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Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species

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Abstract Direct and indirect economic loss in the agricultural sector due to drought is huge. With the advent of molecular-marker technology, research on drought resistance in crop plants has shifted from physiological descriptions of the phenomenon to genetic dissection of the mechanisms involved. Here, we report a comprehensive study of mapping the drought resistance components (osmotic adjustment and root traits) in a doubled-haploid rice (*Oryza sativa* L.) population of 154 lines. A genetic linkage map consisting of 315 DNA markers was constructed. A total of 41 quantitative trait loci (QTLs) were identified for osmotic adjustment and root traits, and individually explained 8–38% of the phenotypic variance. A region on chromosome 4 harbored major QTLs for several root traits. Consistent QTLs for drought responses across genetic backgrounds were detected and should be useful for marker-assisted selection towards the incorporation of a trait of interest into an elite line. Comparative mapping identified three conserved genomic regions associated with various physiological responses to drought in several grass species. These results suggest that these regions conferring drought adaptation have been conserved across grass species during genome evolution and might be directly applied across species for the improvement of drought resistance in cereal crops.

Keywords Quantitative trait loci (QTLs) · Drought resistance · Osmotic adjustment (OA) · Root penetration · Rice (*Oryza sativa* L.)

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Introduction

Drought is by far the leading environmental stress in agriculture both in the U.S. and worldwide. Soils subjected to continuous drought, together with shallow soils subjected to frequent water deficit, make up 45% of the U.S. land surface (Boyer 1982). Understanding the genetic basis of drought resistance in crops is fundamental to enable breeders and molecular biologists to develop new varieties with more drought resistance characters. Many studies on drought resistance have monitored the physiological and biochemical status of stressed plants compared with unstressed plants. Important mechanisms of drought resistance deduced from these studies mainly include the following: (1) drought escape via a short life cycle, photoperiod sensitivity and developmental plasticity; (2) drought avoidance via enhanced water uptake and reduced water loss; (3) drought tolerance via osmotic adjustment (OA) and antioxidant capacity; and (4) drought recovery via desiccation tolerance (Nguyen et al. 1997; Zhang et al. 1999).

With the advent of molecular-marker technology, scanning the whole genome of crops for loci controlling a number of traits of interest is possible. Quantitative trait loci (QTLs) conferring various drought responses in several crops have been identified. QTLs for OA have been mapped in rice (Lilley et al. 1996), barley (Teulat et al. 1998) and wheat (Morgan and Tan 1996). Abscisic acid (ABA) accumulation is a common phenomenon in plants growing under drought, and QTLs for it have been mapped in wheat (Quarrie et al. 1994), maize (Lebreton et al. 1995; Tuberosa et al. 1998) and rice (Quarrie et al. 1997). Stomatal regulation plays an

Table 1 Trait mean values for CT9993, IR62266 and the 154 doubled-haploid lines (DHLs) along with broad-sense heritability (h^2). The heritability was computed as $h^2=\delta^2_g/(\delta^2_g+\delta^2_e/n)$ where δ^2_g and δ^2_e were the estimates of genetic and residual variances, respectively, derived from the expected mean squares of the analysis of variance and n was the number of replications

Trait	CT9993	IR62266	DHLs		h^2
			Mean	Range	
Osmotic adjustment (MPa)	0.47	0.78	0.59	0.24–0.99	62
Root penetration index	0.45	0.17	0.25	0.05–0.62	63
Basal root thickness (mm)	1.08	0.74	0.94	0.71–1.15	57
Penetrated root thickness (mm)	0.99	0.62	0.86	0.59–1.11	58
Root pulling force (kg)	75	45	56	28–102	80
Total root dry weight (g)	2.7	1.4	2.0	1.0–3.8	37
Penetrated root dry weight (g)	0.46	0.14	0.20	0.03–0.54	58
Penetrated root length (cm)	28.9	21.0	24.8	15.5–40.0	40

important role in reducing water loss, and QTLs for stomatal behavior have also been identified in maize (Lebreton et al. 1995) and rice (Price et al. 1997). Roots are the main organs for plant water uptake, and deep root systems help plants maintain better water status under drought. QTLs for root morphology and root penetration ability have been identified in three rice populations (Champoux et al. 1995; Ray et al. 1996; Price and Tomos 1997; Yadav et al. 1997; Zheng et al. 2000). In addition, QTLs associated with other drought-related traits have been mapped in sorghum for pre- and post-flowering resistance to drought (Tuinstra et al. 1996, 1997; Crasta et al. 1999) and in maize for anthesis-silking interval and yield components under drought (Ribaut et al. 1996, 1997).

