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A QTL Cluster for Plant Architecture and Its Ecological Significance in Asian Wild Rice

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The wild progenitor (*Oryza rufipogon*) of Asian rice (*Oryza sativa*) shows a wide range of variations in life-history traits, forming an annual-perennial continuum. A conspicuous feature of an annual type of wild rice is represented by its adaptability to disturbed habitats, and its short stature with many tillers and a prostrate growth habit. The present study was carried out to examine the genetic differentiation between wild annual and cultivated (Japonica type) rice strains by quantitative trait locus (QTL) analysis. In total, 20 adaptive and/or domestication-related traits were evaluated in recombinant inbred lines (RILs). A total of 28 putative QTLs were detected across the genome. Six QTLs responsible for plant architecture were located on the short arm of chromosome 7. The near-isogenic line with the region containing the QTL cluster confirmed that the QTLs exerted a significant effect on the plant architecture in the genetic background of cultivated rice. A similar QTL cluster was also found in another annual strain of a different origin, suggesting that the QTL cluster might be predominant in annual wild rice. Furthermore, a QTL for tolerance to disturbance (simulated trampling) was detected within the region of the cluster on chromosome 7. These results are discussed in relation to their ecological significance in wild annuals of rice.

Key Words: *Oryza sativa*, *Oryza rufipogon*, annual type, domestication, disturbance, plant architecture, quantitative trait locus (QTL).

Introduction

Domestication of cultivated plants is one of the micro-evolutionary processes of adaptation under the influence of human activity (Harlan 1975). The domesticated forms have accumulated genes for adaptation to cultivated fields, which might be associated with the loss of adaptive genes to survive in natural habitats. Such genetic changes should involve a gradual process within a species, resulting in the diversification of an adaptive gene complex between cultivated and wild forms selected under contrasting growth environments. Recent molecular studies using quantitative trait locus (QTL) analysis have contributed significantly to our understanding of genome-wide differentiation between cultivated and wild forms, confirming that numerous genetic changes in morphological and physiological traits have taken place between them (Doebley and Stec 1991, Koinange *et al.* 1996, Xiong *et al.* 1999, Pocet *et al.* 2000, Burke *et al.* 2002).

Studies on domestication-related changes in various crops often showed that the genes responsible for differences from the progenitor were not distributed randomly in the whole genome but were clustered in limited chromosomal regions (Doebley and Stec 1991, Koinange *et al.* 1996, Pocet *et al.* 2000). Regardless of the ultimate cause of clustering of QTLs, which may result from either the pleiotropic effects of a single gene and/or the physical linkage among multiple genes, linked QTLs are simply assumed to preserve the cohesion of adaptive phenotypes for domestication. Such QTL clusters offer selective advantages, especially in cross-pollinated plants such as maize and pearl millet, because frequent hybridization and recombination may destroy the gene combination necessary for domestication (Le Thirry D' Ennequin *et al.* 1999). However, a limited number of clusters has also been found in predominantly self-pollinated plants such as rice (Xiong *et al.* 1999, Cai and Morishima 2002, Li *et al.* 2006a). Recent studies have indicated that a significant linkage disequilibrium persists only within 50 kb or 100 kb in predominantly self-pollinated plants such as *Arabidopsis* and rice, suggesting the occurrence of more frequent recombination in self-pollinated plants than previously expected (Garris *et al.* 2003, Nordborg *et al.* 2005). This indicated that adaptive gene complexes can be maintained in a crop genepool not only by tight linkage but also by other genetic and/or ecological mechanisms.

In Asian rice, the common wild rice, *Oryza rufipogon* Griff., is considered to be the wild progenitor of cultivated rice, *O. sativa* L. (Oka 1988, Chang 1995). *O. rufipogon* tends to show an annual-perennial continuum within a species (Oka and Morishima 1967, Sano and Morishima 1982),

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*Corresponding author (e-mail: onishi@abs.agr.hokudai.ac.jp) while it remains unclear to what extent annual and perennial types within *O. rufipogon* have contributed to the cultivated form (Sano *et al.* 1980, Oka 1988, Chang 1995). In addition, two major varietal groups, Japonica and Indica types, display well differentiated nuclear and organellar genomes as well as morphological and physiological characters (Glaszmann 1987, Oka 1988, Garris *et al.* 2005). Such ecotypes or varietal groups in Asian rice are characterized by contrasting sets of traits and tend to adapt to different environments. Genome-wide surveys using QTL analysis for domestication-related or adaptive traits between cultivated and wild rice have been reported between Indica and the perennial type (Xiong *et al.* 1999, Cai and Morishima 2002), Japonica and the perennial type (Lee *et al.* 2005), Indica and the annual type (Li *et al.* 2006a). Understanding the genetic basis of adaptation to different environments could promote the use of wild relatives as genetic resources for breeding to meet the changes in agricultural practices and demands. The objective of the present study was to analyze the genetic architecture for adaptive differentiation between Japonica type and annual wild rice, which are expected to accumulate the genes for adaptive changes because of their distinct growth environments.

