Theor Appl Genet (2005) 110: 1244–1252
DOI 10.1007/s00122-005-1958-z

ORIGINAL PAPER

Zichao Li · Ping Mu · Chunping Li · Hongliang Zhang · Zhikang Li · Yongming Gao · Xiangkun Wang

QTL mapping of root traits in a doubled haploid population from a cross between upland and lowland *japonica* rice in three environments

Received: 29 June 2004 / Accepted: 7 February 2005 / Published online: 12 March 2005

Abstract To genetically dissect drought resistance associated with *japonica* upland rice, we evaluated a doubled haploid (DH) population from a cross between two *japonica* cultivars for seven root traits under three different growing conditions (upland, lowland and upland in PVC pipe). The traits included basal root thickness (BRT), total root number (RN), maximum root length (MRL), root fresh weight (RFW), root dry weight (RDW), ratio of root fresh weight to shoot fresh weight (RFW/SFW) and ratio of root dry weight to shoot dry weight (RDW/SDW). The BRT was significantly correlated with the index of drought resistance, which was defined as the ratio of yield under the stress of the upland condition to that under the normal lowland condition. A complete genetic linkage map with 165 molecular markers covering 1,535 cM was constructed. Seven additive quantitative trait loci (QTLs) and 15 pairs of epistatic loci for BRT and RN were identified under upland and lowland conditions, and 12 additive QTLs and 17 pairs of epistatic QTLs for BRT, RN, MRL, RFW, RFW/SFW and RDW/SDW were identified under the PVC pipe condition. Four additive QTLs and one pair of epistatic QTLs controlling IDR were also found. These QTLs individually explained up to 25.6% of the phenotypic variance. QTL × environment (Q × E) interactions were detected for all root traits, and the contributions of these interactions ranged from 1.1% to 19.9%. Five co-localized QTLs controlling RFW and RDW, RFW/SFW, RDW/SDW and IDR, BRT and RN, RN, MRL and IDR were found. Four types of QTLs governing BRT and RN were classified by their detection in the upland and lowland conditions. Some common QTLs for root traits across different backgrounds were also revealed. These co-localized QTLs and common QTLs will facilitate marker-assisted selection for root traits in rice breeding programs.

Communicated by D.J. Mackill

Z. Li (✉) · P. Mu · C. Li · H. Zhang · X. Wang
Key Lab of Crop Genomics and Genetic Improvement,
Ministry of Agriculture,
Beijing Key Lab of Crop Genetic Improvement,
China Agricultural University, Beijing 100094, China
E-mail: lizichao@cau.edu.cn
Tel.: +86-10-62731414
Fax: +86-10-62731414

Z. Li · Y. Gao
International Rice Research Institute,
DAPO Box 7777, Manila, Philippines

Z. Li · Y. Gao
Institute of Crop Sciences, Chinese Academy
of Agricultural Sciences, Beijing 100081, China

Introduction

Rice is the most important staple food for more than one-half of the world's population. As the plant is mostly grown under continuously flooded conditions in lowland or irrigated fields, it is the largest water consumer of all the major cereals (Toorchi et al. 2003). However, water shortage is an ever-increasing problem and has become the most serious constraint to rice production and yield stability in many rice-growing areas, particularly in rainfed ecosystems (Nguyen et al. 1997). Severe water shortages have also led to a recent rapid increase in the area cultivated in upland rice in northern China (Wang et al. 2002).

The cultivation of upland rice is a non-flooded alternative to lowland rice culture that can reduce the demand for irrigation water by 50–70% (Wang et al. 2002). Rice varieties adapted specifically to upland conditions are typically characterized by a deep root system, tall stature, thicker stem and fewer tillers. These characteristics are believed to confer adaptability to the upland conditions (Ge 1992; Ling et al. 2002). Ling et al. (2002) reported that the long and thick root system of upland rice contributes greatly to its drought resistance. Additional studies have shown that the ratio of root weight to shoot weight, and root penetration ability are also correlated with drought resistance (Fukai and Cooper 1995; Price et al. 1997). To understand the

genetics of the root system of upland rice, various groups of researchers have studied quantitative trait loci (QTLs) associated with root traits (Champoux et al. 1995; Ray et al. 1996; Price and Tomos 1997; Yadav et al. 1997; Zhang et al. 2001; Kamoshita et al. 2002a). However, most of these studies were conducted at the seedling or early vegetative stages under hydroponic or aeroponic conditions. Consequently, just how the identified QTLs for drought resistance component traits contribute to yield stability at the adult plant stage under water stress conditions remains largely unknown. Moreover, most of the populations previously used for QTL mapping were derived from *indica*/*japonica* crosses, in which many QTLs associated with yield-related traits tend to co-segregate with drought resistance, thereby making it difficult to distinguish the nature of the identified QTLs (Ali et al. 2000).

We report here a genetic dissection, by QTL mapping, of the root system of upland rice. The objective of the present study was to investigate the relationship between different root traits of rice and drought resistance under different water stress conditions and to identify QTLs governing root traits at different developmental stages.

Materials and methods

Plant materials

A doubled haploid (DH) rice population of 116 lines was developed from anther culture of the F_1 plants of a cross between IRAT109 and Yuefu. IRAT109 is an upland tropical *japonica* rice variety from Africa characterized by a thick and long root system, thick culm, high yield, large grains and poor grain quality. Yuefu is a lowland temperate *japonica* variety from Japan with a thin and short root system, high root number and good grain quality. It has been cultivated over a large area in northern China for more than 20 years.

