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Identification of Quantitative Trait Loci Controlling Floral Morphology of Rice Using a Backcross Population between Common Cultivated Rice, *Oryza sativa* and Asian Wild Rice, *O. rufipogon*

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Abstract

Differences in floral morphologies affect pollination behaviour in many flowering plants. In the genus *Oryza*, several differences in the size of floral organs are known. In this study, we focused on the differences in the size of floral organs between common cultivated rice, *Oryza sativa* L. and its wild ancestor, *O. rufipogon*. We compared floral morphologies between cultivated rice *O. sativa* cv. Nipponbare and *O. rufipogon* W630. We first evaluated temporal changes in filament and anther lengths. W630 had longer filaments with rapid elongation within 15 min after spikelet opening. W630 also had longer anthers than Nipponbare, and size of anther was consistent throughout all time examined. We also analysed other six floral traits, and found that W630 had higher stigma and style length, as well as lemma and palea length, but lower lemma and palea width. Quantitative trait locus (QTL) analysis was performed to identify the loci controlling these floral traits, using backcross recombinant inbred lines derived from a cross between Nipponbare and W630. A total of 11 significant QTLs were identified. Of these, two pairs of QTLs for lemma and palea length and one pair for lemma and palea width overlapped, suggesting that common genetic factors may be the reason for the differences in these traits. In addition, we performed QTL analysis for grain size, and found that QTLs for grain size coincided with those for lemma and palea size, indicating that grain size is partly controlled by glume capacity. The QTLs identified in this study will be informative for understanding genetic changes associated with rice domestication.

[#]These two authors contributed equally to this work.

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Keywords

Rice, *Oryza sativa*, *O. rufipogon*, Floral Morphology, Domestication

1. Introduction

Floral morphologies are important factors determining mating systems in many plant species [1]. Plants have evolved their floral morphologies to adapt to environments for efficient propagation. These changes in floral morphologies have also triggered separation of species due to the inhibition of cross hybridization that acts as a reproductive barrier producing diversity of plant species [2]. In flowering plants, floral architecture is widely conserved although species-specific modifications are observed. Extensive molecular genetic studies indicate that the ABC model could explain the genes involved in floral patterning [3] [4].

In the genus *Oryza*, a floral unit is called spikelet, which consists of two interlocking glumes, lemma and palea [5]. Lemma has a long hair-like structure called awn that enhances seed shattering in wild rice, but awns are largely absent in cultivated rice [6]. Inside the spikelet, there are six stamens positioned in a whorl and one pistil in the centre. The stamen is divided into two parts: anther and filament, and the pistil is divided into three parts: stigma, style, and ovary. Differences in the size of these organs are evident in the genus *Oryza*; these may be involved in mating compatibility and pollination behaviour. In particular, the differences in the size of floral organs between common cultivated rice *O. sativa* and Asian wild rice *O. rufipogon* are of interest as the former is known to be domesticated from the latter [7].

Generally, wild rice *O. rufipogon* exhibits outcrossing behaviour, while cultivated rice *O. sativa* has self-pollination behaviour [8]. This difference in pollination behaviour was likely mediated by the selection of the naturally occurring mutations during rice domestication. An interesting change affecting pollination behaviour in rice is a closed panicle trait, which was selected in the early stages of domestication to increase seed harvesting efficiency [9]. In addition to increasing yield, the closed panicle trait was also shown to increase self-pollination, as longer awns on the top of seeds located at the lower part of the panicle block free exposure of the anther at the upper part of panicle.

Apart from the differences in panicle shape, distinct characteristics associated with pollination behaviour are also observed between wild and cultivated rice. Differences in inflorescence morphologies have previously been studied for wild and cultivated rice [10]-[15]. Several QTLs have been identified depending on the parental combinations, and several distinct genes with small effects could cause quantitative variation in these traits. In this study, we investigated the differences in filament length, one of the critical traits associated with pollination behaviour. We also analysed floral morphologies between wild and cultivated rice by focusing on the genetic changes that may have occurred during rice domestication. We used backcross recombinant inbred lines (BRILs) between *O. sativa* Japonica Nipponbare and *O. rufipogon* acc. W630. We performed QTL analysis to detect the loci controlling these floral traits. In addition, we evaluated the grain size and performed QTL analysis to access whether the loci related to grain size are linked to those controlling floral morphologies.