Annual wild rice grows widely in tropical areas and occurs in shallow and temporary swamps as well as in disturbed habitats, while perennial wild rice grows in deeper-water fields and/or stable habitats (Morishima *et al.* 1984, Oka 1988). Disturbance was defined as follows; mechanisms which limit the plant biomass by causing its partial or total destruction (Grime 1979). Annual wild rice can grow near paddy fields where the habitats are markedly disturbed due to trampling and/or grazing (Morishima *et al.* 1984, Oka 1988). A conspicuous feature of an annual type of wild rice is represented by its adaptability to disturbed habitats, and its short stature with many tillers and a prostrate growth habit (Sano and Morishima 1982, Morishima *et al.* 1984, Oka 1988). The architecture of plants should display ecological significances for the gathering and allocation of resources, breeding systems, interactions with biotic and abiotic environmental factors, etc. (Grime 1979, Crawley 1997). In rice, genetic variation in the plant architecture is related to the yielding ability as well as adaptability under different growth environments (Jennings 1966, Jennings and Aquino 1968). In the present study, the QTL analysis between cultivated (Japonica type) and annual wild rice was performed for 20 adaptive and/or domestication-related traits including traits for the plant architecture and disturbance tolerance. We report here that annual strains of wild rice carry a QTL cluster responsible for the plant architecture and tolerance to disturbance on the short arm of chromosome 7.

Materials and Methods

Plant materials

The genetic stocks used consisted of two annual strains of *O. rufipogon* (W107 from India and W630 from Myanmar), two Japonica strains (A58 and T65) of *O. sativa* and a near-isogenic line (NIL), T65*Rc*(W107). A58 (Kokushokuto-2) and T65 (Taichung 65) were cultivated strains from Japan and Taiwan, respectively. T65*Rc*(W107) was derived from successive backcrosses (BC_5F_2) using T65 as the recurrent parent, carrying a fragment including *Rc* (*Red pericarp*) on chromosome 7 of W107 (Eiguchi and Sano 1995). The recombinant inbred lines (RILs) were developed from each A58 × W107 (W107RILs) and A58 × W630 (W630RILs) by the single-seed-descent method. The number of lines used was 79 (F_5) or 89 lines (F_6) for the W107RILs and 99 lines (F_7) for the W630RILs, respectively. To examine all the morphological and physiological traits, two to four plants per RIL and 12 plants of each parent were examined. Seeds were germinated in petri dishes in mid-April and the seedlings were transplanted to plastic pots (13 cm in diameter) filled with soil. The plants were grown in a vinyl greenhouse to avoid low temperatures in Sapporo, Hokkaido, Japan (43°N).

Determinations of morphological and physiological traits

In total, 18 morphological or physiological traits were investigated in the W107RILs and the parents (A58 and W107) as shown in Table 1. At the seedling stage, the length of the leaf sheath (2LSL) and leaf blade (2LBL) was examined for the second leaf. At 10 weeks after germination, leaf sheath length (LSL), tiller number (TN), tiller angle (TA) and leaf angle (LA) were determined. Later, days to heading from germination (HD), culm length (CL), panicle number (PN), panicle length (PL), number of spikelets per panicle (NSP), degree of spreading of panicle (SPR) and degree of shattering in mature seeds (SH) were recorded. CL, PN, PL, NSP, SPR and SH were observed at 40 days after heading. TA and SPR were evaluated visually and given scores from 0 (A58 type; erect) to 4 (W107 type; prostrate or lazy) for TA, and from 0 (A58 type; non-spreading) to 2 (W107 type; spreading) for SPR. SH was scored from 0 (A58 type; non-shattering) to 4 (W107 type; shattering), by gripping the panicles by hand at maturity. In addition, four seed traits, awn length (AWL), seed length (SL), seed width (SW) and apiculus hair length (AHL), were measured in unhulled seeds. The morphology of W630 was similar to that of W107. Only seven traits (LSL, TN, TA, LA, CL, PN and HD) were investigated in 99 lines among the W630RILs to confirm the presence of a QTL cluster for them, as mentioned later.

A58 is a landrace from the northernmost area of rice cultivation in Japan, while W107 and W630 are of tropical origin. Low temperature tolerance (LT) was estimated at the plumule stage in the W107RILs, since it might exhibit a latitudinal cline (Oka 1958). In each RIL, 10 seedlings with a plumule length of 1.5 cm were incubated at 0–1°C for 2 days with two replications, and thereafter the degree of injury was observed after growth at 26°C for 6 days. Each seedling was individually scored from 0 (resistant like A58) to 4 (susceptible like W107), and the average value was used as an index value. LT was evaluated in only 69 lines because of the limited number of seeds.

Determination of disturbance tolerance

The degree of tolerance to disturbances was evaluated by simulated trampling and grazing in 80 lines of the F_7 population in the W107RILs. A randomized block design was adopted with two blocks. Seeds were germinated in petri-dishes in the mid-May and three seedlings were transplanted into a plastic pot (13 cm in diameter). Six plants per RIL per treatment were examined. For the determination of the trampling tolerance (TRA), a hard board was pressed down vertically onto the plants at 40 days after germination (DAG), and the treatment was repeated every four days (five times). Then, the aboveground dry weight (DW) was measured at 70 DAG. For the estimation of grazing tolerance (GRA), each plants was cut at 3 cm above soil at 50 DAG and the DW of regrowth shoots was measured at 70 DAG. The degree of tolerance was quantified using log response ratio (lnRR; Hedges *et al.* 1999) calculated as follows;

ln RR = ln (DW in the disturbance treatment / DW in the control)

DW was measured after live biomass was dried at 80°C for two days.

