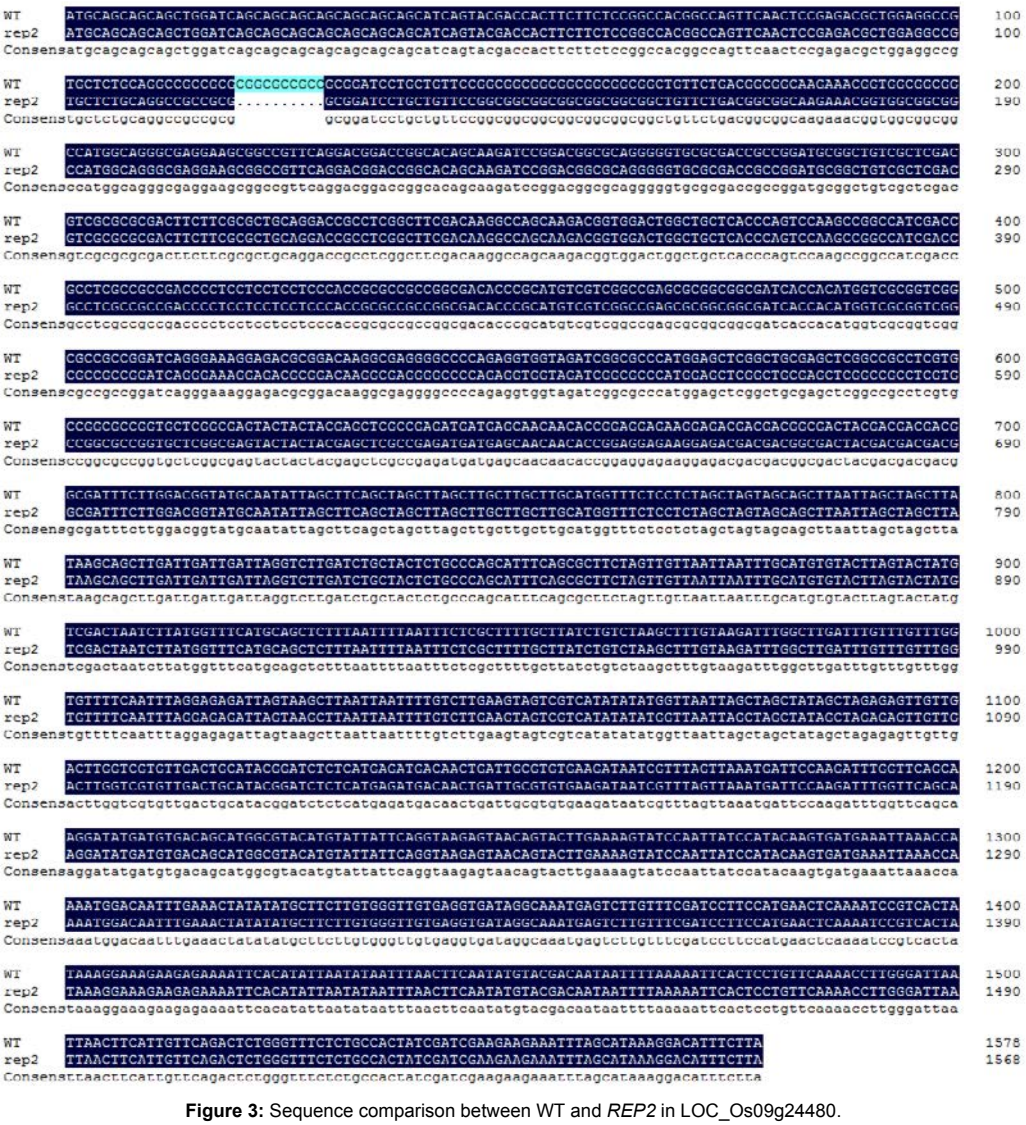
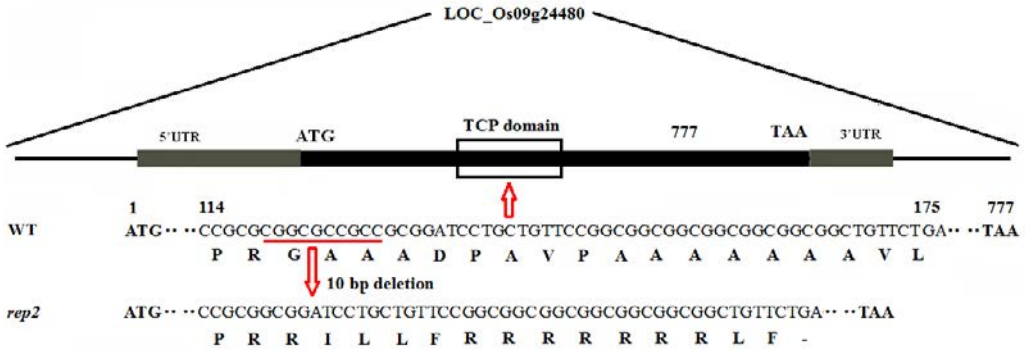


Figure 3: Sequence comparison between WT and *REP2* in LOC_Os09g24480.