2. Materials and Methods

2.1. Plant Materials

A cultivar of *O. sativa* Nipponbare known as a standard Japonica rice with whole genome information [16], and a wild accession of *O. rufipogon* W630 (from Myanmar) were used in this study. The wild accession was kindly provided by the National Institute of Genetics, Japan. A single wild plant of *O. rufipogon* W630 was crossed twice with *O. sativa* Nipponbare as previously described [17], and by the single-seed-descendant method, 159 BRILs were produced at the BC_2F_8 generation [18]. These BRILs theoretically contain 12.5% of the wild genome in the genetic background of *O. sativa* Nipponbare.

2.2. Evaluation of Filament Length

Among floral traits, filament length (FLL) is not stable because of the water absorption of the filament (**Figure 1**). Therefore, the filament elongation of *O. sativa* Nipponbare and *O. rufipogon* W630 was first evaluated with spikelets randomly sampled every five minutes after spikelet opening. Spikelet samples were immediately fixed in acetic-alcohol (acetic acid: ethanol = 1:3) and kept in the fridge at four degrees until investigation. The FLL of each spikelet was calculated from the three randomly chosen filaments. Average lengths of the filaments sampled every five minutes were investigated with ten spikelets after omitting the values of the highest and lowest. Anther length (ANL) was also evaluated using the same samples.

2.3. Evaluation of Floral and Grain Traits

The following six floral and two grain traits were also examined (**Figure 1(c)**): stigma length (SGL), style length (SYL), lemma length (LML) and width (LMW), palea length (PLL) and width (PLW), and grain length (GL) and width (GW). The floral trait values were calculated as the averages of ten flowering spikelets after omitting the values of the highest and lowest. The average GL and GW were measured with five mature seeds after removal of the hulls.

2.4. QTL Analysis for Floral and Grain Traits

The genotypes for 159 BRILs at 180 polymorphic simple sequence repeat (SSR) marker loci between Nipponbare and W630 were previously obtained [18]. With these marker data and the phenotypic data obtained in the present study, QTL analysis for eight floral and two grain traits was carried out by composite interval mapping using WinQTL Cartographer ver. 2.5 software [19]. The presence of a putative QTL was determined based on the optical logarithm of odds (LOD) threshold values with the permutation value set at 1000. The percentages of variation explained by the QTL and the additive effect were also estimated using the software.

3. Results and Discussion

3.1. Temporal Changes in Filament and Anther Lengths after Spikelet Opening

In rice, the filament of male stamen starts to elongate rapidly, immediately after the opening of spikelet. This filament elongation enables the anther to be located at a higher position relative to the stigma, resulting in efficient pollination. In addition, a longer filament as observed in many wild rice species exposes the anther outside the spikelet, hereby increasing the possibility of outcrossing behaviour. To understand filament elongation in rice, we measured temporal changes in FLL after spikelet opening. We sampled spikelets of W630 and Nipponbare every five minutes until 50 minutes after spikelet opening. Rapid elongation of filament was observed for W630 compared to Nipponbare (Figure 1). At all stages, the FLL values of W630 were larger than those of Nipponbare, and the difference in filament elongation between W630 and Nipponbare was greatest at 15 min after spikelet opening (Figure 2). At this stage, both palea and lemma opened, providing the chance for the anther to be exposed outside the spikelet. Thus, W630 may easily expose its anther by rapid elongation during spikelet opening. Then, the elongation of the filament stopped about 35 minutes after spikelet opening for both W630 and Nipponbare. This suggests that the spikelets at 35 - 40 minutes after opening are optimal samples to evaluate fully elongated filament. We also measured ANL of Nipponbare and W630 (Figure 2). During all sampling times, the pattern of the differences in ANL between Nipponbare and W630 was consistent. Considering that the regulation of filament elongation is not clearly known and that no genes controlling filament elongation have been reported to date, the QTLs identified in this study and further genetic studies will provide more information for understanding filament elongation.