Molecular markers used

Total DNAs were extracted from fresh leaves collected from approximately 10 two-week-old seedlings of each RIL according to the method of Murray and Thompson (1980). For map construction, 109 polymorphic markers were selected and used based on reported simple sequence repeat (SSR), sequence tagged site (STS), and cleaved amplified polymorphic sequence (CAPS) markers (http://rgp.dna.affrc.go.jp, http://www.gramene.org). In addition, a total of 150 miniature inverted repeat transposable elements (MITEs) markers were also used in the present study, according to the method of Takagi *et al.* (2003). Furthermore, four gene specific markers (*DFR*, *sd1*, KS-3 and G8008) were added for mapping. The specific primers for amplification were designed based on sequences available in the public data base (DDBJ accession numbers AB003495, AP003561, AP004127 and AP000399); 5′-atg tac ttc gtg tcc aag tca ttg-3′ and 5′-aaa gtc aaa ggt ata tct tgt aaa acg-3′ for *DFR*, 5′-gtg tcg ccg atg ttg atg ac-3′ and 5′-ctc gtc ttc tcc cct gtt aca a-3′ for *sd1*, 5′-ttc ggc cag ccg cac aat att t-3′ and 5′-ttt gct agc att tgc tta tca cca tcg-3′ for KS-3 and 5′-aac ctg att cag caa tat gga ctt-3′ and 5′-aag tag gaa tgt tga cag aca agg-3′ for G8008. To detect polymorphisms, the amplified products were digested with *Hae*III for *DFR*, *Msp*I for *sd1*, *Hin*dIII for KS-3 and *Ssp*I for G8008.

Data analysis

A linkage map was constructed based on the segregation of 263 polymorphic markers (150 MITE markers, 110 co-dominant and 3 dominant PCR-based markers) in the 79 lines of a F_5 population in the W107RILs by using Map Manager QTX (Manly *et al.* 2001) and JoinMap version 3.0 (Van Ooijen and Voorrips 2001). A linkage map was reconstructed in the 89 lines of a F_6 population in the W107RILs using 85 co-dominant PCR-based markers, which were common markers used in the F_5 population to cover the whole genome. The order of the PCR-based markers was fixed based on the published map (McCouch *et al.* 2002, http://rgp.dna.affrc.go.jp, http://www.gramene.org) during the calculation for the marker order and genetic distance. The map distance in centimorgans (cM) was determined using the Kosambi map function (Kosambi 1944). As for the segregation distortion in the RILs, the deviation from Mendelian inheritance was examined by χ^2 test from segregation for 110 co-dominant PCR-based markers.

Before QTL mapping, analysis of variance (ANOVA) and the Kruskal-Wallis test were carried out to determine whether genetic differences in the RILs were significant. Then, QTL analysis was conducted by simple interval mapping and MQM mapping methods using MapQTL version 4.0 (VanOoijen *et al.* 2002). In the first step, putative QTLs were identified by interval mapping. Then, one marker at each putative QTL was selected as a cofactor and the selected markers were used as genetic background controls in the approximate multiple QTL model of MapQTL. To refine the mapping and to identify linked QTLs, cofactor markers at each QTL were moved one by one around the putative QTL position, finally selecting the closest markers to the QTL, i.e., those maximizing the LOD score. The genome-wide LOD thresholds for a significant QTL were estimated by performing the permutation test for each trait (Churchill and Doerge 1994), as implemented in MapQTL. The quantitative trait data of the RILs were permuted 1000 times over the genotypes, and empirical LOD thresholds corresponding to the genome-wide significance at the 5% level were estimated to range between 2.9 and 3.1 for the various data sets. The direction of the genetic effect and the percentage of phenotypic variance explained (PVE) by each QTL, and the total variance explained by all the QTLs affecting a trait (multi-QTL PVE), were obtained using MapQTL in the final multiple-QTL model in which one cofactor marker was fixed per QTL. The tolerance to disturbance was analyzed by using the linkage map from the F_6 population of the W107RILs.

Results

Associations of morphological or physiological traits in the W107RILs

Eighteen morphological or physiological traits examined, excluding 2LBL, were significantly different between the parental strains (Table 1). ANOVA and the Kruskal-Wallis test indicated that the RILs showed a significant genetic variation for all the traits. The values in these traits were continuously distributed between the parental values in the W107RILs, although the four traits (2LBL, AWL, SL and AHL) showed a distinct transgression (Table 1).

