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Mapping of QTLs for Vascular Bundle System and Spike Morphology in Rice, *Oryza sativa* L.

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Summary

We carried out quantitative trait loci (QTL) analysis for three years to identify genes controlling the number of vascular bundles (Vb) in the peduncle and primary rachis branch (Rb) in rice (*Oryza sativa* L.). Sixty-five recombinant inbred lines derived from the cross between a *japonica* variety Asominori and an *indica* variety IR24 were used for QTL mapping and 289 markers were employed to identify QTLs. Nine QTLs for Vb were detected on chromosomes 1 (two regions), 4, 5(two regions), 6 (two regions), 11 and 12. One of the QTLs on chromosome 6 was detected in all 3 tested years. Three QTLs on chromosomes 1, 4 and 12 were identified in 2 years. Most effective QTLs were found on chromosomes 4 and 6, explaining 20-23% of the total variance. The alleles of IR24 increased Vb except for QTL on chromosome 4. One of the 9 QTLs for Vb was located close to the region established as QTLs for the number of spikelet and grain. For the number of Rb, three QTLs on chromosomes 2 and 8 (2 of 4 regions) were commonly detected in all 3 tested years and five others on chromosomes 4, 6 and 8 were found in at least one year. The QTL on chromosome 8 was the most effective and explained 18-25% of the total variance. Except for QTL on chromosome 6, the alleles of Asominori increased Rb. The expression of QTLs was more stable in Rb than in Vb. The QTLs for Vb and Rb and gene loci of diagnostic traits for the differentiation between *indica* and *japonica* types are discussed.

Key Words : *Oryza sativa*, QTL, vascular bundle, rachis branch.

Introduction

The vascular bundle system in plants plays an important role for the transport of photosynthetic products, water, nutrients, and so on. A significant positive correlation between grain yield and the number of vascular bundles or the area of phloem in the peduncle, the first internode below the panicle, have been reported in oat (Housely and Peterson 1982), various species of *Triticum* and *Aegilops* (Evans *et al.* 1970), and winter wheat varieties (Nátrová and Nátr 1993).

Sasahara *et al.* (1982) reported the presence of a wide variation in the number of large vascular bundles in the peduncle in cultivated rice, *Oryza sativa* L., with *indica* varieties having more large vascular bundles and more grains per panicle than *japonica* varieties. Furthermore, Fukuyama and Takayama (1995) revealed that the ratio of the number of large vascular bundles (Vb) in the peduncle to that in the primary rachis branch (Rb), which was called V/R ratio, was quite different between Asian cultivars; Japanese varieties showed a V/R ratio lower than 1.4 with the mean of 1.0, while those from Nepal, Bangladesh and India exhibited a higher V/R ratio than 1.3 with a mean of 1.8. Judging from the phenol reaction, they concluded that the V/R ratio differentiated between *indica* and *japonica* types. Therefore, genetic analysis of the number of Vb and Rb is of great value not only for the improvement of yield potential but also for studies on *indica-japonica* differentiation.

The numbers of Vb and Rb are important quantitative traits agronomically, and are very difficult to analyze based on the classical genetic method. The advent of quantitative trait loci (QTL) analysis has made it possible to map and characterize the polygenes underlying quantitative traits. In rice, QTL analysis has been conducted in many agronomic characters such as heading date, plant height, yield and its related traits (Xiao *et al.* 1996, Lin *et al.* 1996, Fukuta *et al.* 1996, Lu *et al.* 1996, Yano *et al.* 1997, Zhuang *et al.* 1997). At the same time, the variation in the expression of a QTL under various environments has been pointed out (Paterson *et al.* 1991, Stuber *et al.* 1992, Zhuang *et al.* 1997 and Lu *et al.* 1996), which seems to be a serious problem when QTLs are aimed at the marker of selection in breeding program. In this study, we performed QTL analysis for vascular bundles in the peduncle and rachis number in panicles of rice, and examined the stability of the expression of these QTLs during a three-year period using a *japonica* × *indica* recombinant inbred population.