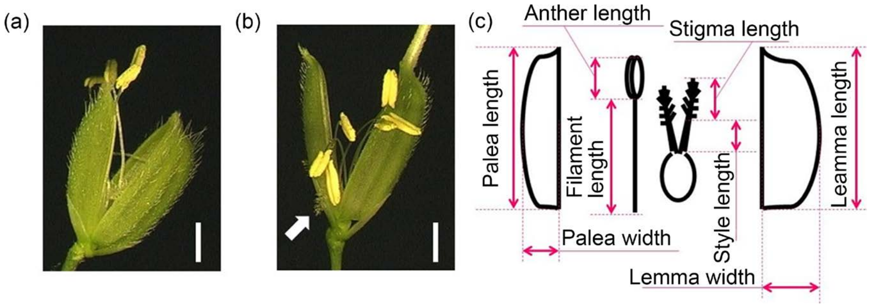

Figure 1. Opening spikelet of cultivated and wild rice and schematic diagrams of eight floral traits analysed in this study. (a) A cultivar of *Oryza sativa* Nipponbare. (b) An accession of Asian wild rice *O. rufipogon*. A white arrow indicates an exposed stigma outside the glume. Bars = 1 mm. (c) Schematic diagrams of the eight floral traits.

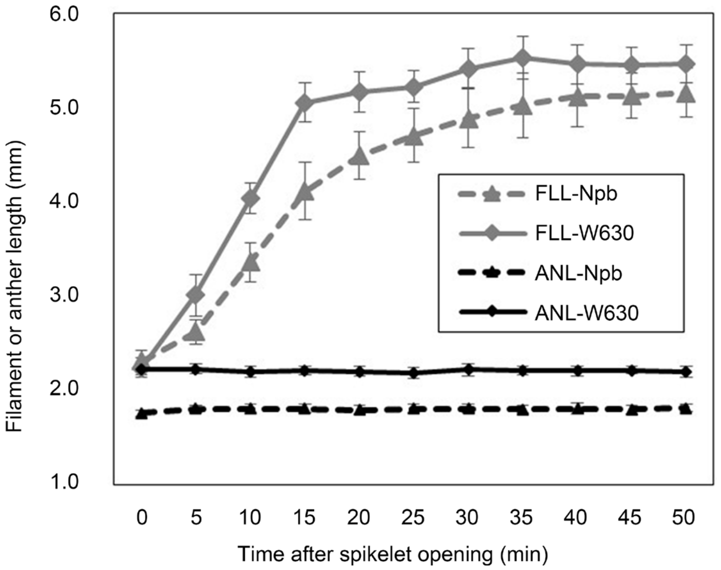

Figure 2. Temporal changes in filament and anther elongation observed with *Oryza sativa* Nipponbare (Npb) and *O. rufipogon* W630. Ten spikelets were evaluated every 5 min until 50 min after spikelet opening. Values are presented as means ± SD. FLL and ANL indicate filament length and anther length, respectively.

3.2. Comparison of Floral Organ Sizes between *O. rufipogon* W630 and *O. sativa* Nipponbare

Using the spikelets collected at 35 - 40 minutes after spikelet opening, we measured eight floral traits between the parental lines, *O. rufipogon* W630 and *O. sativa* Nipponbare (Figure 1). As observed below, the FLL of W630 was longer than that of Nipponbare; 5.71 ± 0.14 and 5.01 ± 0.13 mm for W630 and Nipponbare, respectively (Table 1). The stigma and style of W630 were also larger than those of Nipponbare. During spikelet opening, the stigma of W630 was exposed to outside of the glume, while that of Nipponbare stayed inside the glume (Figure 1(a) and Figure 1(b)). This floral characteristic of wild rice enables it to increase outcrossing rate. In our previous study, outcrossing rate of W630 was estimated to be 10.2% [8]. This higher rate may be partly attributed to FLL, ANL, SGL, and SYL. PLL and LML of W630 were greater than those of Nipponbare, while PLW and LMW of W630 were smaller (Table 1). The same tendency was observed for GL and GW, suggesting that the grain shape is partially affected by the glume shape.

3.3. QTL Analysis for Floral Traits

To understand the genetic basis of the differences in eight floral traits between wild and cultivated rice, we used 159 BRILs previously developed [18]. Each line was genetically fixed and had theoretically 12.5% of the wild genome in the genetic background of Nipponbare. In all the traits, continuous distributions were observed. Furthermore, transgressive segregation was observed for several traits (Figure 3(a)-(h)). Based on these trait data, we performed QTL analysis for eight floral traits using marker genotype data at 180 microsatellite loci. A total of

Table 1. Comparison of floral and grain sizes between *Oryza sativa* Nipponbare and *O. rufipogon* W630.

