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Mapping QTLs influencing rice floral morphology using recombinant inbred lines derived from a cross between *Oryza sativa* L. and *Oryza rufipogon* Griff

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Abstract To understand the genetic basis of floral traits associated with the mating system in rice, we analyzed pistil, stamen and glume traits using a recombinant inbred line population, derived from a cross between an Asian cultivated rice (*Oryza sativa* L.), Pei-kuh, and a wild rice (*Oryza rufipogon* Griff.), W1944. Quantitative trait loci (QTLs) affecting floral morphology were detected by composite interval mapping using a linkage map constructed using 147 markers, mostly RFLPs. A total of 7, 4, 14 and 6 QTLs were detected for traits related to pistil, stamen, and size and shape of the glume, respectively. Comparison of 31 QTLs affecting these organs revealed ten QTLs affecting the different organs in four adjacent regions on chromosomes 2, 4, 5 and 10, but most QTLs (68%) were located separately on the whole chromosomes. Although four QTLs for stigma breadth, anther length and thickness of lemma and palea explained more than 25% of the total phenotypic variance, most QTLs (87%) had smaller effects. These results suggest that quantitative variation observed for pistil, stamen and glume traits is controlled by several distinct genes with small effects.

Keywords Floral trait · Mating system · Asian cultivated rice (*Oryza sativa* L.) · Wild rice (*Oryza rufipogon* Griff.) · QTL analysis

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Introduction

In rice, the floral morphology (pistil, stamen and glume) plays an important role in sexual reproduction processes such as pollination, fertilization and seed setting (Takeoka et al. 1993). This morphology is associated with the mating system of rice (Oka and Morishima 1967). In general, Asian cultivated rice (*Oryza sativa* L.) predominantly shows inbreeding, while its ancestral wild species (*Oryza rufipogon* Griff.) shows partial outcrossing (Oka and Morishima 1997). Virmani (1994) reported that outcrossing is influenced by many floral traits (e.g. size of pistil and stamen, stigma exsertion, angle of glume opening). Between cultivated rice and its wild relatives, these traits exhibit wide variation. Cultivated rice, as a self-pollinated crop, generally has lower stigma exsertion, and smaller pistils and stamens than its wild relatives (Virmani and Athwal 1973). Floral traits suitable for self-pollination in cultivated rice were considered to have been selected for during the process of domestication (Oka and Morishima 1997). The genetic mechanism related to this change of floral morphology, which has

facilitated the change from outcrossing to selfing, is not well-understood.

Several rice floral morphology mutants, such as multiple pistil (*mp1* and *mp2*), leafy hull sterile (*lhs*) and slender glume (*slg*), have been reported (Nagato and Yoshimura 1998). However, relatively little is known about the genetic mechanisms of quantitative variation in floral morphology, such as the size and shape of the floral organs. Virmani and Athwal (1974) hypothesized that stigma, anther length and stigma exsertion were controlled by polygenes, judging from the continuous variation observed in 29 cultivated and wild rice.

Recent advances in DNA marker technology and the construction of linkage maps have enabled the detection of quantitative trait loci (QTLs) controlling complex genetic traits. QTLs for floral morphology were identified in monkeyflower (Bradshaw et al. 1995; Lin and Ritland 1997; Bradshaw et al. 1998), *Arabidopsis thaliana* (Juenger et al. 2000), tomato (Bernacchi and Tanksley 1997; Georgiady et al. 2002) and maize (Veldboom et al. 1994). In rice, Huang et al. (1997) identified four, five and three QTLs for length, width and the length-width ratio of the filled glume using a double-haploid population derived from a cross between an Indica (IR64) and a Japonica cultivar (Azucena). Redoña and Mackill (1998) detected seven, four and three QTLs for length, width and the length-width ratio of the filled glume using an F_2 population derived from a cross between tropical Japonica (Labelle) and an Indica cultivar (Black Gora). Xiong et al. (1999) reported seven QTLs for anther length using an F_2 population derived from a cross between *O. sativa* cv Aijiao Nante and *O. rufipogon* P16. Although these reports suggested that each floral trait was controlled by multiple genes, the relationships among three organs in the spikelet (i.e. pistil, stamen and glume) have not been clarified. To understand the genetic relationships of these organs, a detailed evaluation of the flowering stage is necessary.