Table 1. Twenty traits examined in the present study, and scores of the parents (A58 and W107) and the recombinant inbred lines from A58 × W107 (W107RILs)

Trait	Abbreviation	A58	W107	W107RILs	
				Mean	Min.–Max.
Morphological or physiological traits					
Seedling stage					
Leaf sheath length (cm)	2LSL	4.2 (0.2)	1.3 (0.2)	2.5 (0.6)	1.2–4.6
Leaf blade length (cm)	2LBL	3.2 (0.3)	3.3 (0.1)	3.0 (1.1)	1.2–6.1
Vegetative stage					
Tiller angle	TA	0 (0.0) (erect)	4 (0.0) (prostrate)	1.8 (1.2)	0.0–4.0
Tiller number	TN	12.5 (0.7)	23.3 (3.1)	17.0 (4.9)	9.0–28.5
Leaf sheath length (cm)	LSL	16.8 (0.4)	7.5 (0.5)	9.9 (3.0)	5.3–18.3
Leaf angle (degree)	LA	13.3 (10.4)	60.0 (5.0)	43.6 (23.7)	0.0–100.0
Maturity stage					
Culm length (cm)	CL	78.5 (1.7)	32.8 (3.4)	58.5 (12.5)	32.5–86.5
Panicle number	PN	10.8 (1.8)	28.6 (5.7)	16.1 (4.8)	8.0–35.3
Panicle length (cm)	PL	14.3 (0.4)	9.9 (0.6)	14.0 (2.6)	9.8–21.0
Spikelets per panicle	NSP	61.6 (6.6)	14.3 (1.0)	28.8 (9.8)	8.4–64.5
Spreading of panicle	SPR	0 (0.0) (non-spreading)	2 (0.0) (spreading)	0.5 (0.7)	0.0–2.0
Shattering habit	SH	0 (0.0) (non-shattering)	4 (0.0) (shattering)	2.8 (1.3)	0.0–4.0
Seed traits					
Awn length (cm)	AWL	4.7 (0.4)	6.2 (0.4)	7.4 (1.6)	3.8–11.0
Seed length (mm)	SL	6.3 (0.2)	6.7 (0.1)	6.9 (0.4)	6.0–8.1
Seed width (mm)	SW	3.1 (0.1)	2.6 (0.1)	2.9 (0.2)	2.3–3.5
Apiculus hair length (mm)	AHL	0.63 (0.05)	0.55 (0.05)	0.69 (0.17)	0.38–1.12
Low-temperature tolerance	LT	0 (0.0) (resistant)	4 (0.0) (susceptible)	3.0 (0.9)	0.0–4.0
Days to heading (days)	HD	61.0 (6.2)	128.2 (5.9)	103.7 (16.8)	68.8–159.0
Tolerance to disturbance					
Trampling tolerance	TRA	−0.57 (0.14)	0.16 (0.13)	0.03 (0.14)	−0.28–0.33
Grazing tolerance	GRA	−1.15 (0.26)	−1.02 (0.22)	−0.92 (0.15)	−1.30–−0.58

Standard deviations are shown in parentheses. The parental strains (A58 and W107) significantly differed in all the traits, excluding 2LBL and GRA ($P<0.05$). All the 20 traits showed significant genetic variations among the RILs, based on ANOVA and the Kruskal-Wallis test ($P<0.05$).

Phenotypic correlations between the 18 traits showed that out of 153 possible combinations, 134 were not significant among the RILs, suggesting that the traits were largely controlled by genes segregating independently (Fig. 1). However, there were two groups of traits for plant architecture, within which the traits were positively inter-correlated but between which the traits were negatively inter-correlated. One included LSL, CL, NSP and PL and the other included PN, TA, TN and LA. On the other hand, AWL showed positive correlations with HD and SL but no significant correlations with the other traits. The pattern of significant correlations indicated that only a part of the associations between the parents was maintained after hybridization.

QTL analysis

The linkage map of 12 chromosomes was constructed from 79 lines of W107RILs using 150 MITEs and 113 PCR-based markers (Fig. 2A). Two phenotypic markers, *Rc* and *wx* (*waxy*), were also included in the linkage map. The total length of the map was 1, 302 cM and the average distance

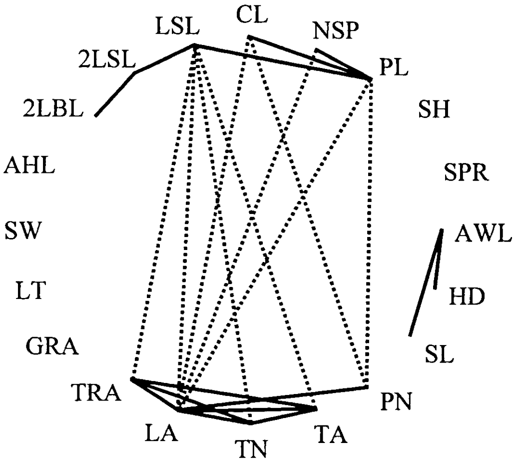

Fig. 1. Pattern of correlations among 20 traits in the W107RILs. Solid and dotted lines indicate positive and negative correlations significant at the 5% level after Bonferroni correction, respectively. Abbreviations of the traits are the same as those shown in Table 1.

between adjacent markers was about 5 cM. The map covered most of the whole genome of rice, based on the reported map (McCouch *et al.* 2002). Among the 110 co-dominant markers, 21 showed a significant deviation from the expected ratio (Fig. 2A). Distortion for segregation was detected on chromosomes 1, 2, 4, 5, 6, 9, 10 and 12, showing a high frequency (16/21) of the excess for the W107-derived alleles. The most severe distortion for segregation was observed in the distal region of the short arm of chromosome 10, and the frequency of the W107-derived allele was 86.7% (Fig. 2A).