Trait	*O. sativa* Nipponbare	*O. rufipogon* W630
Filament length (FLL)	5.01 ± 0.13	5.71 ± 0.14
Anther length (ANL)	1.79 ± 0.05	2.20 ± 0.04
Stigma length (SGL)	0.92 ± 0.09	1.12 ± 0.08
Style length (SYL)	0.56 ± 0.05	0.81 ± 0.06
Lemma length (LML)	6.86 ± 0.12	7.41 ± 0.17
Lemma width (LMW)	2.68 ± 0.11	2.25 ± 0.09
Palea length (PLL)	6.74 ± 0.10	7.30 ± 0.20
Palea width (PLW)	1.51 ± 0.10	1.21 ± 0.07
Grain length (GL)	5.15 ± 0.20	6.05 ± 0.06
Grain width (GW)	2.86 ± 0.06	2.25 ± 0.02

Values are presented as means ± SD in millimetres. Mean values for all traits are significantly different between the parents by t-test ($P < 0.01$).

11 QTLs were detected with significant LOD threshold values calculated for each trait (Table 2).

Among eight floral traits, no significant QTLs were found for ANL. For FLL, a significant QTL was detected on chr. 2 with LOD value of 5.2. Two significant QTLs for SGL were also detected, one of which had high LOD score (15.3) and completely overlapped with the locus detected for SYL as shown in Figure 4. For glume size, significant QTLs for LML and PLL were simultaneously detected in the regions between RM574 and RM440 on chr. 5 and between RM230 and RM502 on chr. 8 (Figure 4). Similarly, significant QTL regions for LMW and PLW overlapped between RM452 and RM29 on chr. 2 (Figure 4). These findings suggest that common genetic factors are responsible for the differences in size of lemma and palea. In a previous study [12], QTL analysis for LML and PLL was performed using recombinant inbred lines derived from a cross between a cultivar of *O. sativa* Pei-kuh and a perennial accession of wild rice *O. rufipogon* W1944. Distinct QTLs were detected on chrs. 4, 8, and 11 for LML and on chrs. 2 and 10 for PLL. They also observed variations in floral traits between annual and perennial wild rice [11]. Therefore, the novel QTLs detected in the present study could also be associated with genetic variations between perennial and annual rice species.

3.4. QTL Analysis for Grain Traits

Grain size is one of the important targets for selection in rice domestication [20] [21]. Compared to wild rice, cultivated rice has larger seeds. Since the grain develops in the spikelet, which consists of a palea and a lemma, the size of spikelet is a major determinant of grain size. Therefore, we also measured GL and GW for the BRILs together with their parents. The average values of GL and GW of Nipponbare were 5.15 mm 2.86 mm, respectively (Table 1). On the other hand, W630 had longer (6.05 mm) and narrower (2.25 mm) seeds than Nipponbare.

Table 2. Putative significant QTLs detected for floral and grain sizes using backcross recombinant inbred lines between *O. sativa* Nipponbare and *O. rufipogon* W630.

Trait	Chr.	Marker	Source [a)]	LOD	PV [b)] (%)	Additive effect
Filament Length (FLL)	2	RM250-RM213	W630	5.2	16.8	2.32
Stigma Length (SGL)	7	RM2-RM11	W630	7.5	18.0	0.99
	8	RM210-RM230	W630	15.3	35.9	1.17
Style Length (SYL)	8	RM210-RM230	W630	4.7	12.9	0.39
Lemma Length (LML)	5	RM574-RM440	W630	6.6	14.6	1.40
	8	RM230-RM502	W630	4.1	9.2	0.96
Lemma Width (LMW)	2	RM452-RM29	Npb	4.3	9.6	0.41
	5	RM122-RM289	Npb	12.6	29.0	0.66
Palea Length (PLL)	5	RM574-RM440	W630	6.0	16.7	1.39
	8	RM230-RM502	W630	4.0	9.3	0.97
Palea Width (PLW)	2	RM145-RM341	Npb	6.4	20.2	0.50
Grain Length (GL)	5	RM574-RM440	W630	9.6	27.0	0.19
	7	RM481-RM125	Npb	4.0	7.7	0.11
	11	RM167-RM202	Npb	3.5	8.4	0.09
Grain Width (GW)	2	RM8-RM263	Npb	8.5	15.0	0.10
	2	RM6-RM250	Npb	3.8	8.1	0.07
	3	RM168-RM571	W630	4.2	7.9	0.08
	5	RM13-RM289	Npb	12.4	38.7	0.14

[a)] Allele source increasing values. [b)] Percentage of variance explained by the QTL.