Phenotypic experiments

The DH lines were planted under three different water regimes: the lowland (flooded), upland (aerobic soil) and PVC-pipe aerobic conditions. In early May of 2001 and 2002, each of the DH lines and the parents were directly sown into two 1.5-m-long rows with a distance of 30 cm between rows and 5 cm between seeds in both the lowland and upland fields (without irrigation before sowing) of the experimental farm of the China Agricultural University, Beijing. A completely random design with two replications was adopted in both years. For the upland experiment, a basal fertilizer level equivalent to 150 kg/ha N, 150 kg/ha P_2O_5 and 150 kg/ha K_2O was applied before planting. An additional 300 kg/ha of urea was applied at the tillering stage in both the upland and lowland experiments.

For the lowland experiment, a 2- to 10-cm layer of surface water was maintained during the growth period. The total water input (irrigation plus rainfall) was about 1,400 mm. The total amount of rainfall that falls, mostly from June to August, is about 300–400 mm in the Beijing region. For the upland experiment, the rice plants encountered severe water stress, mainly before June (tillering stage), due to very little rainfall during this period. Supplementary irrigation was supplied when all of the DH lines had a high degree of leaf rolling at noon in the field. Two supplementary irrigations were provided in May of 2001 and 2002, and the third irrigation was applied only in June of 2001. Approximately 50 mm of irrigation water was applied into the upland field each time. The total water input into the upland field was about 500 mm.

The PVC pipes simulating upland conditions (PVC-pipe culture) were designed to recover the whole root system. The PVC pipes were cut into 100-cm-long segments, and then each segment was cut vertically into two halves. The two halves were then bound together. The inside-diameter of the pipe segment was 15 cm and a plastic membrane was attached inside. The pipes were buried almost completely underground with only 10 cm left above the soil surface and then filled with soil from the rice field. Each DH line was sown into five hills with two to three seeds per hill in each pipe. After 1 month, the plants were thinned to one plant per hill. The plants were sown on 17 July 2001 and sampled on 1 October 2001 (76 days after sowing); in 2002, they were sown on 15 May and sampled on 1 August [78 days after sowing (DAS)]. The management of the pipe-culture was the same as that of the upland experiment. After the roots were washed, the root traits were measured.

Trait measurements

The plants were sampled at maturity (upland and lowland field) and the late-tillering stage (PVC-pipe culture). Grain yield, basal root thickness and total root number under lowland and upland conditions were measured. In the PVC-pipe culture, seven root traits, including basal root thickness (BRT), total root number (RN), maximum root length (MRL), root fresh weight (RFW), root dry weight (RDW), ratio of root fresh weight to shoot fresh weight (RFW/SFW), and ratio of root dry weight to shoot dry weight (RDW/SDW) were measured or calculated. Under the upland and lowland conditions, five plants from each row were harvested for yield assessment, then the root system was removed and washed, and the thickness of five roots per plant was measured under the microscope (the mean of five thick roots represents the BRT of the plant). The number of roots per plant was counted. The IDR (index of drought resistance) was calculated from the ratio of average yield per plant under upland conditions to average yield per plant under lowland conditions. With the PVC-pipeculture, all of the plants were used as samples for measurement.

Molecular marker analysis

For restriction fragment length polymorphism (RFLP) analysis , genomic DNA of each DH line was extracted, digested and hybridized as described by McCouch et al. (1988). Five restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I and *Bam*HI) were used to digest the genomic DNA. Enzymes that revealed polymorphisms for the RFLP markers in the parental survey were then used to genotype the 116 DH lines. The Amersham ECL kit (non-isotope system; Amersham, UK) was used for Southern blotting and hybridization. The RFLP probes were kindly provided by the Japanese Rice Genomic Program.

The preparation of the primer sequences of the microsatellites (SSRs) followed the method of Temnykh et al. (2000). The PCR reaction was conducted in a volume of 20 µl containing 30 ng genomic DNA, 10 µmol primers, 2 µl 10× buffer and 1 U *Taq* DNA polymerase. The PCR amplification was performed on a PCR system with 30 cycles. Following PCR amplification, 6 µl of 3× loading buffer was added to the amplified products. The amplified products were denatured at 95°C for 5 min, then loaded on 8% denatured polyacrylamide gels in 0.5× TBE buffer. After electrophoresis, the gels were stained with silver to detect polymorphisms (McCouch et al. 2001).

Genetic linkage map construction and QTL analysis

The genetic linkage map was constructed using the software MAPMAKER/EXP VER.3.0 (Lander et al. 1987; Lincoln et al. 1993). Linkage groups were created with a LOD score of 3.0 and a recombination fraction of 0.4 using the GROUP command. The order of the linkage groups was determined using the COMPARE, TRY and RIPPLE commands.

QTL analysis, including additive QTLs, epistatic QTLs and QTL × environment (Q × E)interactions, was carried out using mixed linear model approaches conducted with QTLMAPPER VER.1.0 (Zhu 1998; Wang et al. 1999). A threshold probability of $P \leq 0.005$ and $P \leq 0.001$ was used for the additive and epistatic QTLs, respectively. The LOD score of 2.4 was used as another threshold to declare the presence of a putative QTL. The percentage variation explained (general contribution) by the QTL, and the additive effect, epistatic effect and QTL × E interaction effect of QTLs were also estimated by QTLMAPPER VER.1.0.