Cai and Morishima (2002) detected 6, 1 and 20 QTLs for the width and length-width ratio of the filled glume and anther length, respectively, using recombinant inbred lines (RILs) derived from a cross between *O. sativa* cv Pei-kuh and *O. rufipogon* W1944. This population could be expected to show wide segregation for floral traits, allowing detection of loci responsible for differences in mating system between cultivated rice and its wild relatives. In this study, we attempted to elucidate the genetic mechanism associated with three spikelet organs of rice using QTL analysis of this population. Pistil, stamen and the angle of glume opening are recognized to affect the outcrossing (Virmani 1994). Previous studies showed that the size or shape of the glume is associated with the size and exsertion of the stigma, which contributed to the outcrossing (Virmani and Athwal 1973; Kato and Namai 1987). Therefore, we considered the size and shape of the glume as traits indirectly related to outcrossing.

Materials and methods

Population and cultivation

A set of 102 F_8 RILs was used for QTL analysis of the floral traits. The RILs were developed by a single-seed descent method from an F_2 population between a Taiwanese Indica cultivar (*O. sativa*), Pei-kuh, and an Asian wild rice (*O. rufipogon*), W1944, which is a perennial and exhibits partial outcrossing (Cai and Morishima 2002). Five plants of each RIL and parental lines were grown using pots in the field at the Agricultural and Forestry Research Center at the University of Tsukuba, Japan, in 1998. Three vigorously growing plants for each line were selected and photographed. At the flowering stage, three blooming spikelets were randomly selected from each plant and a total of nine spikelets were photographed to measure the maximal angle of glume opening. Since the opening period of each glume was less than 1 hour on average, this was the maximum number possible. Simultaneously, 15 other spikelets from each plant were randomly collected for evaluation of other traits and a total of 45 spikelets were fixed in acetic-alcohol (acetic acid:ethanol = 1:3). Most measured traits showed high heritability (data not shown). Therefore, the average of nine individuals was used as the value of each line in all floral traits except for the rate of exserted stigma.

Pistil and stamen

Stigma length (STL), stigma breadth (STB), style length (SYL) and anther length (ANL) of the fixed nine spikelets were measured using a micrometer under a stereomicroscope. The rate of exserted stigma (RES) from the spikelet was evaluated from average scores of 27 other spikelets (score, 0 = no exserted sigma, 1 = exserted stigma).

Size and shape of the glume

The maximal angle of glume opening (AGO) was measured using the image analysis software package, NIH Image (http://rsb.info.nih.gov/nih-image/, National Institutes of Health, USA).

To measure the size and contour shape of the lemma and palea, the nine fixed spikelets of each line were photographed. The length and thickness of lemma (LML and LMT) and palea (PLL and PLT) were determined using the NIH Image. Contour shape was quantitatively evaluated by the shape analysis software package, SHAPE (Iwata 1998), using standardized elliptic Fourier descriptors (Kuhl and Giardina 1982; Furuta et al. 1995). In each lemma and palea, the coefficients of standardized elliptic Fourier coefficients were calculated from a total of 936 image samples consisting of 102 RILs and parental lines. Subsequently, these coefficients were summarized by principal component analysis (PCA). From the PCA results, we visualized the contour shapes that can be accounted for by each principal component (Fig. 1). These shapes indicated that the first, second and third PCA scores of the contour shape of lemma (1CSL, 2CSL and 3CSL) and palea (1CSP, 2CSP and 3CSP) corresponded to the length-thickness ratio, the degree of asymmetry and the location of the centroid in each trait, respectively. We used the first to third PCA scores of each line as the phenotypic value of contour shape in lemma and palea for phenotypic and QTL analysis.

QTL analysis

We used a linkage map constructed by Cai and Morishima (2002) using MAPMANAGER 3.0 (Manly 1993) based on the segregation data set of 147 markers in the F_6 RILs. To determine the primary linkage group, we referred to the known maps (Causse et al. 1994; Harushima et al. 1998).

The chromosomal locations of putative QTLs were determined by composite interval mapping (CIM) of QTL Cartographer 2.0

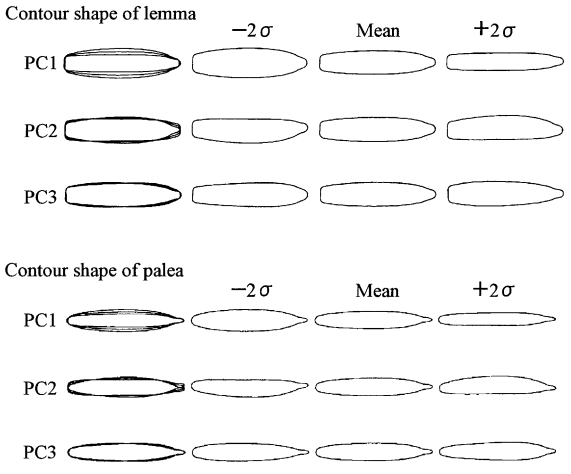

Fig. 1 Contour shapes of the lemma and palea drawn using the elliptic Fourier coefficients estimated under three typical values of each principal component score. Each column shows that the score takes either $+2\sigma$, mean or -2σ. The leftmost column shows the overlaid drawings of the three cases. *PC1*: the first principal component, corresponds to the length-thickness ratio. *PC2*: the second principal component, corresponds to the degree of asymmetry. *PC3*: the third principal component, corresponds to the location of the centroid