Among the BRILs, continuous distributions of GL and GW were observed (Figure 3(i) and Figure 3(j)). The QTL analysis revealed that three and four loci were significantly associated with GL and GW, respectively (Table 2). The putative region of the most significant QTL for GL overlapped with those for LML and PLL on chr. 5 (Figure 4). As for GW, two QTLs with high LOD scores of 8.5 on chr. 2 and 12.4 on chr. 5 were respectively detected in the same regions of *GW2* and *qSW*5, previously identified major QTLs [22] [23]. They also overlapped with those for LMW and PLW on chr. 2 and for LMW on chr. 5 (Figure 4). These results suggest that the Nipponbare alleles at *GW2* and *qSW5* have widening effects on both floral and grain sizes as compared with wild rice. In addition, our results confirmed that the *qSW5* gene does not control inner glume size (PLW in this study) of rice spikelets [23]. The QTLs for floral sizes detected between cultivated and wild rice may have been involved in rice domestication to improve grain sizes.

4. Conclusion

In this study, we evaluated variations between *O. sativa* Nipponbare and *O. rufipogon* W630 for eight floral and two grain traits. We identified a total of 11 and 7 significant QTLs for floral and grain traits. Overlapped QTL regions were

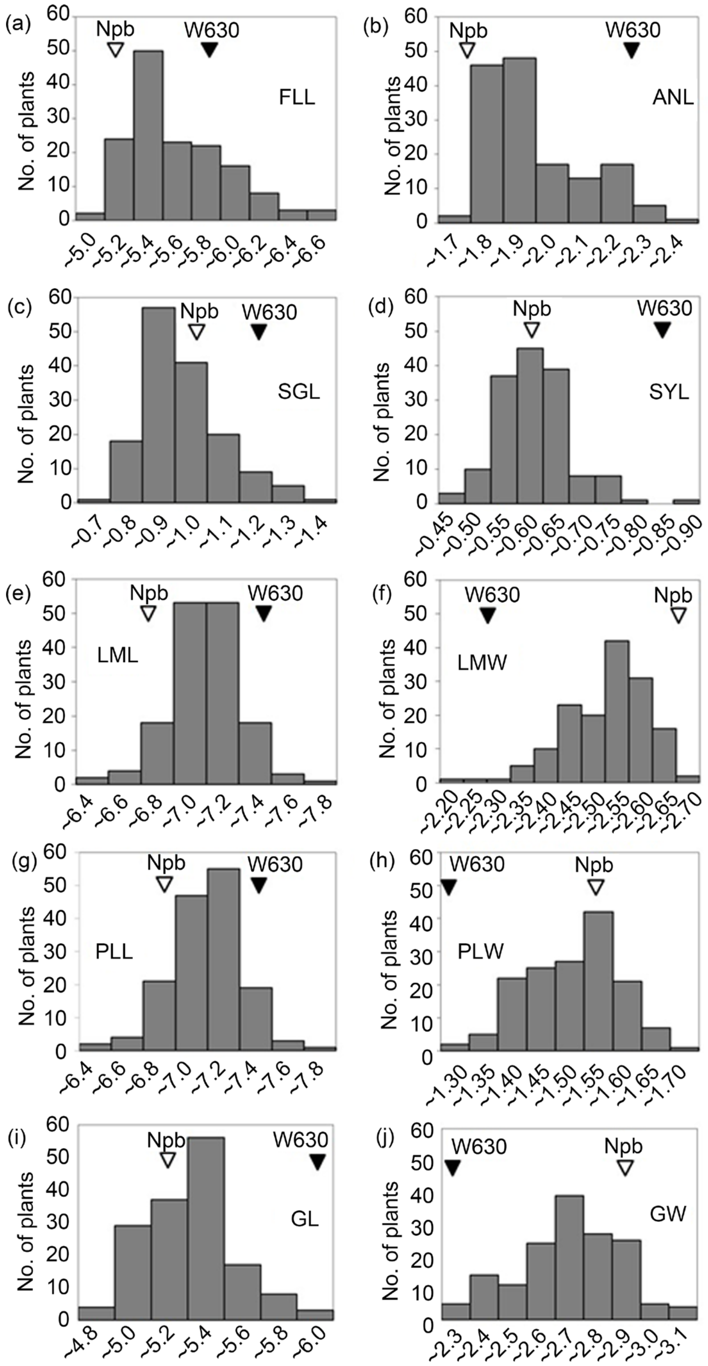