In the present study, an integrated genetic linkage map has been constructed with various DNA markers using a rice population, and comparative mapping of QTLs for drought resistance components has been investigated across genetic backgrounds. The rationale for selecting OA and root traits is that a well-developed deep root system can facilitate water uptake from lower soil layers, and OA can help maintain the turgor of both shoots and roots as plants experience water deficits (Nguyen et al. 1997; Zhang et al. 1999). To our knowledge, this is the first comprehensive report to dissect such complex physiological and morphological traits related to drought resistance in any crop. A partitioning of drought resistance into components and a comparative QTL analysis would contribute to a better understanding of the genetic basis for drought-resistance in plants and facilitate the rapid development of drought resistant varieties. Additionally, comparative mapping of QTLs for drought resistance across species would provide insights into genome evolution and a basis for interpreting genetic information between cereals.

Materials and methods

Plant population

A doubled-haploid (DH) population was developed from a cross between CT9993–5-10–1-M (abbreviated as CT9993, an upland *japonica* type possessing a deep and thick root system and low OA) and IR62266–42–6-2 (abbreviated as IR62266, an *indica* type with a shallow root system and high OA) at Centro Internacional de Agricultura Tropical and the International Rice Research Institute in 1992. The anther culture-derived doubled-haploid lines (DHLs) were originally grown at the Ubon Rice Research Center, Thailand, for the phenotypic characterization of major agronomic traits (e.g., maturity period and canopy structure). A total of 154 DHLs randomly selected from this population were used in the present study.

Phenotyping of drought resistance components

Experiments on the phenotyping of OA and root traits were conducted in a temperature-controlled greenhouse at Texas Tech University, Lubbock, Texas. Phenotyping of OA was repeated two times with three and four replications, respectively. Plant growth and water-stress treatment followed the protocol established earlier (Babu et al. 1999). The osmotic potential of leaf samples was measured with a vapor pressure osmometer (Zhang and Kirkham 1995). Osmotic adjustment capacity was calculated as the difference in osmotic potential at full turgor between well-watered and re-hydrated plants (Babu et al. 1999; Zhang et al. 1999).

Phenotyping of root traits was repeated three times with three, five and five replications, respectively. A root-penetration screening system, the wax-petrolatum layer system developed by Yu et al. (1995) to stimulate soil compactions, was used to characterize root traits according to the modified procedure described by Zheng et al. (2000). At 50 days after sowing (DAS), the number of roots penetrated through the wax-petrolatum layer (PRN, penetrated root number), the total root number (TRN) at the stem base, the penetrated root length (PRL), the penetrated root thickness (PRT) and the basal root thickness (BRT) were recorded. Penetrated roots and total roots were dried for determination of the penetrated root dry weight (PRDW) and the total root dry weight (TRDW). BRT was measured on roots 2-cm below the soil surface and PRT was measured on penetrated roots immediately below the wax layer. Root penetration ability is expressed as the root penetration index (RPI), which was calculated as the ratio of PRN to TRN per plant.

Evaluation of root pulling force (RPF) was conducted in the field at the Ubon Rice Research Center (latitude 15°19′52.35′′ N, longitude 104°40′55.15′′ E, altitude 110 m). Seeds were sown in May, 1996, in a loamy sand soil. A randomized complete block design with four replications was used. Plot size was 4 rows x 11 hills (25 cm between rows and 25 cm between hills) with one plant per hill. The experiment was conducted in rainfed conditions with supplementary irrigation as needed. RPF was determined at the flowering stage (Ekanayake et al. 1985). Two hills per plot in each replication were randomly selected for determination of RPF.