(Basten et al. 1994), because Zeng (1994) suggested that CIM could be superior to simple interval mapping for estimating the position and effect of QTLs from the results of their simulation analysis. For CIM, we used a likelihood ratio of 11.5, corresponding to a LOD score of 2.5, by manual input, since a higher threshold in a small population may underestimate putative QTLs and show bias towards genes with a large effect (Yano and Sasaki 1997). We analyzed the results of 1,000 permutation tests for each trait using the 5% level of significance (Churchill and Doerge 1994) to ensure the false positive (type-I error) rate for QTL detection. Putative QTLs were assumed to be located in the vicinity of the peak of the LOD score, and the additive effect and phenotypic variance explained by each QTL were estimated at the peak of the LOD score. The detected QTLs were designated as recommended by McCouch et al. (1997).

Results and discussion

Variation in floral traits

All traits of *O. rufipogon* W1944, except for LMT, 2CSL, 3CSL and 3CSP, had larger values than those of *O. sativa* cv Pei-kuh (Fig. 2). In particular, the anther length of W1944 (6.50 mm) was about four times that of Pei-kuh (1.68 mm). A wide continuous variation was recognized in all traits of RILs. STL, ANL, LML, PLL, 1CSL, 1CSP, 2CSL and 2CSP were within the range of the parental values, while transgressive segregation was observed for RES, STB, SYL, LMT, PLT, AGO, 3CSL and 3CSP. Most traits showed nearly normal distributions, but RES segregated widely from 0 to 100%, showing a significant deviation from normal distribution. Therefore, RES was transformed using the arcsine function prior to QTL analysis.

Table 1 Correlation coefficients among 16 floral traits in RILs derived from Pei-kuh × W1944[a]. *RES*: rate of exserted stigma, *STL*: stigma length, *STB*: stigma breadth, *SYL*: style length, *ANL*: anther length, *AGO*: maximal angle of glume opening, *LML*: lemma length, *PLL*: palea length, *LMT*: lemma thickness, *PLT*: palea thickness, *1–3CSL*: first to third principal component scores of contour shape of lemma, *1–3CSP*: first to third principal component scores of contour shape of palea. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$

Floral trait	Pistil				Stamen	Glume size					Glume shape				
	RES	STL	STB	SYL	ANL ANL	AGO	LML	PLL	LMT	PLT	1CSL	1CSP	2CSL	2CSP	3CSL
STL	0.397**														
STB	0.076	0.483**													
SYL	0.449**	0.309**	0.142												
ANL	0.299**	0.176	0.145	0.243*											
AGO	0.399**	0.034	0.035	0.041	0.288**										
LML	0.086	0.355**	0.280**	0.137	0.306**	–0.108									
PLL	0.089	0.384**	0.277**	0.147	0.368**	–0.040	0.959**								
LMT	–0.428**	–0.186	0.094	–0.066	–0.308**	–0.267**	–0.040	–0.034							
PLT	–0.354**	–0.138	0.110	–0.060	–0.323**	–0.208*	–0.012	–0.011	0.929**						
1CSL	0.402**	0.358**	0.068	0.131	0.430**	0.147	0.602**	0.574**	–0.804**	–0.736**					
1CSP	0.338**	0.380**	0.115	0.127	0.482**	0.131	0.627**	0.660**	–0.717**	–0.726**	0.935**				
2CSL	0.082	0.040	–0.010	0.016	–0.051	0.083	–0.086	–0.103	–0.076	0.014	–0.033	–0.098			
2CSP	–0.059	0.003	0.049	–0.008	0.014	–0.040	0.007	0.033	0.082	0.034	–0.020	0.034	–0.869**		
3CSL	0.019	0.060	–0.034	–0.055	–0.201*	0.029	–0.062	–0.148	0.025	0.008	–0.037	–0.113	–0.051	–0.052	
3CSP	0.100	–0.123	–0.058	0.167	0.120	–0.042	0.074	0.025	–0.013	0.048	0.066	–0.027	–0.030	0.137	–0.682**