Figure 3. Frequency distributions of eight floral and two grain traits in the backcross recombinant inbred lines between *Oryza sativa* Nipponbare and *O. rufipogon* W630: (a) filament length (FLL); (b) anther length (ANL); (c) stigma length (SGL); (d) style length (SYL); (e) lemma length (LML); (f) lemma width (LMW); (g) palea length (PLL); (h) palea width (PLW); (i) grain length (GL); (j) grain width (GW). Triangles indicate the average values of parental lines.

detected for LML and PLL, and for LMW and PLW, suggesting that common genetic factors may have caused the differences in these traits. In addition, major QTLs for spikelet length (LML and PLL) and width (LMW and PLW), were detected in the similar QTL regions for GL and GW, respectively. These

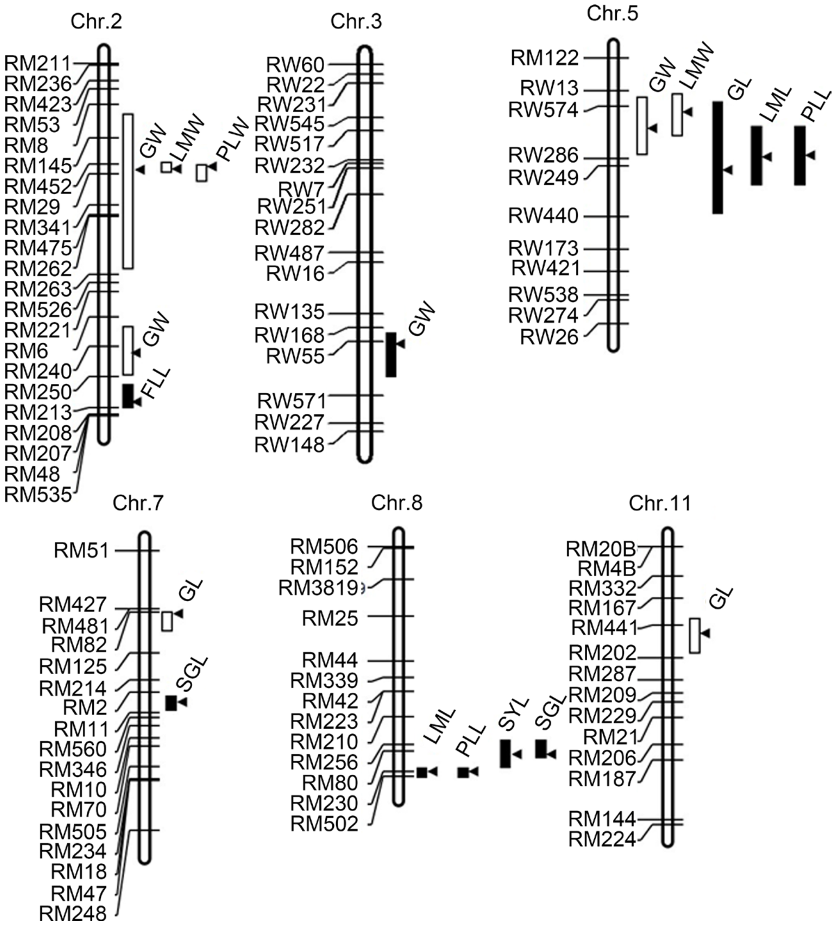

Figure 4. Chromosome positions of the QTLs detected for seven floral and two grain traits using the backcross recombinant inbred lines between *Oryza sativa* Nipponbare and *O. rufipogon* W630. QTL positions are represented as bars with the LOD peak shown by triangles. The abbreviations of the traits are explained in **Table 2**. Black and white bars indicate the allele effects of W630 and Nipponbare to increase the trait values, respectively.

results indicate that spikelet size is an important factor that controls grain size. Segregating populations at further backcross generation will facilitate identification of the candidate QTLs. Considering that wild *O. rufipogon* has a large genetic diversity, other loci could be discovered when different parental combinations are employed. These genetic materials will be useful for identifying novel loci involved in the diversity of floral morphologies in rice.

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