DNA marker analysis

Genomic DNA was isolated from leaves collected at 45 DAS according to McCouch et al. (1988) and used to evaluate RFLP (restriction fragment length polymorphism), AFLP (amplified frag-

Table 2 Putative QTLs detected for osmotic adjustment (MPa), root penetration index, root thickness (mm), root pulling force (kg), root dry weight (g), and root length (cm) by interval mapping via MapMaker/QTL in a doubled-haploid population of 154 lines from CT9993 and IR62266 in rice. Individual QTLs are designated with the italicized abbreviation of the trait and the chromosome number. When more than one QTL affecting a trait is identified on the same chromosome, they are distinguished by decimal numbers

Traits	QTLs	Chr#[a]	Interval	LOD	R^2 (%)[b]	Effect[c]
Osmotic adjustment	*oa1.1*	1	ME2_12-RG140	4.6	12.9	0.10 (I)
	oa2.1	2	RM263-R3393	3.0	8.9	0.08 (I)
	oa3.1	3	EM17_1-C63	3.5	9.9	0.09 (I)
	oa8.1	8	G2132-R1394A	2.9	8.3	0.08 (I)
	oa9.1	9	EM14_6-ME4_13	2.9	8.3	0.09 (I)
			Best multiple-QTL model:		**30.5**	
Root penetration index	*rpi3.1*	3	EM19_4-EM13_1	3.0	10.9	0.06 (I)
	rpi4.1	4	EM14_5-ME2_13	2.9	8.3	0.07 (I)
	rpi4.2	4	RG939-RG476	3.4	11.0	0.06 (C)
	rpi12.1	12	ME6_12-RG9	3.6	10.3	0.06 (C)
			Best multiple-QTL model:		**32.7**	
Basal root thickness	*brt2.1*	2	TGMSP2-ME9_7	4.3	12.0	0.11 (I)
	brt3.1	3	EM19_11-RZ474	3.1	10.0	0.06 (C)
	brt4.1	4	RG939-RG476	14.0	37.6	0.12 (C)
	brt8.1	8	RZ997-EM14_1	3.81	10.8	0.08 (C)
	brt9.1	9	ME2_17-C711	7.7	20.7	0.08 (C)
	brt2.1	12	ME10_3-ME6_6	3.2	9.2	0.07 (C)
			Best multiple-QTL model:		**52.6**	
Penetrated root thickness	*prt1.1*	1	ME6_4-EM18_10	3.0	9.3	0.06 (I)
	prt1.2	1	RG957-RG345	3.2	9.2	0.06 (C)
	prt2.1	2	ME2_7-EMP2_7	4.5	12.6	0.07 (C)
	prt2.2	2	ME9_7-K706	3.2	16.0	0.11 (I)
	prt4.1	4	RG939-RG476	10.7	31.3	0.12 (C)
	prt6.1	6	R2549-RG716	3.0	9.0	0.06 (C)
	prt7.1	7	RG417-EM17_3	2.9	8.5	0.06 (C)
	prt9.1	9	RG553-EM14_6	6.6	18.0	0.09 (C)
	prt9.2	9	ME9_6-K985	6.2	18.5	0.10 (C)
	prt12.1	12	RG9-ME10_1	4.6	14.5	0.08 (C)
	prt12.2	12	ME4_5-ME10_8	4.4	12.3	0.08 (C)
			Best multiple-QTL model:		**53.0**	
Root pulling force	*rpf2.1*	2	EM13_3-RG158	3.2	9.0	9.1 (C)
	rpf3.1	3	EM11_9-CDO20	6.0	16.5	14.5 (I)
	rpf3.2	3	EM13_1-R2170	4.2	11.9	10.3 (I)
	rpf4.1	4	RG214-RG620	7.3	19.9	13.4 (C)
	rpf5.1	5	RM164-EM15_5	3.5	12.7	11.1 (I)
	rpf11.1	11	ME2_6-RM21	3.3	10.3	11.1 (I)
			Best multiple-QTL model:		**50.1**	
Total root dry weight	*trdw1.1*	1	RG109-EM11_11	3.3	9.3	0.34 (C)
	trdw2.1	2	ME2_7-EMP2_7	3.7	11.0	0.36 (C)
	trdw4.1	4	RG190-EM15_3	4.6	12.9	0.41 (C)
	trdw6.1	6	ME4_11-ME7_5	3.0	8.6	0.31 (C)
	trdw10.1	10	RG257-ME5_16	7.1	20.2	0.51 (C)
			Best multiple-QTL model:		**34.1**	
Penetrated root dry weight	*prdw4.1*	4	RG939-RG476	3.5	11.5	0.07 (C)
	prdw9.1	9	R41-ME2_10	5.4	16.8	0.09 (C)
	prdw12.1	12	RG9-ME10_1	4.1	13.2	0.07 (C)
			Best multiple-QTL model:		**26.0**	
Penetrated root length	*prl11.1*	11	G1465-C950	5.8	17.0	3.75 (C)