For the 18 morphological or physiological traits, 27 significant QTLs were detected by MQM mapping (Fig. 2A and Table 2). No significant QTLs were detected for PL, NSP, AWL and SL. The number of QTLs detected for each trait ranged from 1 to 5, and each QTL explained 7.3 to 44.9% of the variance of the trait (Table 2). The detected QTLs were distributed across the genome, although they were absent on chromosome 2 or 8. Among the 27 QTLs detected, 15 were present on chromosomes 1 and 7 (Fig. 2A). Especially, a distinct cluster was detected on the short arm of chromosome 7, including six QTLs responsible for the plant architecture (TA, TN, LSL, LA, CL and PN).

Another cluster of QTLs, which was found on the long arm of chromosome 1, affected 2LSL, 2LBL, CL, SH and HD, although no association among these traits was observed among the RILs except for an association between

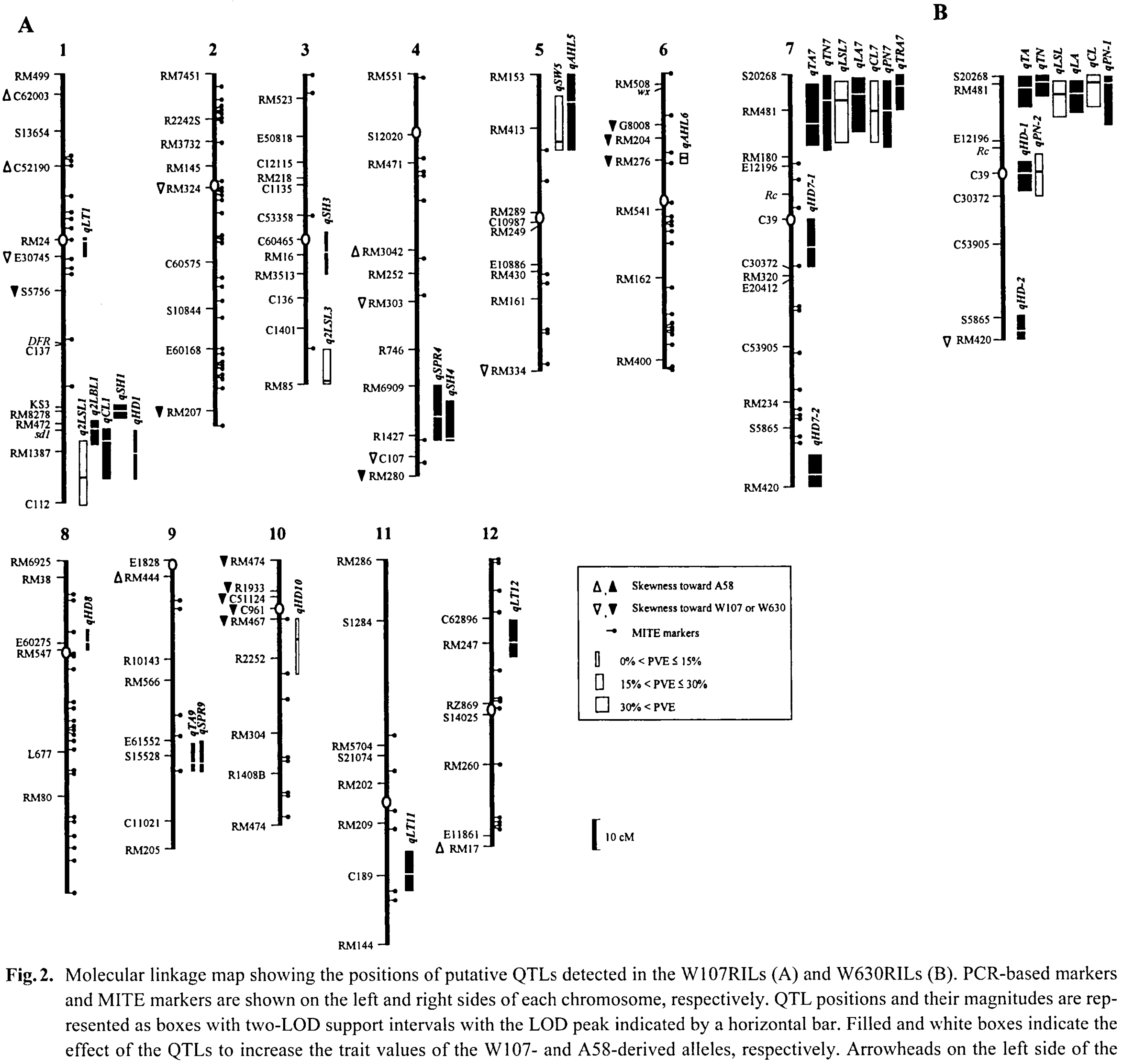

Fig. 2. Molecular linkage map showing the positions of putative QTLs detected in the W107RILs (A) and W630RILs (B). PCR-based markers and MITE markers are shown on the left and right sides of each chromosome, respectively. QTL positions and their magnitudes are represented as boxes with two-LOD support intervals with the LOD peak indicated by a horizontal bar. Filled and white boxes indicate the effect of the QTLs to increase the trait values of the W107- and A58-derived alleles, respectively. Arrowheads on the left side of the PCR-based markers indicate a deviation from Mendelian inheritance. The white and filled arrowheads indicate significant deviations at 5% and 1%, respectively. The oval symbol in each chromosome represents the centromere region estimated based on the YAC-base physical map of the *O. sativa* cultivar Nipponbare (http://rgp.dna.affrc.go.jp).