Results

Phenotypic values of the DH population and its parents

The analysis of variance (ANOVA) for all root traits was carried out in 2001 and 2002 for the three experimental conditions separately. Significant differences for all of the traits measured were found between the parents IRAT109 and Yuefu and among the DH lines. No significant differences were found between the RN, BRT, MRL, RFW and RDW of 2001 and those of 2002, but significant differences were found between the RFW/SFW and RDW/SDW over the two years (data not shown). This result indicated that shoot weight was more easily influenced by environment than were the root traits.

As expected, the upland parent IRAT109 had significantly larger values than the lowland parent Yuefu for all of the measured root traits under all three culture conditions in both years, except that the parents did not differ for RN under the upland condition and Yuefu had many more roots than IRAT109 under the lowland condition (Table 1). The mean RN of the DH lines showed the same tendency. For BRT, the differences were small for IRAT109, Yuefu and the DH lines under the upland and lowland conditions. The yield of Yuefu was 19.4 g per plant, which was significantly higher (26%) than that of IRAT109 under the lowland condition. However, the yield of Yuefu under the upland condition was 80% less than that under the lowland condition, while IRAT109 showed a 9% yield increase under the same situation. DH lines grown under the upland condition had on average a 30% lower yield than those grown under the lowland condition (Table 1), indicating that water stress was a severe restriction under the upland condition. There was considerable variation and transgressive segregation for all of the traits measured, except for BRT, among the DH lines. For BRT, those DH lines with the highest values were equivalent to that of IRAT109 under the upland and PVC-pipe conditions. The DH population showed an approximately normal distribution for all of the measured traits, indicating that the population was suitable for QTL mapping for these traits (data not shown).

Correlation analysis between root traits and upland yield, lowland yield and IDR

Genetic correlation coefficients were obtained from data gathered over 2 years (2001 and 2002) with two replications and were based on variance and covariance analyses (Table 2). While some significant correlations were observed, these were mostly very low. The results revealed that RNs under the three conditions were positively correlated with lowland yield but that under lowland and PVC-pipe conditions, they were negatively correlated with IDR. RN appeared to have influenced yield to a greater extent under the lowland condition than under the upland and PVC-pipe conditions. Under upland and PVC-pipe conditions, BRT was positively correlated with upland yield and IDR. These results demonstrated that RN under PVC-pipe culture was similar to that under lowland condition and that BRT under PVC-pipe culture was similar to that under upland condition. On the other hand, BRT played the

Table 1 Summary statistics of the parents, IRAT109 (P1), Yuefu (P2) and the doubled haploid (DH) population for root number (RN), basal root thickness (BRT), maximum root length (MRL), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (*SFW*), shoot dry weight (SDW), RFW/SFW, RDW/SDW, plant yield and index of drought resistance (IDR) evaluated under the lowland, upland and pipe conditions during a 2-year period

Trait	Cultivated condition	2001						2002					
		IRAT109	Yuefu	*t*-value	*P*	DH lines		IRAT109	Yuefu	*t*-value	*P*	DH lines	
						Mean	Range					Mean	Range
RN	Upland	130	108	5.5	0.057	113	50–314	130	133	−2.71	0.112	134	74–281
	Lowland	164	267	−69	0.004	228	104–362	199	394	−129	0.002	229	134–407
	Pipe	34	31	1.5	0.187	45	26–71	36	30	2.9	0.1	49	20–89
BRT (mm)	Upland	2.85	1.85	12.3	0.026	2.17	1.49–3.04	2.91	1.48	96.3	0.003	2.11	1.30–2.80
	Lowland	3.03	2.16	18.4	0.017	2.43	1.95–3.09	2.50	2.17	4.71	0.066	2.32	1.83–2.85
	Pipe	2.47	1.78	27.8	0.011	2.17	1.39–2.70	2.60	1.34	21	0.015	2.00	1.43–2.56
MRL (cm)	Pipe	64.0	23.6	85	0.007	61.0	35.0–85.0	89.0	45.0	87	0.006	66.4	22.0–131.0
RFW (g)	Pipe	3.12	1.03	7046	0	2.67	1.31–6.99	2.62	0.80	85	0.007	2.56	1.05–5.03
RDW (g)	Pipe	0.62	0.10	17.6	0.018	0.56	0.23–1.49	0.47	0.09	759	0.001	0.46	0.14–0.82
RFW/SFW	Pipe	0.53	0.38	31	0.01	0.42	0.15–0.56	0.39	0.23	159	0.002	0.29	0.10–0.43
RDW/SDW	Pipe	0.47	0.31	31	0.01	0.36	0.35–0.63	0.26	0.23	59	0.005	0.24	0.15–0.37
Yield per plant (g)	Upland	16.1	3.4	249	0.001	7.7	1.6–16.4	17.2	5.4	113	0.003	9.4	0.65–25.1
	Lowland	16.0	21.1	−2.5	0.121	12.7	6.2–24.2	14.7	17.7	−61	0.005	10.7	4.3–20.3
IDR		1.01	0.16	34.2	0.009	0.67	0.11–1.95	1.17	0.20	65	0.005	0.72	0.22–2.30

Table 2 Genetic correlation coefficients between root traits and upland yield, lowland yield and IDR

Root traits	Condition	Upland yield	Lowland yield	IDR
RN	Upland	−0.01	0.26**	0.12
	Lowland	−0.20*	0.49**	−0.19*
	Pipe	0.01	0.23*	−0.25**
BRT	Upland	0.33**	−0.12	0.25**
	Lowland	0.10	0.11	0.01
	Pipe	0.34**	0.01	0.28**
MRL	Pipe	0.16	−0.01	0.19*
RFW	Pipe	0.11	0.16	0.05
RDW	Pipe	0.11	0.19*	0.09
RFW/SFW	Pipe	0.13	0.08	−0.04
RDW/SDW	Pipe	0.15	0.05	0.04

*Significant at the 5% level; **significant at the 1% level

most important role in plant yield under the upland condition.