[a] Chromosome number
[b] Phenotypic variation explained by detected QTLs
[c] Letters I and C in parentheses indicate that positive or favorable alleles for the effects are from IR62266 and CT9993, respectively

ment length polymorphism) and microsatellite markers. RFLP was done essentially by following the procedure of Causse et al. (1994). Ten restriction enzymes (*Apa*I, *Eco*RI, *Eco*RV, *Bam*HI, *Bgl*II, *Dra*I, *Hind*III, *Kpn*I, *Sca*I and *Xba*I) were used to digest DNA for RFLP analysis. AFLP was performed as described by Vos et al. (1995) with minor modification (Bai et al. 1999). Microsatellite primer pairs (Research Genetics Inc. USA) were used to amplify the simple-sequence-length polymorphic DNA according to Chen et al. (1997). DNA bands were visualized via silver staining as described by Panaud et al. (1996).

Map construction and QTL analysis

The genetic linkage map was constructed using Mapmaker Macintosh V2.0. AFLP markers were assigned to chromosomes

based on their linkage to microsatellite or RFLP anchor markers (Causse et al. 1994; Cho et al. 1998; Harushima et al. 1998). Data from repeated experiments were pooled together and the means for each trait were used to identify QTLs by employing MapMaker/QTL (Lincoln et al. 1993). The threshold of the LOD score used for declaring the presence of QTLs was 2.8, which was derived based on the total map distance and the average distance between markers according to Lander and Botstein (1989). Tests for independence of QTLs were conducted when two or more QTLs of the same trait were located on the same chromosome, as described by Paterson et al. (1988). For the best multiple-QTL model, a maximum of seven QTLs is allowed in MapMaker/QTL. If more than seven QTLs were detected for one trait, the QTLs which explain the highest values of phenotypic variation (R^2) were selected for the model.

Results

Phenotypic variation

The mean trait values for the two parents and the 154 DHLs, along with the broad-sense heritabitility, are listed in Table 1. The frequency distribution of phenotypes for these traits evaluated in this study approximately fit normal distributions (data not shown). The two parents, CT9993 and IR62266, differed statistically for all traits examined ($P<0.05$). The heritability varied with traits, ranging from 37 to 80%, with RPF having the highest and TRDW having the lowest values.

Parental polymorphism and genetic linkage map

The percentage of polymorphism detected by RFLP between the two parents CT9993 and IR62266 varied with the restriction enzyme, *Eco*RV having the highest (23%) value. The AFLP data were generated using 19 *Eco*RI/*Mse*I primer pairs. The percentage of polymorphism detected by AFLP between the two parents was 20%. Compared with the RFLP and AFLP techniques, microsatellites detected the highest percentage (48%) of polymorphism between the two parents.

The overall frequency distribution of the percent CT9993 alleles for 315 markers and 154 DHLs is close to the expected 50%. An integrated molecular genetic linkage map consisting of 153 AFLPs, 145 RFLPs and 17 microsatellites was constructed, which covered 1,788 cM in length using the Kosambi function with an average distance of 5.7 cM between adjacent markers (Fig. 1). The map orientation was based on the molecular linkage map of rice given by Singh et al. (1996). The map length for individual chromosomes or the whole genome was comparable to that of Cho et al. (1998) and Harushima et al. (1998).