Materials and Methods

Plant materials

A total of 65 recombinant inbred (RI) lines were used. They were constructed by Tsunematsu *et al.* (1996) from

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the cross between a *japonica* variety, Asominori, and an *indica* variety, IR 24, with a single seed descent method. The RI lines were grown at Joetsu (37 6'N, 138 15'E) in 1995, and Niigata (37 55'N, 139 3'E) in 1996 and 1997; the generations were of F_9, F_{10} and F_{11}, respectively. Thirty plants of each RI line and their parents were cultivated in 1995 and 5 plants in 1996. After heading, 3 panicles were collected from longer tillers of a plant randomly selected under the apprehension of heterozygosity of RI lines. In 1997, 5 plants of RI lines and the parents were grown and the panicle was taken from the longest tiller of each plant. The transverse hand section with 1mm thickness was made at 1cm below the neck of panicle and the number of Vb was observed under a dissecting microscope, and Rb of the same panicle was counted. The ratio of the number of Vb to that of Rb was regarded as the V/R ratio.

The heritability (h^2) in the broad sense for the numbers of Vb and Rb and the V/R ratio was estimated from the analysis of variance for the data in the 1997 trial, since each RI line included 5 plants. The h^2 was calculated as V_L/V_T, where V_L and V_T indicate the variance among RI lines and the total variance, respectively.

RFLP linkage map and QTL analysis

The RFLP linkage map of Asominori/IR 24 and the RFLP segregation data of RI lines were provided by the Japanese Rice Genome Research Program (Tsunematsu *et al.* 1996). Although the RFLP map had 375 markers, some of them were located on the same locus. Then, we used 289 subsets of the markers without overlapping, for which the average interval of adjacent two loci were 4.4 cM.

The QTLs for the numbers of Vb and Rb and the V/R ratio were analyzed using the interval analysis of a computer program, QGENE (Nelson 1997). In this method, the log-likelihood (LOD) score indicates the strength of the data supporting the hypotheses about the existence of the QTL for the numbers of Vb and Rb and the V/R ratio. An LOD score of the marker of 2.0 or more in any of the 3 tested years, was considered to indicate that the locus of RFLP marker was linked with the QTL for the character. We used the nomenclature of putative QTL according to McCouch *et al.* (1997), where the chromosome number and locus number on the same chromosome are designated after the abbreviated capital letters of the character. The proportion of the total phenotypic variation explained by each QTL was calculated as an iR^2 value (iR^2 = ratio of the sum of squares explained by the QTL to the total sum of squares).

Results

Phenotypic variation

Fig. 1 shows the parental means for the numbers of Vb, Rb and the V/R ratio, and the distributions of RI lines in 1995, 1996 and 1997. In Asominori, there were no differences in the number of Vb among the 3 years (10.3-10.8), while IR 24 bore more Vb (24.2) in 1996 than in 1995 and 1997 (20.0 and 19.4). In the number of Rb, Asominori varied from 9.0 to 10.2 and IR 24 from 11.7 to 12.8, both parents being fewer in 1995 than in the other 2 years. The V/R ratio of Asominori ranged from 1.04 to 1.15 in the three years and those of IR 24 from 1.60 to 1.89.

Segregation for the three characters in RI lines showed a continuous distribution in all examinations. As shown in Fig. 1, the transgressive segregations were observed in Rb in all three years to the direction of both low and high number. In 1997, the transgressive segregants were observed with a higher number of Vb and higher V/R ratio. According to the analysis of variance, the difference in the three characters among RI lines was