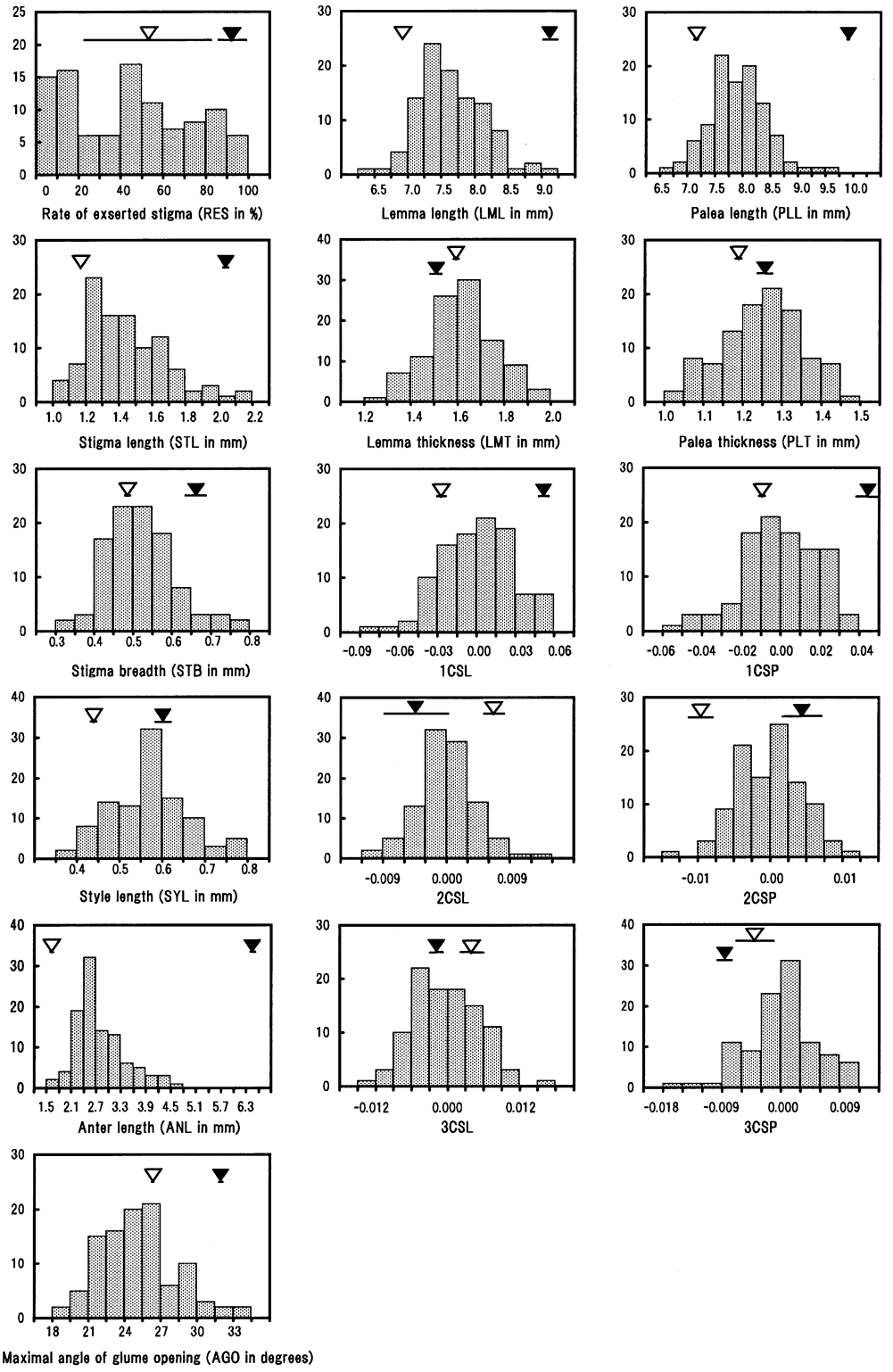

Fig. 2 Frequency distributions of 16 floral traits in RILs derived from a cross between Pei-kuh and W1944. The *inverted triangles and horizontal bars* indicate the values of the averages and the standard deviation of parental lines, respectively. The *white and black triangles* correspond to Pei-kuh and W1944, respectively. *1–3CSL*: the first to third principal component scores of the contour shape of the lemma, *1–3CSP*: the first to third principal component scores of the contour shape of the palea

Phenotypic relationships among floral traits

Phenotypic correlations among the pistil, stamen and glume are shown in Table 1. STL showed a significant correlation with STB (0.48) and SYL (0.31), while SYL and STB did not show any significant correlation. Among traits related to glume size, highly significant correlations were observed between LML and PLL, and between LMT and PLT. However, no significant correlation was observed between LML and LMT, or between PLL and PLT. These results suggest that the lengths of lemma and palea are controlled by the same genetic factors, but that