Identification of QTLs for root traits and IDR

Of the 220 RFLP markers and 216 SSR markers analyzed, 94 (44.8%) and 71 (33.2%), respectively, detected polymorphism. An integrated genetic linkage map consisting of 94 RFLP and 71 SSR markers was constructed, which was 1,535 cM long, with an average distance of 9.3 cM between adjacent markers. QTLs associated with BRT, RN, MRL, RFW, RDW, RFW/SFW, RDW/SDW and IDR were detected (Table 3, Fig.1).

For BRT, one additive QTL and nine pairs of epistatic QTLs were detected under the lowland condition, three additive QTLs and five pairs of epistatic QTLs were detected under the upland condition, and three pairs of epistatic QTLs were detected under the PVC-pipe condition. The variance explained by epistatic QTLs was higher than that explained by additive QTLs under all three conditions. For RN, there were more epistatic QTLs under the upland and PVC-pipe conditions than under the lowland condition. No QTLs were identified to be common between the upland and lowland conditions or between the PVC-pipe and upland conditions. For MRL, two pairs of epistatic QTLs were detected, while five additive QTLs and one pair of epistatic QTLs were detected for RFW. Three additive QTLs and three pairs of epistatic QTLs were detected for RDW. There was one additive QTL and one pair of epistatic QTLs for RFW/SFW. For RDW/SDW, three additive QTLs and three pairs of epistatic QTLs were detected. The QTL *rrsd3* was common to both RFW/SFW and RDW/SDW with high LOD scores of 14.6 and 17.3, respectively. Four additive QTLs and one pair of epistatic QTLs were found for IDR. Two of the additive QTLs, *idr6b* and *idr6a*, had a high general contribution of 11.6% and 13.8%, respectively.

Co-localization of QTLs

Intervals on five chromosomes were found to contain multiple QTLs (Table 4). This co-localization of QTLs may be the genetic basis of the phenotypic correlation. These QTLs could be helpful in marker-assisted selection (MAS), but unwanted traits might also be selected during MAS due to the co-localization of QTLs. Fine mapping for these regions is needed in order to overcome this problem and improve the efficiency of MAS.

The QTL × E interactions for root traits and IDR

Q × E interactions over the 2 years were also detected. For Q × E interaction involving BRT, one additive QTL and two pairs of epistatic QTLs were detected under the upland condition, and one additive QTL and two pairs

Table 3 QTLs associated with RN, BRT, MRL, RFW, RDW, RFW/SFW, RDW/SDW and IDR detected in the IRAT109/Yuefu DH population under the three environmental conditions tested