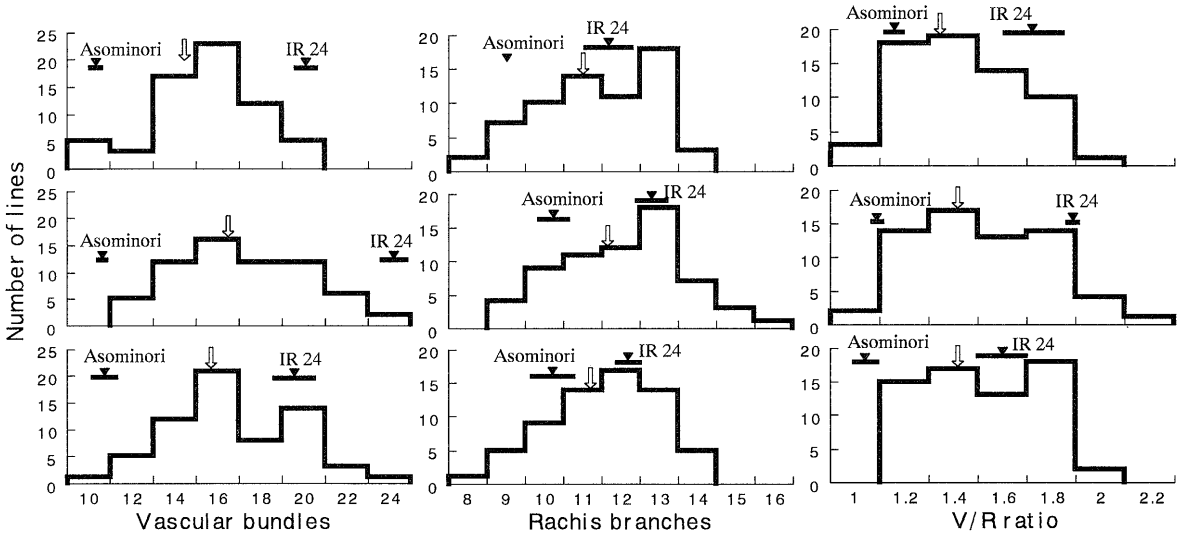

Fig. 1. The distributions of the number of large vascular bundles in the peduncle (left column) and primary rachis branch(middle column) and the V/R ratio(right column) observed in the three years in RI lines. Solid arrows and bars show the means and SE of the parents. Means of RI lines are indicated by white arrows. Uppermost: distribution in 1995, Middle: in 1996, and Bottom: in 1997.

highly significant in all 3 years (data not shown). The h^2 for the numbers of Vb and Rb and the V/R ratio were as high as 0.707, 0.606 and 0.655, respectively.

Mapping QTLs for Vb and Rb

A total of 9 QTLs for the numbers of Vb were detected and tentatively named *qNVB-1-1, qNVB-1-2, qNVB-4-1, qNVB-5-1, qNVB-5-2, qNVB-6-1, qNVB-6-2, qNVB-11-1* and *qNVB-12-1*, and these QTLs were apart from the nearest marker with the distance of 0.0-4.7 cM (Table 1, Fig. 2). Only one QTL, *qNVB-6-2*, was commonly detected through all 3 years, and 3 (*qNVB-1-1*, *qNVB-4-1* and *qNVB-12-1*) of the 9 QTLs were detected in 2 of the 3 years. The *qNVB-1-1* and *qNVB-1-2* were 44 cM apart from each other, while two QTLs on chromosomes 5 and 6 were close to each other with the distance of 4 and 16-17 cM, respectively (Table 1, Fig. 2). Phenotypic variations explained by the putative QTLs ranged from 13.3 to 22.9% in 1995, from 12.8 to 21.4% in 1996 and from 12.6 to 20.0% in 1997. The most effective QTL differed between years; in 1995, the highest variance explained was observed at *qNVB-4-1* (22.9%), while in 1996 and 1997, *qNVB-6-2* was highest (21.4 and 20.0%, respectively, Table 1). According to multiple regression, the total variance explained by the QTLs was calculated to be 48.7% in 1995(5 QTLs), 64.0% in 1996(7 QTLs) and 33.1% in 1997(2 QTLs). Except for *qNVB-4-1*, the alleles of IR 24 increased the number of Vb in all three years, their additive effect ranging from 0.96 to 1.64.