Table 2. Putative 28 QTLs for 15 traits detected by MQM mapping

Trait	QTL	Nearest markers	LOD	Direction of effect	PVE (%)	Multi-QTL PVE (%)
2LSL	*q2LSL1*	R1387	3.1	A	15.6	
	q2LSL3	RM85	5.2	A	26.0	34.0
2LBL	*q2LBL1*	*sd1*	4.3	W	22.7	
TA	*qTA7*	RM481	9.8	W	44.7	
	qTA9	S15528	3.5	W	12.8	48.4
TN	*qTN7*	RM481	4.2	W	24.5	
LSL	*qLSL7*	RM481	7.9	A	40.6	
LA	*qLA7*	RM481	7.7	W	44.9	
CL	*qCL1*	*sd1*	4.0	W	19.0	
	qCL7	RM481	3.7	A	17.4	33.0
PN	*qPN7*	RM481	3.0	W	16.2	
SPR	*qSPR4*	R1427	3.9	W	21.2	
	qSPR9	S15528	3.0	W	14.7	30.1
SH	*qSH1*	RM8278	10.9	W	36.2	
	qSH3	RM16	3.8	W	10.2	
	qSH4	R1427	7.1	W	20.8	60.7
SW	*qSW5*	R413	3.2	A	19.2	
AHL	*qAHL5*	RM413	4.0	W	23.0	
	qAHL6	RM276	4.5	A	21.6	28.9
LT	*qLT1*	RM24	3.9	W	13.1	
	qLT11	C189	6.0	W	22.2	
	qLT12	RM247	6.8	W	27.0	53.5
HD	*qHD1*	RM1387	3.4	W	10.0	
	qHD7-1	C39	9.3	W	28.1	
	qHD7-2	RM420	9.9	W	32.2	
	qHD8	RM547	3.0	W	7.3	
	qHD10	R2252	4.2	A	13.2	63.3
TRA	*qTRA7*	RM481	4.8	W	26.6	

For each QTL, the nearest PCR-based marker to the LOD peak is listed. Direction of effect indicates whether the A58 (A) or W107 (W) allele increases the trait value. PVE: percentage of variance explained; Abbreviations are the same as those shown in Table 1.

2LSL and 2LBL (Fig. 2). Regarding the culm length, *qCL1* was detected near *sd1* on chromosome 1, in addition to *qCL7* on chromosome 7. The W107-derived QTL at the *qCL1* locus increased the culm length, although that W107 was short-statured.

Effects of QTL cluster on chromosome 7

To determine whether the QTL cluster found on the short arm of chromosome 7 was present in other annual wild strains, QTL analysis was performed for the W630RILs. W630 from Myanmar showed a short-stature and a prostrate growth habit, producing many tillers and panicles, as observed in W107 from India. The common nine molecular markers were used for map construction of chromosome 7. Although the map length of chromosome 7 was shorter in the W630RILs (Fig. 2B), significant QTLs for seven traits (TA, TN, LSL, LA, CL, PN and HD) were also observed at similar positions as those in the W107RILs. In addition, another QTL for PN was detected near the centromere (Fig. 2B and Table 3). The higher values of LOD scores and PVE than those detected in the W107RILs suggested the existence of distinct effects on those traits in the W630RILs.

The phenotypic effects of the QTL cluster were also investigated in T65*Rc*(W107). The genotyping of T65*Rc*(W107) revealed that the region from S20268 to C39 was integrated into T65, showing that T65*Rc*(W107) carried a whole region for the QTL cluster detected on chromosome 7. In T65*Rc*(W107), the values of LSL and CL were lower, while the values of LA, TN, TA and PN were higher than those of T65 (Table 4 and Fig. 3). In addition, in T65*Rc*(W107), the leaf blades were significantly shorter at 10 weeks after germination (17.5 ± 0.7 cm for the eighth leaf) than those of T65 (23.3 ± 1.0 cm), revealing that the introgressed fragment carried a gene(s) for short leaves at the vegetative stage (Fig. 3). The late heading of T65*Rc*(W107) indicated that the introgressed fragment harbored *qHD7-1* near C39. These tendencies were consistent with the results of the QTL analysis, indicating that the short arm of chromosome 7 derived from W107 affected the plant architecture in different backgrounds of the cultivated strains.

W107 tended to show a higher tolerance to disturbance (TRA) than A58, while the difference in GRA was not significant between A58 and W107 (Table 1). TRA was positively correlated with LA, TN and TA and negatively correlated with LSL in the RILs, suggesting that the differences in the plant architecture was highly associated with TRA (Fig. 1). A significant QTL for TRA was detected in the region of the QTL cluster on the short arm of chromosome 7 in the W107RILs (Fig. 2A), indicating its ecological role in annual wild rice.