Table 2 Putative QTLs for 13 floral traits in rice

Trait	QTL	Chr.	Franking markers	LOD	R^{2d}	A[e]
Rate of exserted stigma (RES)	*qRES-5*	5	*APAGE1 – RG480*	2.76	9.8	7.160
	qRES-10	10	*G37 – C16*	3.15	24.8	10.446
Stigma length (STL)	*qSTL-4*	4	*R288 – G162*	3.38	19.6	0.106
	qSTL-6	6	*G12 – NT*	3.45	10.5	0.076
Stgma breadth (STB)	*qSTB-4*	4	*R1427 – RG214*	3.00	22.8	0.046
	qSTB-12	12	*RZ257 – G124a*	3.57*	30.5	0.049
Style length (SYL)	*qSYL-6*	6	*G12 – NT*	4.14*	23.3	0.044
Anther length (ANL)	*qANL-1*	1	*CDO118 – RB1*	5.12*	27.8	0.373
	qANL-2	2	*BCD349 – Amp1*	2.57	8.1	0.192
	qANL-5	5	*C43 – APAGE1*	2.96	12.8	0.227
	qANL-9	9	*RZ792 – C506*	3.97	17.4	0.277
Maximal angle of glume opening (AGO)	*qAGO-2*	2	*RG322 – G275*	3.02	12.2	1.277
	qAGO-8	8	*RZ143 – C400a*	2.53	13.4	–1.255
	qAGO-9	9	*C506 – RZ792a*	3.04	11.8	–1.171
Lemma length (LML)	*qLML-4*	4	*R288*	3.16	20.6	0.231
	qLML-6	6	*RZ588 – R2171*	3.19	11.0	0.172
	qLML-8	8	*G278 – G104*	2.97	23.6	0.252
	qLML-11	11	*G24 – RZ141*	2.83	14.7	0.202
Palea length (PLL)	*qPLL-2*	2	*RG322 – G275*	5.26*	21.4	0.277
	qPLL-10	10	*G37 – C16*	2.84	10.4	–0.173
Lemma thickness (LMT)	*qLMT-5*	5	*C43 – APAGE1*	4.67*	21.9	–0.072
	qLMT-8	8	*R1394A – G2132*	5.34*	30.5	–0.083
Palea thickness (PLT)	*qPLT-2*	2	BCD349	3.00	9.9	–0.036
	qPLT-5	5	*C43 – APAGE1*	3.09	18.0	–0.044
	qPLT-8	8	*R1394A – G2132*	3.47	28.6	–0.055
1CSP[a]	*q1CSP-5*	5	*C43 – APAGE1*	3.33*	17.3	0.008
	q1CSP-7	7	*RZ387*	3.86	13.9	0.007
	q1CSP-11	11	*G24 – RZ141*	2.84	12.4	0.007
2CSL[b]	*q2CSL-1*	1	*RB1 – G393*	3.62*	13.1	–0.002
	q2CSL-12	12	*G148*	2.84	11.2	–0.002
3CSL[c]	*q3CSL-9*	9	*BCD1087 – G103*	2.71	16.1	0.003

[a] *1CSP*: first principal component of the contour shape of the palea
[b] *2CSL*: second principal component of the contour shape of the lemma
[c] *3CSL*: third principal component of the contour shape of the lemma
[d] Percent phenotypic variance explained by each QTL
[e] Additive effect of the allele from W1944 compared with Pei-kuh
* Putative QTLs with significant LOD scores on 1,000 permutation tests at the 5% level

the factors contributing to thickness are different from those controlling length. The significant correlations were recognized between two pistil traits (STL and STB) and two traits related to glume size (LML and PLL), ranging from 0.28 to 0.38. The ANL was significantly correlated with five traits related to glume size (LML, LMT, PLL, PLT and AGO), respectively. The two scores of glume shape (1CSL and 1CSP) showed a significant correlation with STL, ANL, LML, PLL, LMT and PLT. These correlations suggest that pistil, stamen and glume share some genes, or their genes are tightly linked to each other on the genome.

Quantitative trait loci for floral traits

A total of 7, 4, 14 and 6 QTLs for traits related to pistil, stamen, and the size and shape of the glume, were distributed on all chromosomes except 3 (Table 2, Fig. 3). The phenotypic variance explained by each QTL (R^2) ranged from 8.1 to 30.5%.