Table 1. Location and effect of putative QTLs for the number of large vascular bundles (Vb) in the peduncle and primary rachis branch (Rb) in the recombinant inbred lines of Asominori/ IR 24

Trait	Year	QTL	cM	Marker interval[1]		Distance[2]	LOD of peak	Additive[3] effect	Varience explained (%) Single[4]	Maltiple[5]
Number of Vb	1995	*qNVB-1-1*	103	*R2159*	***C178***	0.9	2.29	− 0.96	14.8	
		qNVB-1-2	147	*C955*	***R3192***	4.7	2.14	− 1.20	13.3	
		qNVB-4-1	30	*XNpb114*	***R2783***	0.2	3.89	1.22	22.9	48.7
		qNVB-6-2	86	***C962***	*XNpb170*	1.6	2.61	− 1.09	16.6	
		qNVB-11-1	79	***C1350***	*C794A*	0.1	2.38	− 0.99	16.0	
	1996	*qNVB-1-1*	103	*R2159*	***C178***	0.9	2.00	− 1.18	13.3	
		qNVB-4-1	30	*XNpb114*	***R2783***	0.2	2.09	1.21	12.8	
		qNVB-5-1	73	***C128***		0.0	2.07	− 1.17	13.7	
		qNVB-5-2	77	*R2117*	***C1402***	0.5	2.48	− 1.28	16.7	64.0
		qNVB-6-1	70	*G1314A*	***XNpb12***	3.5	2.57	− 1.52	16.6	
		qNVB-6-2	86	***C962***	*XNpb170*	1.6	3.51	− 1.64	21.4	
		qNVB-12-1	57	***R367***	*C3029B*	3.0	2.59	− 1.49	17.4	
	1997	*qNVB-6-2*	87	***C962***	*XNpb170*	2.6	3.38	− 1.51	20.0	33.1
		qNVB-12-1	58	***R367***	*C3029B*	4.0	2.07	− 1.27	12.6	
Number of Rb	1995	*qNRB-2-1*	56	***XNpb67***	*C621*	0.1	2.43	0.61	13.9	
		qNRB-4-1	53	***XNpb49***	*XNpb271*	1.7	2.07	0.62	16.2	
		qNRB-4-2	64	*C891*	***C975***	0.6	2.23	0.60	15.5	
		qNRB-6-1	65	***G1314A***	*XNpb12*	6.8	2.39	− 0.78	12.6	55.1
		qNRB-8-1	5	*XNpb397*	***R2027***	0.8	3.51	0.75	21.9	
		qNRB-8-2	15	*XNpb56*	***R2382***	5.9	3.74	0.86	21.1	
		qNRB-8-3	23	***XNpb126***	*C621C*	0.7	4.26	0.82	24.9	
		qNRB-8-4	29	***C621C***	*R727*	1.2	3.85	0.78	22.7	
	1996	*qNRB-2-1*	56	***XNpb67***	*C621*	0.1	2.10	0.61	10.7	
		qNRB-4-1	51	*C621B*	***XNpb49***	0.3	2.23	0.65	17.4	40.1
		qNRB-8-1	5	*XNpb397*	***R2027***	0.8	3.01	0.75	19.0	
		qNRB-8-4	31	***R727***	*C1115*	0.6	2.30	0.68	14.6	
	1997	*qNRB-2-1*	57	***XNpb67***	*C621*	1.1	2.92	0.62	15.0	
		qNRB-8-1	6	***R2027***	*G1149*	0.2	2.92	0.61	18.2	31.4
		qNRB-8-4	31	***R727***	*C1115*	0.6	2.14	0.55	14.2	

[1] Gothic letter indicates the nearest marker.

[2] Distance from the nearest marker to putative QTL.

[3] + and − mean that the alleles of Asominori and IR 24 increase respectively the number of Vb or Rb.

[4] Variance explained by the nearest marker.

[5] Variance calculated by the multiple regression of all QTLs.