Trait	Environment	QTL[a]	Interval	QTL[a]	Interval	A/AA[b]	LOD	R^2 (%)[c]	AE_1/AAE_1[d]	R^2 (%)[c]
BRT	Lowland	*brt11d*	RM224-G181			0.049	7.44	4.7	−0.06	12.7
		brt1a	C161A-RM243	*brt12a*	RM101-RM260	0.095	2.91	4.9		
		brt1b	RM259-RM84	*brt11a*	C3-R2918	−0.047	4.13	1.2		
		brt1c	C813-C955	*brt3b*	R2247-C746	−0.065	6.45	2.3		
		brt1e	RM5-RM302	*brt6b*	C1520-RM225	−0.083	3.53	3.7		
		brt2b	G1314A-R26	*brt7b*	C847-C39	0.089	4.77	4.3		
		brt3a	R2247-C746	*brt10c*	RM184-RM311	−0.054	4.68	1.6		
		brt5b	RM161-R521	*brt12d*	R617-S826	−0.130	3.28	9.2		
		brt6a	C607-C1004	*brt12c*	N869-R1709	0.047	5.23	1.2	−0.06	3.4
		brt7a	R1488-C1008	*brt11a*	C3-R2918	−0.134	8.93	9.8	0.10	11.3
	Upland	*brt1d*	C742-C904			−0.126	5.77	6.4	−0.12	1.1
		brt7c	RM47-RM172			0.103	3.24	4.2		
		brt11b	OSR1-RM202			0.112	5.66	5.0		
		brt1d	C742-C904	*brt5a*	RM146-R569	−0.095	8.47	3.3		
		brt1e	RM5-RM302	*brt6b*	C1520-RM225	−0.160	6.45	10.8	−0.14	6.4
		brt2b	G1314A-R26	*brt10d*	R716-R1933	−0.068	6.94	1.7		
		brt2c	RM208-RM48	*brt12c*	N869-R1709	0.123	3.10	5.5		
		brt5a	RM146-R569	*brt12b*	RM270-RM235	−0.166	5.90	7.4	−0.16	1.9
	Pipe	*brt3*	G51-RM231	*brt11a*	C3-R2918	−0.086	3.45	13.0		
		brt10a	RM311-RM216	*brt11b*	RM287-RM209	−0.051	7.44	4.6		
		brt10b	RM311-RM216	*brt12d*	R617-S826	−0.064	3.09	7.2		
RN	Lowland	*rn1b*	RM243-RM259			15.330	4.92	9.1		
		rn3	RM231-RM175			14.040	3.33	7.7		
	Upland	*rn7a 39-*RM214 0.967 0.480 14.46 3.14 14.1759 0.0000*	C39-RM214			3.140	14.18	11.7	−5.60	3.7
		rn1c	C904-RM306	*rn6a*	R1954-C607	5.520	17.82	15.7		
	Pipe	*rn1a*	C161A-RM243	*rn5*	RM161-R521	3.297	3.44	5.1		
		rn1b	RM243-RM259	*rn6b*	RM276-RM253	−2.226	5.92	2.0	3.00	8.1
		rn2	RM208-RM48	*rn4*	RM348-RM349	3.190	3.29	4.0		
		rn7	OSR22-RM11	*rn11*	RM287-RM209	3.216	5.50	5.2	2.80	7.2
MRL	Pipe	*mrl2*	RM208-RM48	*mrl5*	C282-R1838	4.363	2.67	6.2		
		mrl3	R2247-C746	*mrl8*	G187-RM310	8.147	3.14	21.5		
RFW	Pipe	*rfw1b*	C813-C955			0.583	5.59	11.0	−0.55	19.9
		rfw1c	RM306-RM5			−0.447	3.00	6.5		
		rfw2	G1327-RM263			0.568	4.07	10.5	−0.39	9.6
		rfw3	RM60-C814			−0.331	2.35	3.6		
		rfw10	C1166-R2174			0.645	3.56	13.5		
		rfw1a	RM259-RM84	*rfw7*	RM18-RM47	0.243	5.49	5.6		
RDW	Pipe	*rdw7*	RM47-RM172			−0.034	2.42	2.1		
		rdw9	R79-R2638			−0.102	3.77	18.5	−0.06	13.6
		rdw11b	RM224-G181			0.035	2.79	2.2		
		rdw2	R712-G21	*rdw8*	C1107-R2976	−0.064	3.32	3.3		
		rdw5a	R566-R2289	*rdw11b*	RM224-G181	−0.026	4.18	5.4		
		rdw11a	C6-OSR1	*rdw12*	RM101-RM260	0.179	5.23	25.6		
RFW/SFW	Pipe	*rrsf3*	G51-RM231			0.032	14.56	1.9	0.04	5.8
		rrsf2	RM341-RM208	*rrsf6*	R1962-G1314	−0.046	3.00	3.3		
RDW/SDW	Pipe	*rrsd3*	G51-RM231			0.029	17.32	7.0	0.02	8.0
		rrsd6b	RM253-RM314			−0.019	4.44	3.0		
		rrsd11a	RM229-RM21			−0.018	8.85	2.7	−0.02	6.6
		rrsd2	R3393-C747	*rrsd4*	C107-C734	−0.014	2.82	1.2		
		rrsd3	G51-RM231	*rrsd8*	OSR30-RM152	−0.018	9.54	2.0		
		rrsd5	RM146-R569	*rrsd6a*	C1004-R1962	?0.013	3.04	1.1		
IDR		*idr2*	RM208-RM48			0.087	2.87	2.0	0.06	1.9
		idr3b	G51-RM231			0.144	5.99	5. 5	0.17	3.8
		idr6b	RM276-RM253			0.116	2.42	11.6		
		idr6a	C1004-R1962			0.120	6.34	13.8	0.07	8.3
		idr3a	RM60-C814	*idr7*	R2401-R1488	−0.156	2.70	8.7	−0.11	2.3

[a] QTLs are denoted by trait abbreviations plus chromosomal number

[b] "A" is the additive effect of a QTL. A positive value indicates that the IRAT109 genotype has a positive effect on the trait. "AA" is the effect of additive-by-additive interaction between two QTLs; a positive value indicates that the parental two-locus genotypes have a positive effect on the trait and that the recombinants have negative effects

[c] R^2 is the percentage of the phenotypic variations explained by A, AA, AE and AAE, respectively

[d] E_1, Environment of 2001; E_2, Environment of 2002; $AE_1/AAE_1 = -AE_2/AAE_2$

of epistatic QTLs were detected under the lowland condition. For RN, one QTL was found showing Q × E interaction under the upland condition but none was found under the lowland condition. Under the PVC-pipe condition, no Q × E interaction was detected for BRT and MRL. Two QTLs controlling RFW and RDW were detected for Q × E interaction, explaining 19.9% and 13.6% of the variance, respectively. Therefore, we concluded that QTLs for MRL and BRT were less affected by the environment than those for RFW and RDW. For IDR, three additive QTLs and one pair of epistatic QTLs showed Q × E interactions between the 2 years. On the basis of our results it was clear that the QTLs governing IDR were easily affected by environment.

Detection of QTLs for BRT and RN across the upland and lowland conditions

In order to examine the QTLs observed under different conditions, the QTLs identified for BRT and RN under the upland and lowland conditions were re-analyzed