Discussion

Asian rice includes wild, weedy and cultivated forms and the species-complex was found to be well differentiated in the nuclear genome based on restriction fragment length polymorphisms (Xiao *et al.* 1998) and the presence of short interspersed elements (Cheng *et al.* 2003), while frequently occurring spontaneous forms (formerly designated as *O. sativa* f. *spontanea* or *O. sativa* var. *fatua*) have been suggested to result from introgression between these taxa (Oka and Chang 1959, Oka 1988, Bres-Patry *et al.* 2001). The complex genetic structure was also observed within the cultivated forms, in which a number of varietal groups, including Japonica and Indica types, showed a significant genetic differentiation (Glaszmann 1987, Garris *et al.* 2005). Therefore, the genetic divergence observed at present should be reflecting the historical processes, including domestication, varietal differentiation as well as adaptive differentiation in the wild population.

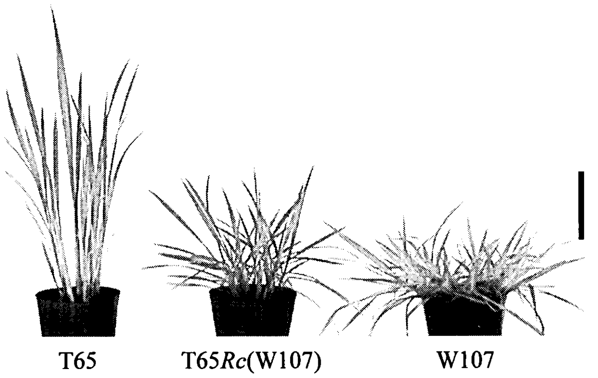

Fig. 3. Comparison of the plant stature of T65, T65*Rc*(W107) and W107, at 10 weeks after germination. T65*Rc*(W107) carried an introgressed segment at least from S20268 to C39 (Fig. 2). Bar, 10 cm.

The seed-shattering trait is taxonomically the most diagnostic trait for distinguishing domesticated forms from their wild progenitor. A simple genetic base was reported in various crops, such as barley (Takahashi 1955), pearl millet (Ponecet *et al.* 2000), common bean (Koinange *et al.* 1996) and buckwheat (Matsui *et al.* 2003), suggesting that the change to the non-shattering habit exerted a more pronounced effect on the domestication processes than the other genetic changes. Recent genome-wide QTL analysis between cultivated and wild rice, however, has revealed the complex nature of the seed-shattering habit, with QTLs detected on eight chromosomes of the rice genome (Xiong *et al.* 1999, Cai and Morishima 2002, Thomson *et al.* 2003, Lee *et al.* 2005, Li *et al.* 2006a). In the present study, three QTLs were detected on chromosomes 1, 3 and 4, where shattering QTLs had been observed more frequently than on other chromosomes. Recently, the seed shattering genes on chromosomes 1 and 4 have been isolated, suggesting their unique nucleotide variations among rice taxa (Konishi *et al.* 2006, Li *et al.* 2006b). The relative importance of these genes needs to be studied to understand the domestication processes of Asian rice.

Table 3. Putative QTLs for the seven traits detected on chromosome 7 in the W630RILs

Trait	QTL	Nearest markers	LOD	Direction of effect	PVE (%)
TA	*qTA*	RM481	15.0	W	55.2
TN	*qTN*	RM481	19.1	W	60.5
LSL	*qLSL*	RM481	17.1	A	61.0
LA	*qLA*	RM481	21.8	W	75.1
CL	*qCL*	RM481	8.3	A	30.3
PN	*qPN-1*	RM481	7.9	W	31.9
	qPN-2	C39	5.4	A	18.3
HD	*qHD-1*	C39	11.2	W	38.0
	qHD-2	RM420	7.3	W	21.9

For each QTL, the nearest marker to the LOD peak is listed. Direction of effect indicates whether the A58 (A) or W630 (W) allele increases the trait value. PVE: percentage of variance explained; Abbreviations are the same as those shown in Table 1.

Table 4. Phenotypic differences in the seven traits between T65*Rc*(W107) and T65

Trait	T65*Rc*(W107)	T65
TA	2.5 (0.5)	1.0 (0.0)
TN	15.4 (1.8)	11.1 (1.8)
LSL	7.5 (0.5)	10.8 (0.8)
LA	78.2 (10.3)	17.5 (4.5)
CL	34.9 (2.7)	52.3 (0.8)
PN	9.8 (0.8)	6.5 (1.6)
HD	132.7 (7.5)	117.8 (4.2)

All the seven traits are significantly different at the 1% level between T65*Rc*(W107) and the recurrent parent (T65). Standard deviations are shown in parentheses. Abbreviations are the same as those shown in Table 1.

Heading date and low-temperature tolerance may play a role in the local adaptation of rice. So far, 14 QTLs have been reported for photoperiod sensitivity in rice (Lin *et al.* 2003). The QTLs for HD detected in the present study corresponded to those previously reported, except for a minor QTL on chromosome 1. Regarding low-temperature tolerance at the plumule stage, three QTLs were detected on chromosomes 1, 11 and 12 between A58 and W107, while one QTL on chromosome 8 was found between wild (perennial) and cultivated (Indica) rice (Cai and Morishima 2002). Thus, genetic changes related to local adaptability might have accumulated under different growth conditions.