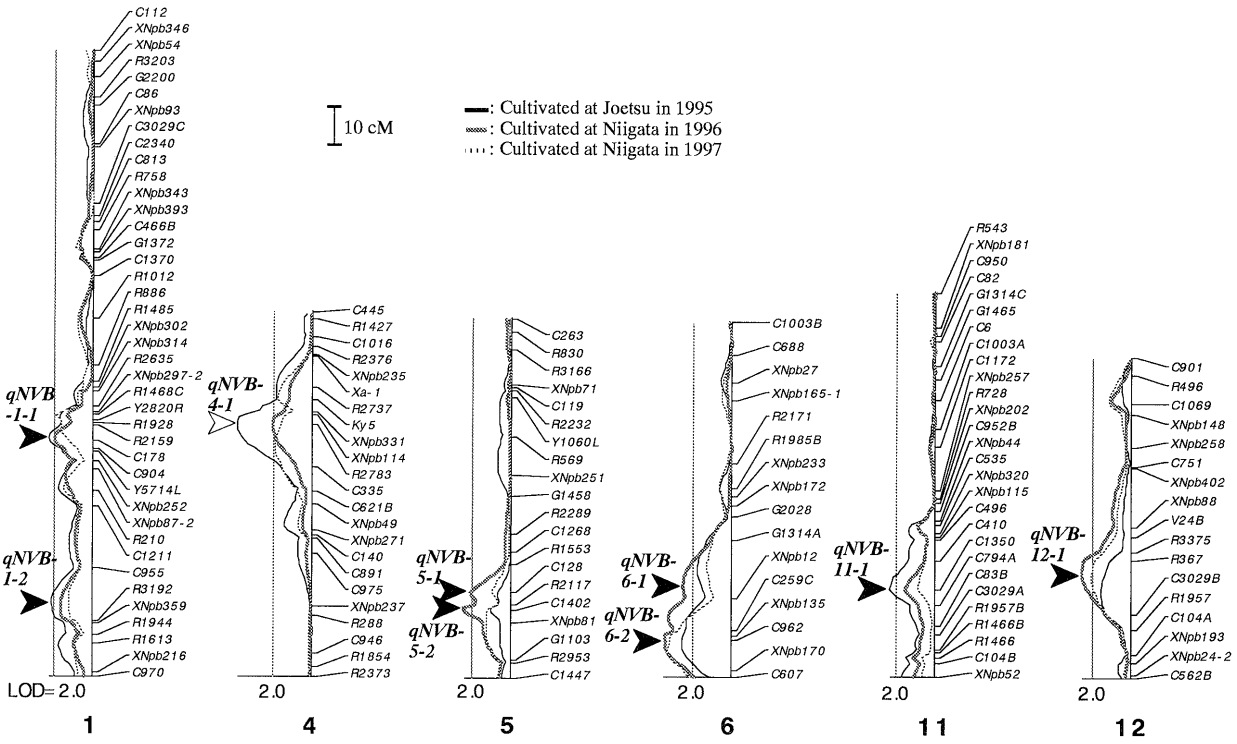

Fig. 2. Interval analysis for the number of large vascular bundles in the peduncle using RI lines derived from the cross between Asominori (*japonica*) and IR 24 (*indica*). Arrowheads indicate the location of each putative QTL, and open and darkened-heads indicate that *japonica* and *indica* alleles increase Vb, respectively.