QTLs for components of drought resistance

A total of 41 putative QTLs have been identified for OA and the root traits evaluated in this study (Table 2 and Fig. 1). Five QTLs associated with OA were detected on chromosomes 1, 2, 3, 8 and 9. Thirty six QTLs for root traits were identified on all 12 chromosomes. The number of QTLs identified for each trait varied from 1 to 11 with the phenotypic variation (R^2) ranging from 8 to 38%. The IR62266 parental line contributed all the favorable alleles for OA, whereas CT9993 contributed 75% of favorable alleles for the root traits.

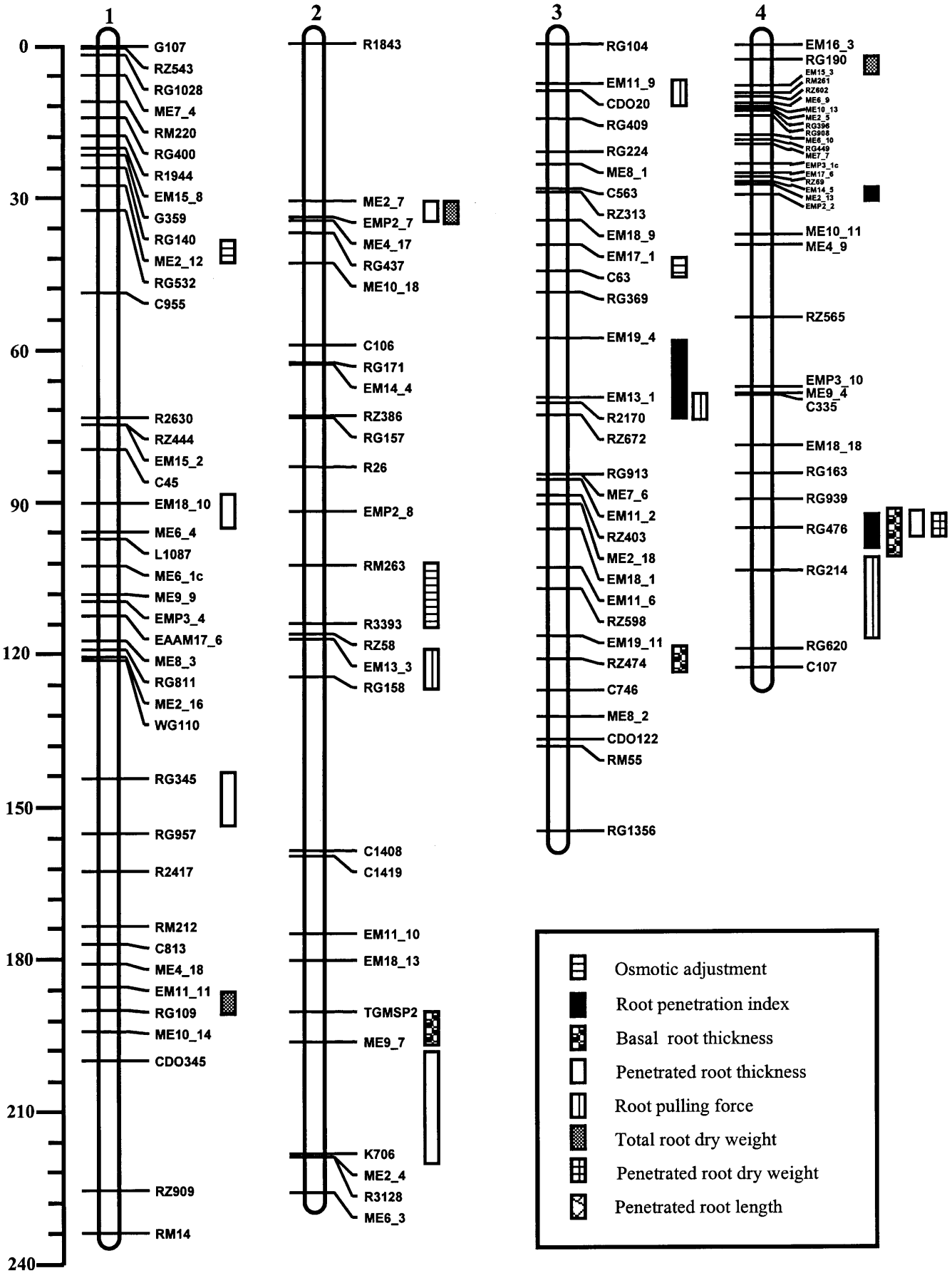
QTLs for OA did not overlap with any of the QTLs for root traits. This result is supported by the correlation analysis between traits. We observed that the root penetration index and OA were not statistically correlated ($P>0.05$). This result implies that molecular mechanisms of drought tolerance via OA, and drought avoidance via a good root system, are different. In addition, when comparing the locations of these QTLs, we found that the genomic region RG939-RG476-RG214 on chromosome 4 (Fig. 1) harbored QTLs for RPI (*rpi4.2*), RPF (*rpf4.1*), BRT (*brt4.1*), PRT (*prt4.1*), and PRDW (*prdw4.1*). Moreover, the CT9993 parental line contributes all these favorable alleles. Therefore, this region may contain either one gene (more likely) derived from CT9993 having pleiotropic effects on root traits or more genes conferring several different root traits, respectively.