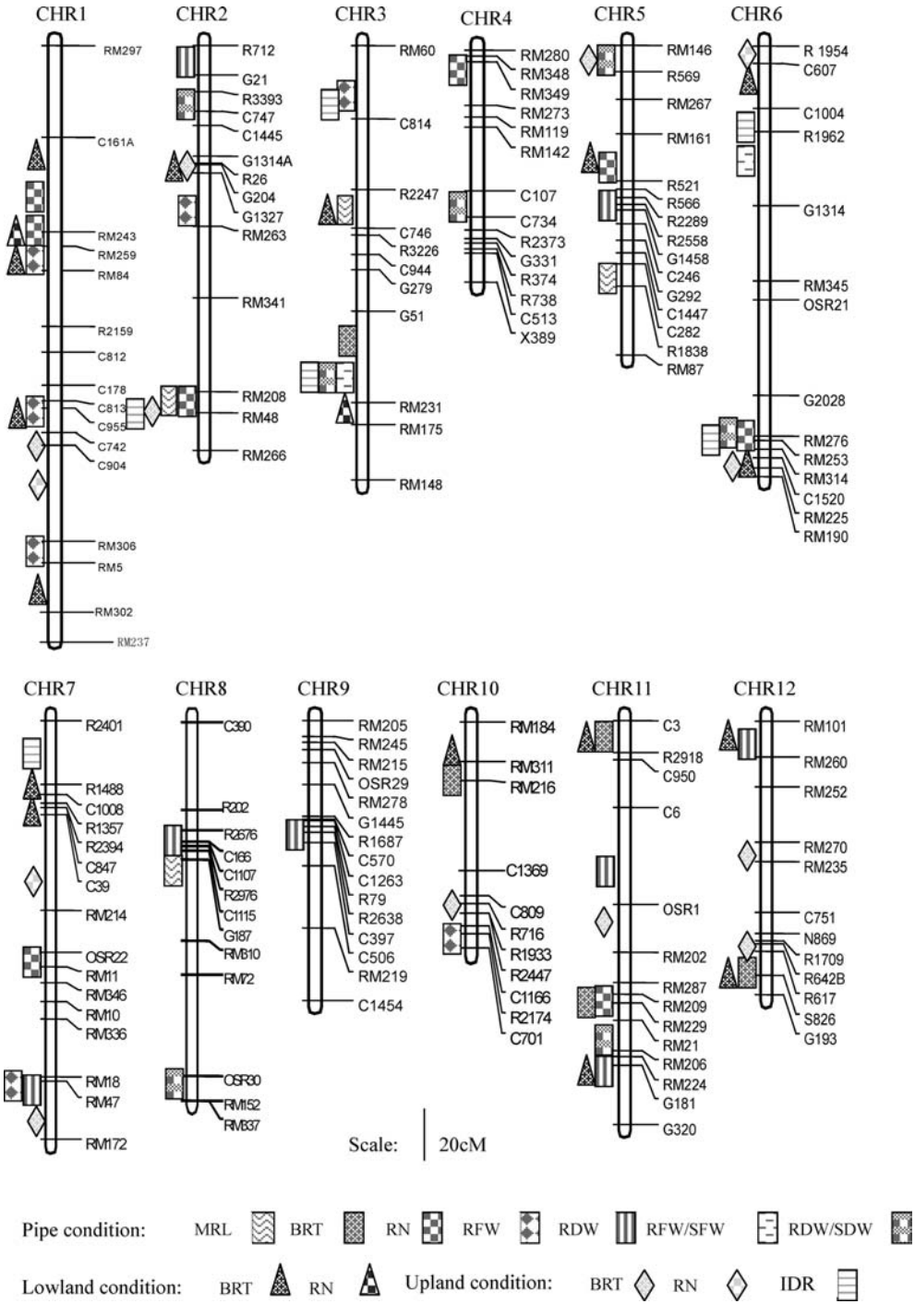

Fig. 1 QTL linkage map for root traits under three growing conditions

Table 4 Co-localization of QTLs for different traits

Chromosome	QTL	Interval	Traits
2	*rn2, mrl2, idr2*	RM208-RM48	RN, MRL, IDR
3	*rrsf3, rrsd3, idr3b*	G51-RM231	RFW/SFW, RDW/SDW, IDR
6	*rn6b, rrsd6b, idr6b*	RM253-RM314	RN, RDW/SDW, IDR
7	*rfw7, rdw7*	RM18-RM47	RFW, RDW
11	*brt11c, rn11*	RM287-RM209	BRT, RN

Table 5 LOD scores and effects of QTLs for BRT and RN under the upland and lowland conditions

Trait	QTL type	QTL	Marker interval	QTL	Marker interval	Upland		Lowland	
						LOD	Effect	LOD	Effect
BRT	Additive QTL	*brt11d*	RM224-G181			2.0	0.052	7.4	0.049
	Epistatic QTL	*brt1c*	C813-C955	*brt3b*	R2247-C746	2.1	−0.048	6.5	−0.06
		brt1e	RM5-RM302	*brt6b*	C1520-RM225	6.5	−0.16	3.5	−0.08
		brt3a	R2247-C746	*brt10c*	RM184-RM311	2.1	0.033	4.7	−0.05
		brt2b	G1314A-R26	*brt7b*	C847-C39	2.0	−0.05	4.8	0.089
RN	Additive QTL	*rn1b*	RM243-RM259			0.6	4.63	4.9	15.33

Table 6 Common QTL regions for root traits across different genetic backgrounds and environments

Traits	Chromosome	The present study (J/J)[a]	Champoux et al. (1995) (I/J)[b]	Ray et al. (1996) (I/J)	Price and Tomos (1997) (I/J)	Zhang et al. (2001) (I/J)	Kamoshita et al. (2002b) (I/I)[c]
BRT	1	RM5-RM302	RG811				
	3	RM231-RM175			RG191		RG1356-R1925
	3	R2247-C746				RZ474-C746	
	10	RM311-RM216	RZ892				
	12	RM101-RM260	RZ397				
	12	RM270-RM235				RZ261	
RDW	3	RM60-C814					PC73M7
	7	RM47-RM172	RG351				
	12	RM101-RM260	RG9				
MRL	3	R2247-C746			C746		
RN	1	RM243-RM259		RG811-RG140			
	4	RM348-RM349		RG620-RG214			
	11	RM287-RM209		RG16-RG211			