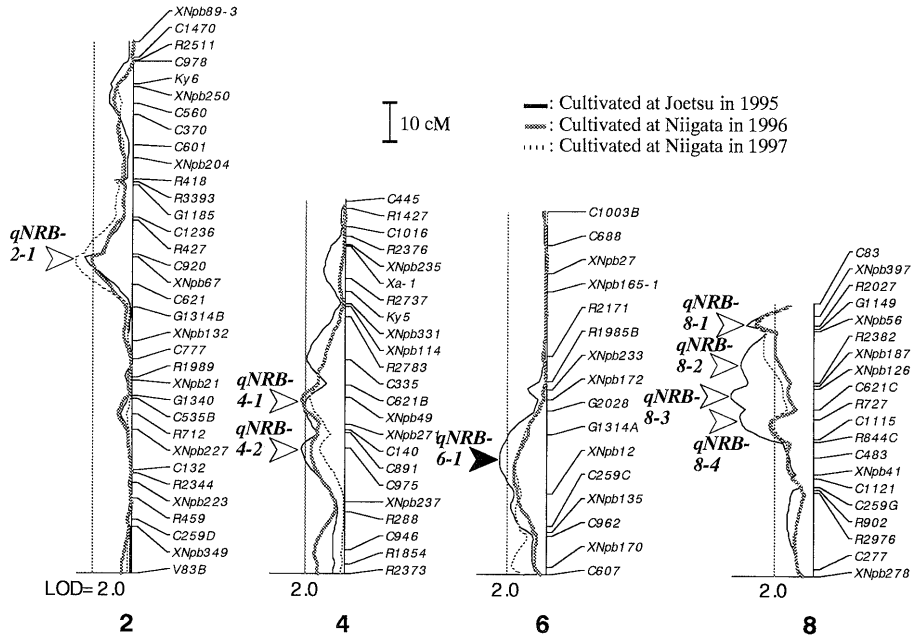

Fig. 3. Interval analysis for the number of primary rachis branches using RI lines derived from the cross between Asominori (*japonica*) and IR 24 (*indica*). Arrowhead indicates the location of putative QTLs, and open and darkened-heads indicate that *japonica* and *indica* alleles increase Rb, respectively.

The QTLs for the numbers of Rb were detected on 8 locations; *qNRB-2-1*, *qNRB-4-1*, *qNRB-4-2*, *qNRB-6-1*, *qNRB-8-1*, *qNRB-8-2*, *qNRB-8-3* and *qNRB-8-4* (Table 1, Fig. 3). These QTLs were 0.1 to 6.8 cM apart from the nearest marker. Each QTL explained the variance from 10.7% to 24.9% in the 3 years, and all together explained the variance from 31.4% to 55.1%. At the QTL, *qNRB-6-1*, the alleles of IR 24 increased Rb with the additive effect of 0.78, while at the remaining 7 QTLs, the alleles of Asominori increased Rb with the additive effect from 0.55 to 0.86. Three QTLs, *qNRB-2-1*, *qNRB-8-1* and *qNRB-8-4* were com-

monly detected in all 3 years, and *qNRB-4-1* was common in 2 of the 3 years (1995 and 1996). The QTL, *qNRB-6-1*, was detected only in 1995. Most effective QTLs based on the variance explained were *qNRB-8-3* with the value of 24.9% in 1995 and *qNRB-8-1* with 19.0 and 18.2% in 1996 and 1997, respectively.

Finally, we tried to map the QTLs for the V/R ratio. A total of 8 QTLs were detected on chromosome 1, 3, 5(two regions), 8(three regions) and 11(data not shown). However, seven QTLs excluding that on chromosome 3 were respectively very close to the QTLs for the number of Vb and Rb; *qNVB-1-2*, *qNVB-5-1* and *2*, *qNRB-8-2*, *3* and *4* and *qNVB-11-1*. Also, the alleles on the QTLs for the V/R ratio showed the same gene action as those for the number of Vb and Rb. The QTL on chromosome 3 for the V/R ratio was found only in 1996, and the peak LOD score, 2.21, was slightly higher than the threshold.

Discussion

QTLs for vascular bundle system

This is the first report on the QTLs for the number of vascular bundles in the peduncle of rice, and a total of 9 QTLs were detected on chromosomes 1(two regions), 4, 5(two regions), 6(two regions), 11 and 12. Among them, *qNVB-5-1* and *qNVB-5-2* on chromosome 5 were only 4 cM apart from each other. Tanksley (1993) described that two or more polygenes closer together than approximately 20 cM will usually appear as a single QTL. However, both of the QTLs for the number of Vb exhibited the same pattern of LOD peaks in three tested years, although non-significant values were included. Therefore, the QTLs on chromosome 5 appears to consist of not one but two QTLs. In the region between *XNpb12* and *C259C* on chromosome 6, a significant peak appeared in 1996, but disappeared in 1995 and 1997. Therefore, we presume that the peak in this region involved no QTLs.