Pistil

QTLs for all pistil traits were detected on chromosomes (chrs.) 4, 5, 6, 10 and 12. W1944 alleles of all QTLs contributed to an increase in each trait and most QTLs showed a small effect. Two QTLs for RES, explaining 9.8 and 24.8% of total variance, were detected on chrs. 5 and 10, respectively. Two QTLs for STL, explaining 19.6 and 10.5% of total variance were detected on chrs. 4 and 6, respectively. Two QTLs for STB were detected on chrs. 4 and 12. The *qSTB-12* showed a large R^2 (30.5%), while the *qSTB-4* explained 22.8% of total variance. One QTL for SYL was detected on chr. 6, explaining 23.3% of total variance. The QTL for STL was located together with the QTL for SYL on chr. 6 (*G12 – NT*), while other QTLs for the pistil traits were distributed on different chromosomal regions.

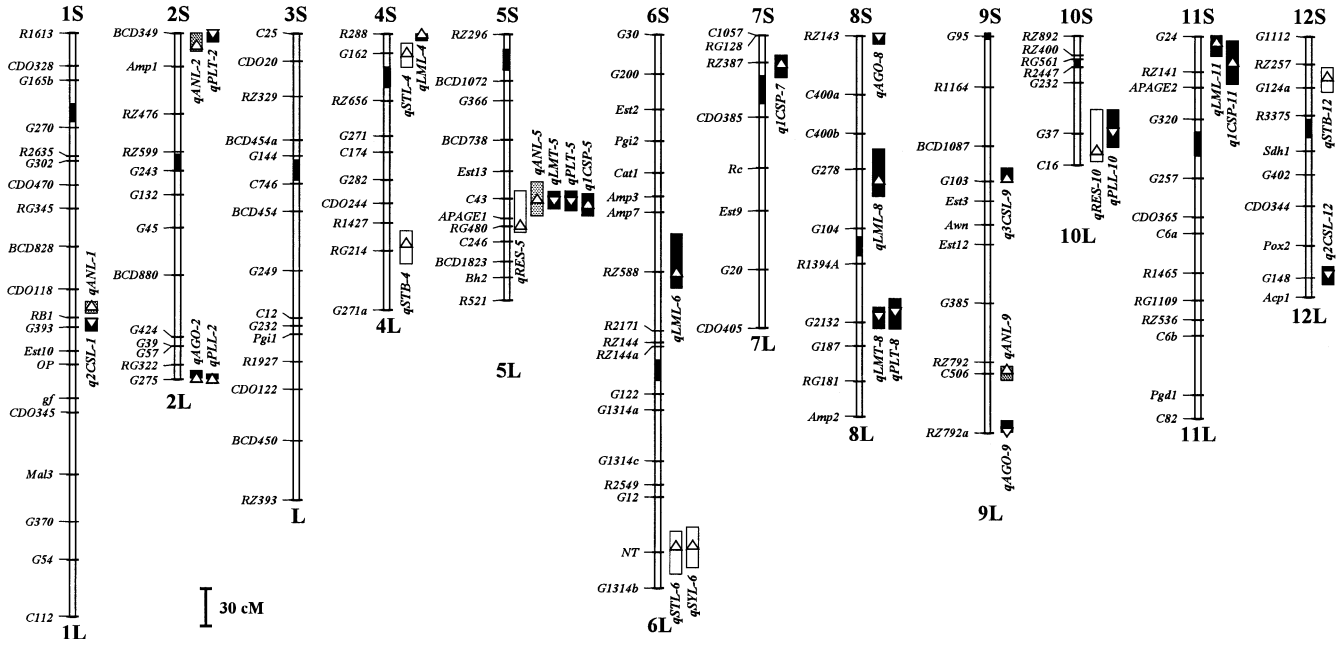

Fig. 3 Mapping of QTLs for floral traits on the rice linkage map. The linkage maps consisted of 147 markers including 121 RFLPs, 17 isozymes, two protein markers (*APAGE1, 2*), one RAPD marker (*RB1*) and six morphological markers (*OP, NT, Awn, gf, Bh2* and *Rc*) (Cai and Morishima 2002). The orientation of each chromosome was obtained from known maps (Causse et al. 1994; Harushima et al. 1998). The *black regions* on chromosomes represent the estimated centromere regions (Singh et al. 1996). The numbers, *S* and *L*, indicate each short and long chromosome arm, respectively. Markers are indicated on the left of each chromosome. *Triangles and boxes* on the right of each chromosome represent LOD peaks of putative QTLs and their one-LOD support intervals (Lynch and Walsh 1998), respectively. *White, gray and black boxes* indicate pistil, stamen and glume QTLs, respectively. *Upward and downward triangles* indicate that the presence of the W1944 and Pei-kuh alleles increase each trait, respectively