The cohesion of the parental differences was markedly reduced in the RILs, suggesting that the genes, which determined the trait differences between the parents, underwent recombination after hybridization. However, significant associations were maintained in some of the morphological traits, indicating that genes controlling the plant architecture were not randomly distributed on the chromosomes. The presence of a QTL cluster for plant architecture on the short arm of chromosome 7 was a distinct feature. Although similar QTLs, such as those for plant height, tiller number and tiller angle, had been observed in the cross between Indica and annual wild rice (Li *et al.* 2006a), a QTL with a large effect for leaf angle (LA) was also detected within the cluster in the present study, in which it was confirmed that the chromosomal segments, including the cluster, affected the plant architecture in the genetic background of cultivated rice. However, such a cluster has not been reported in perennial wild rice (Xiao *et al.* 1998, Xiong *et al.* 1999, Cai and Morishima 2002, Septiningsih *et al.* 2003, Thomson *et al.* 2003). This discrepancy suggests that the QTL cluster on chromosome 7 might be peculiar to annual wild rice and that perennial wild rice might carry similar allele(s) to those of cultivated rice. Regarding the origin of the phenotype for cultivated rice, these results suggested at least two possibilities: 1) mutation(s) in the QTL cluster on chromosome 7 occurred during the domestication process, 2) the cultivated allele(s) were derived from perennial wild rice, which pre-existed in wild rice before the domestication. Further investigations on the allelic distribution in wild rice should be conducted to understand the evolutionary significance of the QTL cluster.

Seed production is a critical trait for the survival of wild annuals as well as cultivated rice. In cultivated rice, short stature is considered to be negatively associated with competitive ability but positively correlated with yielding ability under fertile soil and less competitive conditions (Jennings 1966, Jennings and Aquino 1968). It is generally recognized that the introduction of the semi-dwarf gene (*sd1*) on chromosome 1 was crucial to the 'Green Revolution' (Hedden 2003). Spectacular increases in yield associated with the *sd1* gene were achieved due to a higher harvest index (reproductive allocation) as well as lodging resistance, enabling the plants to respond to nitrogen application under controlled weeds. Reproductive allocation was positively correlated with the short stature in wild rice strains, and the semi-dwarf annuals tended to display higher values than the tall perennials grown in closed habitats (Sano and Morishima 1982). However, W107 showed a QTL for tall stature at or near the *sd1* locus in the present study, although A58 have exhibited wild-type allele at the *sd1* locus. A similar QTL for tall stature was also detected near the *sd1* locus in the W630RILs (unpublished data). Furthermore, we have recently observed, using PCR method, that 30 annual wild strains did not carry *sd1* (Nagano *et al.* 2005). Accordingly, the gene(s) controlling the short stature on the short arm of chromosome 7 might play a role in the high yielding potential in less competitive and open habitats where annual wild rice grows.

The distinct set of character associations for plant architecture should confer adaptability under different growth conditions in rice (Jennings 1966, Sano and Morishima 1982). The QTL cluster on chromosome 7 could largely explain the differences in plant architecture between cultivated and wild annual rice. The cluster of QTLs might result from the pleiotropic effects of a single gene and/or the physical linkage of multiple genes. Although tight linkage of QTLs is assumed to prevent the destruction of the gene complexes, it might be insufficient because linkage disequilibrium decays within 100 kb even in self-pollinated rice plants (Garris *et al.* 2003). A QTL for disturbance tolerance (TRA) was also located in the QTL cluster for plant architecture on chromosome 7, suggesting that the plant architecture might be functionally related to the disturbance tolerance. Although it remains to be determined which genetic component(s) within the cluster contributed to the disturbance tolerance, the QTL cluster controlled the co-adapted traits of annual wild rice grown in disturbed habitats. Therefore, the QTL cluster should have been maintained in wild annual rice through selection under disturbed environments.

Numerous intergrading hybrids, referred to as spontaneous forms, are observed near or within rice fields in tropical areas, as mentioned above. Introgressive hybridization between cultivated and wild forms is considered to enable cultivated forms to address abiotic and biotic challenges (Harlan 1975, Jarvis and Hodgkin 1999). However, genetically connected populations must avoid the destruction of adaptive gene complexes after hybridization, because extensive gene flow may lead to homogenization and extinction of the wild progenitor (Ellstrand *et al.* 1999). The cohesive mode of inheritance observed in the present study suggests that it could affect the co-existence of cultivated and wild forms, since a set of adaptive traits rapidly reappeared after hybridization and persisted through disruptive selection; otherwise, various co-adapted traits (including plant architecture and disturbance tolerance) would quickly disappear after hybridization. Nevertheless, most of the genes on the genome, except for the QTL cluster, tended to be exchanged, suggesting that subdivided populations act as a genetic reservoir keeping the adaptive differentiation. This fact may indicate that frequent introgressive hybridization between subdivided populations is indispensable for cultivated rice under changing environments, implying the importance of genetic variations preserved in populations of wild relatives.

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