The start codon (ATG) and stop codon (TAA) are indicated. Black and gray boxes indicate the ORF region and untranslated region (UTR) of the *REP2* gene, respectively. There was a 10-bp deletion at the ORF region of *rep2*, and arrow showed the mutation site.

Figure 4: The structure of the *REP2* gene (LOC_Os09g24480) and the mutation site.

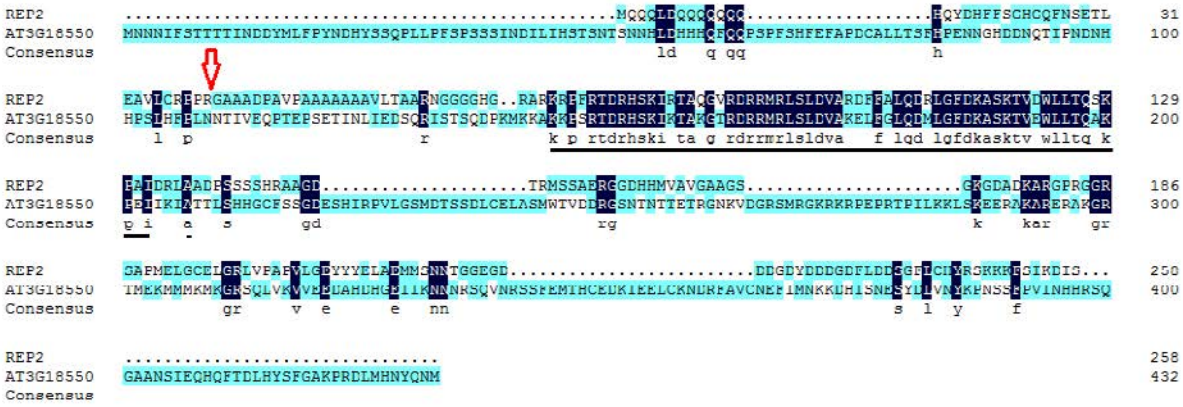


Figure 5: Alignment of amino acid sequences for TCP family protein domain in plant orthologous group. The positions of two conserved motifs are indicated above the sequences, and the conserved domain is underlined with a straight. Identical and similar amino acids are shaded dark blue. The red arrow indicates deletion position in the *rep2* mutant.

many similar characters. These results indicated that the *rep2* was allelic to *rep1*. The *rep2* is not only an epigenetic allele described in rice but also exhibits unique qualities compared with the *rep1*. For example, the *rep2* mutant shows different traits compared with WT (Table 1). Interestingly, we observed that the seed setting rate of the *rep2* mutant (48.4%) was lower than that of WT (92.6%) (Table 1). The previous observations showed that the *rep2* mutant had normal stamen and pistil, and why the *rep2* mutant had lower seed setting rate? We further observation showed that the *rep2* mutant had at least 3 stamens exposed outside during flowering and pollination (Figure 2c). Therefore, we speculate that stamens of the *rep2* mutant exposed outside, which are difficult to reach on the pistil stigma, and lead to the rate of decline.

REP2 is a TCP family member

In this article, using a map-based cloning strategy we isolated the *REP2* gene, which encoded a putative protein and belonged to a plant-specific TCP transcription factor family. These TCP family members have previously only been identified in angiosperms and have been shown to be essential in specifying plant morphology [39]. For example, *TB1* in maize [40], *OsTB1* [41], *REP1* [40] in rice, and *AtTCP12/BRC1* proteins in Arabidopsis [42] are associated with controlling zygomorphic floral development. However, a key question is whether there are TCP genes that control the diversification of floral asymmetry in grasses. Through genetic and molecular studies, some researchers have addressed this point in rice [33]. Therefore, this finding theref ore extended the function of the TCP gene family members in defining the diversification of floral morphology in grasses, and suggested that a common conserved mechanism controlling floral zygomorphy by *CYC*-like genes existed in both eudicots and monocots [33]. In summary, the *rep2*, which is allelic to *rep1*, plays an important role in establishing palea identity and controlling the diversification of floral asymmetry in rice. Therefore, further molecular study on the *REP2*-like proteins in the relative grass family will help us to facilitate elucidate whether the conserved pathway exists in controlling other grass floral zygomorphy.

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