Stamen

Four QTLs for ANL were distributed on chrs. 1, 2, 5 and 9. Although the *qANL-1* showed the large total variance (27.8%), the other three QTLs had a small effect, ranging from 8.1 to 17.4% of the R^2. All QTLs increased anther length at the wild rice allele. Three out of four QTLs were identified in the vicinity of QTL regions previously detected for ANL using simple interval mapping based on a LOD score > 3.0 (Cai and Morishima 2002). Moreover, The two QTLs, *qANL-5* and *qANL-9*, were also identified near regions where Xiong et al. (1999) detected two of the seven QTLs for ANL using simple interval mapping based on a LOD score > 2.4. The results from this study, together with those reported by Xiong et al. (1999) and Cai and Morishima (2002), suggest that the variation in anther length between cultivated rice and its wild relatives was controlled by a relatively large number of QTLs compared to pistil traits. Comparative mapping among different populations is needed to confirm this hypothesis, since different field conditions and thresholds were used to detect QTLs in these studies.

Glume size

QTLs for all traits related to glume size were detected on chrs. 2, 4, 5, 6, 8, 9, 10 and 11. Four QTLs for LML were identified on chrs. 4, 6, 8 and 11, ranging R^2 from 11.0 to 23.6%. Two QTLs for PLL, explaining 21.4 and 10.4% of total variance were detected on chrs. 2 and 10, respectively. The two regions where *qPLL-2* and *qLML-4* were located are in agreement with a previous study which mapped glume length QTLs in chrs. 2 and 4, respectively (Redoña and Mackill 1998). *qPLL-10* was identified near regions where two glume-length QTLs reported by Huang et al. (1997) and Redoña and Mackill (1998) were in the long-arm region of chr. 10. Although the lengths of lemma and palea were positively correlated (r = 0.96), their QTLs were not located together. From comparison of QTL analyses between the lengths of lemma and palea, some regions where those QTLs were detected were recognized with suggestive QTLs for another trait, which were not revealed in this study (data not shown). These suggest that the lengths of lemma and palea are affected by multiple genes with small effects. Two QTLs for LMT, explaining 21.9 and 30.5% of total variance, were detected on chrs. 5 and 8, respectively. Three QTLs for PLT, explaining 9.9 to 28.6% of total variance were detected on chrs. 2, 5 and 8. The QTLs for LMT and PLT were located together on chrs. 5 (*C43 – APAGE1*) and 8 (*R1394A – G2132*). These QTLs are likely to play a role in the development of glume thickness.

These results indicate that the formation of lemma and palea is partially controlled by common chromosomal regions. However, all QTLs for length and thickness were

not detected in the same chromosomal regions, suggesting that these two components are controlled by different genes. This relationship of QTLs for traits related to glume size confirms the phenotypic correlations observed among them.

Three QTLs for AGO, explaining 11.8 to 13.4% of total variance were detected on chrs. 2, 8 and 9. The presence of W1944 alleles at *qAGO-2* resulted in an increased maximal angle of glume opening, while the presence of Pei-kuh alleles at*qAGO-8* and*qAGO-9* also increased it. The *qAGO-2* was linked with *qPLL-2* on chr. 2 (*RG322 – G275*), while the other two QTLs mapped to unique locations. AGO was weakly correlated with only two glume traits (LMT and PLT) at the phenotypic level. This result indicates that AGO is principally controlled by different and unique genetic factors.

Glume shape

A total of six QTLs with small effects, ranging from 11.2 to 17.3 of R^2 were detected for 1CSP, 2CSL and 3CSL on chrs. 1, 5, 7, 9, 11 and 12, but not for the other three traits of glume shape. Three QTLs were detected for 1CSP on chrs. 5, 7 and 11, ranging from 12.4 to 17.3% of R^2. The presence of W1944 alleles at all QTLs resulted in an increased 1CSP. *q1CSP-5* was identified in the interval *C43 – APAGE1* on chr. 5, as were *qLMT-5* and *qPLT-5*. *q1CSP-11* mapped along with *qLML-11* in the interval *G24 – RZ141* on chr. 11. The *q1CSP-7* is likely to be related only with glume shape, since this is not associated with any other QTL for glume size. Two QTLs for 2CSL, which were detected on chrs. 1 and 12, explained 13.1 and 11.2% of total variance, respectively. One QTL for 3CSL, explaining 16.1 of total variance, was detected on chr. 9. These three QTLs were independently located for the QTLs of 1CSP. This observation suggests that each glume shape is completely controlled by different genes.