[a] *J*/*J*, *Japonica*/*Japonica* cross
[b] *I*/*J Indica*/*Japonica* cross
[c] *I*/*I Indica*/*Indica* cross

using a relatively high value of threshold ($P \leq 0.05$) as described by Li et al. (2003). The results revealed that there were more QTLs for BRT than for RN under both upland and lowland conditions and that these QTLs could be classified into four types (Table 5) based on their effects, which could be expressed under different environmental conditions. (1) Some QTLs were detected only under the upland condition but not under the lowland condition even under a relatively high threshold value; examples of these QTLs include such additive QTLs as *brt1d*, *brt7c*, *brt11b*, *rn3* and *rn7a* and epistatic QTLs as *brt1a-brt12a*, *brt1b-brt11a brt5b-brt12d* and *brt6a-brt12c*. Most of the QTLs for BRT and RN detected under the upland condition belonged to this category. These results indicated that these QTLs could be expressed only under upland conditions. (2) A few QTLs were detected under both upland and lowland conditions, such as the epistatic QTLs *brt1e* and *brt6b*. (3) Some QTLs were expressed under the lowland condition but only weakly expressed under the upland condition (additive QTLs *brt11d*, *rn1b*; epistatic QTLs *brt1c-brt3b*, *brt3a-brt10c* and *brt2b-brt7b*). (4) Approximately one-half of the total QTLs for BRT and RN identified under the lowland condition were detected only under the lowland condition, such as *brt1a-brt12a*, *brt1b-brt11a*, *rn3,* etc.

Discussion

The advantage of the *japonica-japonica* (J-J) population

The populations that have been described in previous reports on QTLs for drought resistance have been ones mainly derived from *indica-japonica* crosses. While

these *indica-japonica*-derived populations possess a higher polymorphism, they often show distorted segregation. In the present study, a *japonica* × *japonica* population was used under the premise that the polymorphism level between intra-subspecies rice cultivars would be lower. However, in the present study, the polymorphism rates between the two parents (44.8% and 33.2% for RFLP markers and SSR markers, respectively) were relatively higher than those found in other intra-subspecies populations (17% between two *indica* parents) (Ali 2000) because they were from geographically distant regions. One of the parents, IRAT109, is a tropical *japonica*, and the other, Yuefu, is a temperate *japonica*. They are quite different with respect to some traits related to drought resistance. Therefore, this kind of population was suitable for QTL mapping of drought tolerance traits.

Comparison with previous studies on QTLs for root traits

The QTL regions for root traits in this study were compared with the results of Champoux et al. (1995), Price and Tomos (1997), Zhang et al. (2001), Kamoshita et al (2002a) and Ray et al. (1996) (Table 6). However, for QTLs linked to markers not present in all the six populations, it was necessary to use the maps developed by Temnykh et al. (2000) and McCouch et al. (2001) with a high density of molecular markers to compare the QTLs among the different populations. Since inconsistent map distances between markers in different maps hampers precision, our results should be taken as being approximate.

There were six common QTL regions for BRT: one QTL region of RM231-RM175 on chromosome 3 for BRT was identified in three different populations and another five QTL regions on chromosomes 1, 3, 10 and 12 were identified in two different populations. For RDW, three common QTL regions were found. One QTL region of RM60-C814 on chromosome 3 was similar to that found by Kamoshita et al. (2002a), but QTL regions of RM47-RM172 on chromosome 7 and RM101-RM260 on chromosome 12 were similar to the results presented by Champoux et al. (1995). For MRL, one QTL region of R2247-C746 on chromosome 3 was similar to that found by Price and Tomos (1997). Three common QTL regions for RN were found both in the results of Ray et al. (1996) and in the present study (Table 6). These demonstrate show that there were more common QTL regions for BRT than for other root traits and more common QTL regions between Champoux et al.' s results and the present study than with the other studies.

More QTLs controlled BRT at the maturity stage than at the early vegetative stage. However, for RN, there were fewer QTLs at the maturity stage than at early vegetative stage (Tables 3, 6). Four additive QTLs and 14 pairs of epistatic QTLs for BRT were detected at the maturity stage in the field experiments (Table 3), but only three pairs of epistatic QTLs were detected at the later tillering stage in the pipe experiment (76 DAS) (Table 3) in the present study. There were 6, 3 and 18 QTLs for BRT found in the studies of Zhang et al. (2001) (50 DAS), Price and Tomos (1997) (28 DAS) and Champoux et al. (1995) (38 DAS), respectively (Table 6). For RN, there were three additive QTLs and one pair of epistatic QTLs at the maturity stage (Table 3), but at the early vegetative stage, four pairs of epistatic QTLs were detected in the present study and 19 QTLs were detected in the study of Ray et al. (1996) (33 DAS).

QTLs for molecular MAS in rice drought resistance breeding

We found BRT and MRL to be positively correlated with IDR and RN to be negatively correlated with IDR. These results are similar to those reported in previous studies (Ge 1992; Ling et al. 2002). Therefore, a deeper root system with high MRL, high BRT and low RN should be the breeding objective when selecting for drought-resistant plants. MAS for these root traits would be extremely useful because they cannot be measured directly.