For the number of Rb, a total of 8 QTLs were detected on chromosomes 2, 4(two regions), 6 and 8(four regions) in this study. Among them, *qNRB-4-1* and *qNRB-4-2* were only 11 cM apart from each other, but in this region between *C621B* and *XNpb237*, an aspect of the LOD curves was coincident in all three tested years, though non-significant values were included. Thus, we concluded that there were at least two QTLs on chromosome 4. The QTLs on chromosome 8 were more complicated; *qNRB-8-1* and *qNRB-8-4* were 24-26 cM apart and LOD peaks were significant in all 3 years, while *qNRB-8-2* were only 10 or 14 cM apart from *qNRB-8-1* or *qNRB-8-4*, and the LOD score in 1996 was not significant. Also, *qNRB-8-3* was only 6 cM apart from *qNRB-8-4*, and LOD scores in 1996 and 1997 were not significant. Although we considered that 4 QTLs were on chromosome 8, high resolution mapping in this region or a more powerful mapping method will be needed to assess the precise number of QTLs for the number of Rb.

As mentioned, the 8 putative QTLs for the V/R ratio detected in this study were very close to the QTLs for the number of Vb and Rb, or significant in only one year. From these results, we could not specify the QTLs affecting alone the V/R ratio. However, it may be possible that the QTLs for V/R ratio exist in a different cross combination.

The heritability for Vb and Rb were estimated in this study to be 0.707 and 0.606, respectively, and the total variance explained by putative QTLs ranged from 33.1% to 64.0% in Vb, and from 31.4% to 55.1% in Rb. From these results, we could expect that 47 to 91% of the total genetic variation in the number of Vb were explained by 9 QTLs and 52 to 91% by 8 QTLs for Rb. Thus, other QTLs for both characters will remain undetected.

In this study, the degree of expression of the putative QTLs was largely influenced by the year tested, although the cultivation sites were almost the same, Joetsu and Niigata. Among a total of 17 QTLs for Vb and Rb, only one for Vb(*qNVB-6-2*) and three for Rb(*qNRB-2-1*, *qNRB-8-1* and *qNRB-8-4*) were detected through all three trials. On the other hand, 3 QTLs for Vb(*qNVB-1-1*, *qNVB-4-1*, *qNVB-12-1*) and a QTL for Rb(*qNRB-4-1*) were detected in two years, and the remaining 5 QTLs for Vb (*qNVB-1-2*, *qNVB-5-1* and *-2*, *qNVB-6-1* and *qNVB-11-1*) and 4 QTLs for Rb (*qNRB-4-2*, *qNRB-6-1*, *qNRB-8-2* and *qNRB-8-3*) were detected in only one year. This implies that the expression of the QTL is more stable in Rb than Vb. In fact, the parental *indica* variety of RI lines, IR 24, was highly variable in the number of Vb among 3 years as compared with that of Rb. In *japonica*, the development of Vb in Rb is synchronized with that of Vb in the peduncle, and the Vbs in both organs are connected with each other, resulting almost in the same number of Vb in the peduncle and Rb (Kawahara and Chonan 1960, Kawahara *et al.* 1968). On the other hand, in *indica* the vascular bundles enter from the primary rachis branches(V_P) and from the secondary rachis branches (V_S). Since V_P and V_S are independently connected with the Vb in the peduncle without fusion, the V/R ratio becomes higher in *indica* (Fukushima and Akita 1997). As is well known, the number of secondary branches is considerably affected by the environmental condition. Therefore, it seems that unstable development of V_S to the secondary branch results in low expression of the QTLs for Vb, although further investigation on the morphogenesis of Vb and Rb, especially in *indica*, is needed.