Relationships among QTLs for different organs

To understand the genetic bases of relationships among the three different organs, we compared the locations of QTLs for them. Of 31 QTLs affecting the three organs, ten were located together in four specific regions on chrs. 2, 4, 5 and 9. Two regions were associated with both stamen and glume. These were in the interval *BCD349 – Amp1* on chr. 2 (*qANL-2* and *qPLT-2*) and *C43 – APAGE1* on chr. 5 (*qANL-5, qLMT-5, qPLT-5* and *q1CSP-5*). In addition, the interval *R288 – G162* on chr. 4 was related with both pistil (*qSTL-4*) and glume (*qLML-4*). Veldboom et al. (1994) and Xiao et al. (1996) observed that QTLs for correlated traits are often found in the same regions. Although this trend was observed in the three regions above, *qRES-10* and *qPLL-10* mapped together on chr.10 (*G37 – C16*), but did not exhibit significant phenotypic correlations. To confirm whether these phenomena are due to the pleiotropic effect of a single gene or to close linkage between two genes, fine mapping using near-isogenic lines is necessary.

Comparison of the locations of QTLs affecting pistil, stamen and glume revealed that most QTLs are located on different chromosomal regions, although some are adjacent to those for other floral traits. This observation suggests that phenotypic variations of the three organs are primarily controlled by genes unique to each organ, while some regions that are associated with more than one organ partially affect those organs. From the viewpoint of floral trait differentiation, the lemma and palea originate from the tunica cell, while the stamen develops from the corpus cell of the apical meristem. Differentiation of the pistil differs from that of other organs, since it is formed when the entire apex bulges after the apex has become very flat (Takeoka et al. 1993). The different patterns of development among the three organs agree with our hypothesis that phenotypic variation of each organ is primarily controlled by different genes. However, further study is needed to elucidate how both unique and overlapping QTLs for these traits are involved in the development of spikelet organs in rice.

QTLs for the mating system between cultivated and wild rice

This study revealed that the floral traits affecting differences in the mating system between cultivated rice and its wild relatives were the result of multiple QTLs having small effects. Bernacchi and Tanksley (1997) identified most major QTLs for several floral traits related to pollination near the region where a self-incompatibility locus was detected, using a backcross population of the self-compatible tomato and its wild self-incompatible relative. Bradshaw et al. (1998) detected one major QTL explaining more than 25% of total variance in 9 out of 12 floral traits that distinguish between bird-pollinated and bee-pollinated monkeyflowers. They suggested that major genes contributing to floral morphology are partly associated with reproductive isolation between the two sympatric species. From the viewpoint of floral morphology associated with the mating system between outcrossing to selfing, Georgiady et al. (2002) also reported that four of the six traits distinguishing between selfing and outcrossing in currant tomato had a QTL with a major effect. However, Lin and Ritland (1997) found that quantitative traits responsible for differences in the selfing rate between the outbreeding and inbreeding yellow monkeyflowers were primarily controlled by QTLs having small effects. They argued that the majority of the variation in traits related to mating system were most likely due to alleles with a small effect. Our findings in rice are consistent with this observation. The results of this study, and those of Lin and Ritland (1997) using an LOD threshold of 2.5 and 2.4, respectively, were different from those reported by Georgiady et al. (2002). This discrepancy might be attributed to their high threshold of QTL detection. They detected 18 QTLs using an LOD

threshold of 2.4, and finally five significant QTLs remained through 1,000 permutation tests at a 5% level. Although we also estimated the putative QTLs using the permutation test, the LOD threshold of 2.5 was used for QTL detection to avoid missing the QTL with a small effect according to the hypothesis that quantitative variation of floral traits affecting the mating system is primarily controlled by genes with a small effect (Lin and Ritland 1997). Our data indicate that minor genes play a more important role than major genes in rice floral morphology that characterizes outcrossing and selfing types. Anther length is a typical example. The ANL of *O. rufipogon* was four times that of *O. sativa* in this study, and several ANL QTLs with small effects were detected. More information about other rice populations will be required to understand the role of floral trait QTLs in the evolution of mating systems in rice.

Utilizing QTLs of floral morphology

Cultivated rice is an autogamous crop but, in heterosis breeding, parental lines need to have floral structures suitable for outcrossing, like those of its wild relatives in order to increase yields of hybrid seeds. For instance, AGO is an important trait for influencing the outcrossing rate in rice (Rangaswamy and Kumar 1995). However, it may be difficult to introduce the wild rice alleles using phenotypic selection for AGO in a large number of plants during the flowering time because of the short duration of glume opening (Virmani and Athwal 1973; Parmar et al. 1979). AGO was also controlled by multiple QTLs in our study. Therefore, when we breed or study floral traits such as AGO, the markers closely linked to the target QTLs shown here will be a useful tool for marker-assisted selection or map-based cloning.

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