Some QTLs for BRT, MRL, RFW and RDW had high general contributions and no Q × E interactions. Two pairs of epistatic QTLs, *brt5b* and *brt12d*, and *brt3* and *brt11a*, for BRT had a general contribution of 9.2% and 13.0%, respectively. One pair of epistatic QTLs, *mrl3* and *mrl8*, for MRL had a high general contribution of 21.5%, and an additive QTL, *rfw10*, for RFW and a pair of epistatic QTLs, *rdw11a* and *rdw12*, for RDW had general contributions of 13.5% and 25.6%, respectively. Three QTL locations, RM208-RM48 on chromosome 2, G51-RM231 on chromosome 3 and RM276-RM253 on chromosome 6, were found to control both root traits and IDR. All of these QTLs would be useful for drought resistance breeding in rice.

Acknowledgements We thank Prof. Zuomin Yang for reading the manuscript. This work was supported by the State Key Basic Research and Development Plan of China (2003CB114301), the National Natural Science Foundation of China (30250009), the Hi-Tech Research and Development Program of China (2004AA211190 and 2003AA207040).

References

Ali ML, Pathan MS, Zhang J, Bai G, Sarkarung S, Nguyen HT (2000) Mapping QTLs for root traits in a recombinant inbred population from two *indica* ecotypes in rice. Theor Appl Genet 101:756–766

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

Fukai S, Cooper M (1995) Development of drought-resistant cultivars using physio-morphological traits in rice. Field Crops Res 40:67–86

Ge SL (1992) The comparison and classification of several morphological criteria of root of paddy rice and upland rice in different cultural methods. PhD thesis, Beijing Agricultural University, Beijing

Kamoshita A, Wade LJ, Ali ML, Pathan MS, Zhang J, Sarkarung S, Nguyen HT (2002a) Mapping QTLs for root morphology of a rice population adapted to rainfed lowland conditions. Theor Appl Genet 104:880–893

Kamoshita A, Zhang JX, Siopongco J, Sarkarung S, Nguyen HT, Wade LJ (2002b) Effect of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. Crop Sci 42:255–265

Lander ES, Green P, Abraham SJ, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Li ZK, Yu SB, Lafitte HR, Huang N, Courtois B, Hittalmani S, Vijayakumar CHM, Liu GF, Wang GC, Shashidhar HE, Zhuang JY, Zheng KL, Singh VP, Sidhu JS, Srivantaneeyakul S, Khush GS (2003) QTL × environment interactions in rice. I. Heading date and plant height. Theor Appl Genet 108:141–153

Lincoln SE, Daly MJ, Lander ES (1993) Constructing genetic linkage maps with MAPMAKER/EXP VER. 3.0: a tutorial and reference manual, 3rd edn. Technical Report, Whitehead Institute for Biomedical Research, Cambridge, Mass.

Ling ZM, Li ZC, Yu R, Mu P (2002) Agronomic root characters of upland rice and paddy rice (*Oryza sativa* L.). J Chin Agric Univ 7:7–11

McCouch SR, Kochert G, Yu Z, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

McCouch SR, Temnykh S, Lukashova A, Coburn J, DeClerck G, Cartinhour S, Harrington S, Thomson M, Septiningsih E, Semon M, Moncada P, Li J (2001) Microsatellite markers in rice: abundance, diversity, and applications. In: Khush GS, Brar DS, Hardy B (eds) Rice genetics IV. Proc 4th Int Rice Genet Symp. Science Publ, New Delhi/ International Rice Research Institute, Los Baños, Philippines, pp 117–135

Nguyen HT, Babu RC, Blum A (1997) Breeding for drought resistance in rice: physiology and molecular genetics consideration. Crop Sci 37:1426–1434

Price AH, Tomos AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). II: mapping quantitative trait loci using molecular markers. Theor Appl Genet 95:143–152

Price AH, Virk DS, Tomos AD (1997) Genetic dissection of root growth in rice (*Oryza sativa* L.). I: a hydroponic screen. Theor Appl Genet 95:132–142

Ray JD, Yu L, McCouch SR, Wang G, Nguyen HT (1996) Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). Theor Appl Genet 92:627–636

Temnykh S, Park WD, Ayres N (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.). Theor Appl Genet 100:697–712

Toorchi M, Shashidhar HE, Gireesha TM, Hittalmani S (2003) Performance of backcrosses involving transgressant doubled haploid lines in rice under contrasting moisture regimes: yield components and marker heterozygosity. Crop Sci 43:1448–1456

Wang DL, Zhu J, Li ZK, Paterson AH (1999) A computer software for mapping quantitative trait loci with main effects, epistatic effects and QTL × environment interactions. User manual for QTLMAPPER VER.1.0. Texas A&M University, College Station

Wang HQ, Bouman BAM, Zhao DL, Wang CG, Moya PF (2002) Aerobic rice in northern China: opportunities and challenges. In: Bouman BAM, Hengsdijk H, Hardy B, Bindraban PS, Tuong TP, Ladha JK (eds) Water-wise rice production. International Rice Research Institute Los Baños, Philippines, pp 143–154

Yadav R, Courtois B, Huang N, McLaren G (1997) Mapping genes controlling root morphology and root distribution in a double-haploid population of rice. Theor Appl Genet 94:619–632

Zhang J, Zheng HG, Aarti A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD, Sarkarung S, Blum A, Nguyen HT (2001) Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. Theor Appl Genet 103:19–29

Zhu J (1998) Mixed model approaches for mapping quantitative trait loci. Hereditas 20[Suppl]:137–138