The variation in the expression of QTLs between years observed in this study indicates that the detection of QTL for the traits such as Vb, Rb, yield and its components may need a trial across several years.

Relationships between QTLs for Vb system and other traits or genes

Since the vascular bundles transport photosynthates, their number may be reflected in the yield or its components. Sasahara *et al.* (1982) described that the *indica* varieties had more Vbs in the peduncle and also more grains per panicle than the *japonica* varieties, and suggested that many Vbs in *indica* variety were related to a higher increasing rate of ear weight. The QTLs for the number of spikelets have been reported to be located between *G122* and *G1314A* on chromosome 6(Lu *et al.* 1996). Since *G122* is located nearby *G2028* on chromosome 6(Harushima *et al.* 1998), it is possible that the QTLs for the number of spikelets are located between *G2028* and *G1314A* used in this study as the markers, this region being near the QTL for the number of Vb, *qNVB-6-1*, detected in this study. Moreover, the QTL for the grain number per panicle has been reported by Lu *et al.* (1996) to be located between *G294* to *G329* on chromosome 6, which are located near *R688* and *C259C,* respectively(Harushima *et al.* 1998), and *R688* is the same locus as *XNpb233* in the map of Tsunematsu *et al.* (1996). Therefore, the region from *C259C* to *XNpb233* covers the putative QTL for the number of Vb, qNVB-6-1. Further investigation will be needed to decide whether the effects of these QTLs for the numbers of spikelets and grains per panicle are due to pleiotropism or close linkage of the QTL for Vb, because the materials and most of the molecular markers used are different in each experiment.

The same is pointed out in the case of QTLs for the number of Rb detected in this study; *qNRB-8-1*, *qNRB-8-2*, *qNRB-8-3* and *qNRB-8-4* may be the same or closely linked to the QTL for the number of primary rachis identified by Lin *et al.* (1996). However, it is difficult to know precisely their relations because of the difference in markers used. On the other hand, the QTL analysis by Yoshimura *et al.* (1998) could be directly compared with the results of the present study because of the same RI population used as the materials. According to Yoshimura *et al.* (1998), one of the QTLs for heading date, dth-A8b, is located on *R727*, where the QTL for Rb, *qNRB-8-4*, is detected with the distance of 0.6 cM. This indicates that QTLs for both heading date and Rb are identical or closely linked. It is possible that the rachis number is smaller at an earlier heading date.

Since the number of Vb and the V/R ratio clearly differentiate among *indica*, tropical-*japonica* and temperate-*japonica* types (Fukuyama *et al.* 1996), the QTLs for the number of Vb and also Rb, which affect the V/R ratio, may by related to the phylogeny of cultivated rice, or with the *indica-japonica* differentiation. Some genes for diagnostic characters for classification of *indica* and *japonica* types have been located by conventional methods or using molecular markers. A gene for the phenol reaction of the kernel, *Ph*, is located between *R2376* and *C600* on chromosome 4 (Lin *et al.* 1994), which is close to *qNVB-4-1*. Another diagnostic trait, mesocotyl elongation in a dark condition, is conditioned by the QTLs linked with *G175* on chromosome 3, *G1091* on chromosome 6 and *G320* on chromosome 11 (Katsuta-Seki *et al.* 1996). *G1091* is located between *XNpb12* and *C259C* on chromosome 6 (Kurata *et al.* 1994, Tsunematsu *et al.* 1996, Yoshimura *et al.* 1997). Therefore, it is likely that the QTLs for *qNVB-6-1*, *qNVB-6-2* and *qNRB-6-1* are closely linked with that for mesocotyl elongation.

The relationship of the gene loci above mentioned will show the possibility that the genetic factors included in *indica-japonica* differentiation, if any, may be located near the genes for diagnostic characters and also the QTLs for the Vb system, or some of the latter may directly influence the differentiation itself. Since many genes are concerned with the *indica-japonica* differentiation, as mentioned by Morishima and Oka (1981), further investigation for these genes will be needed to resolve the mechanism of cultivated rice differentiation.

Acknowledgments

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