Comparison of QTLs for root penetration ability and root thickness

Root penetration ability and root thickness are two important parameters contributing to drought resistance. To determine if there are any common QTLs for root penetration ability and root thickness across rice genetic backgrounds, the results from this study were compared with those of other reports available in the literature.

For RPI, the major QTL *rpi4.2* (RG939-RG476-RG214) on chromosome 4 of the present study was common to the QTL (RG214-RG476) identified in the CO39/Moroberekan (Ray et al. 1996) and the QTL (RG214) identified in the IR64/Azucena (Zheng et al. 2000) populations. We also identified two QTLs for RPI with 2.0<LOD<2.8 located in the RZ543-G107 and EM11_10-EM18_13 regions of chromosomes 1 and 2, respectively. We further observed that the QTL (RZ543-G107) was common to the QTL (RG472) identified in the IR64/Azucena population (Zheng et al. 2000) be-

Fig. 1 The molecular genetic linkage map of rice based on 154 doubled-haploid lines derived from a cross between CT9993 and IR62266 in rice. Chromosome numbers are indicated above each chromosome. Distances are given in Kosambi centiMorgans. The *letters* before the numbers in the marker names indicate the category of mapped clones as follows: *RM*, rice microsatellites; *EM and ME*, AFLPs markers; *other letters*, RFLPs. *R1G1* was a cDNA fragment cloned via a differential display procedure homologous to water stress-induced mRNA in rice. *TGMSP2* was the DNA marker tightly linked to the thermo-sensitive genic male-sterile gene in rice. The positions of QTLs are indicated by *vertical bars* beside chromosomes. The bar length is drawn to be equal to the length as detected for the QTL in the MapMaker/QTL ▶


1
2
3
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0
30
60
90
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150
180
210
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G107
RZ543
RG1028
ME7_4
RM220
RG400
R1944
EM15_8
G359
RG140
ME2_12
RG532
C955
R2630
RZ444
EM15_2
C45
EM18_10
ME6_4
L1087
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RM212
C813
ME4_18
EM11_11
RG109
ME10_14
CDO345
RZ909
RM14
R1843
ME2_7
EMP2_7
ME4_17
RG437
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EM17_1
C63
RG369
EM19_4
EM13_1
R2170
RZ672
RG913
ME7_6
EM11_2
RZ403
ME2_18
EM18_1
EM11_6
RZ598
EM19_11
RZ474
C746
ME8_2
CDO122
RM55
RG1356
EM16_3
RG190
ME10_11
ME4_9
RZ565
EMP3_10
ME9_4
C335
EM18_18
RG163
RG939
RG476
RG214
RG620
C107
Osmotic adjustment
Root penetration index
Basal root thickness
Penetrated root thickness
Root pulling force
Total root dry weight
Penetrated root dry weight
Penetrated root length

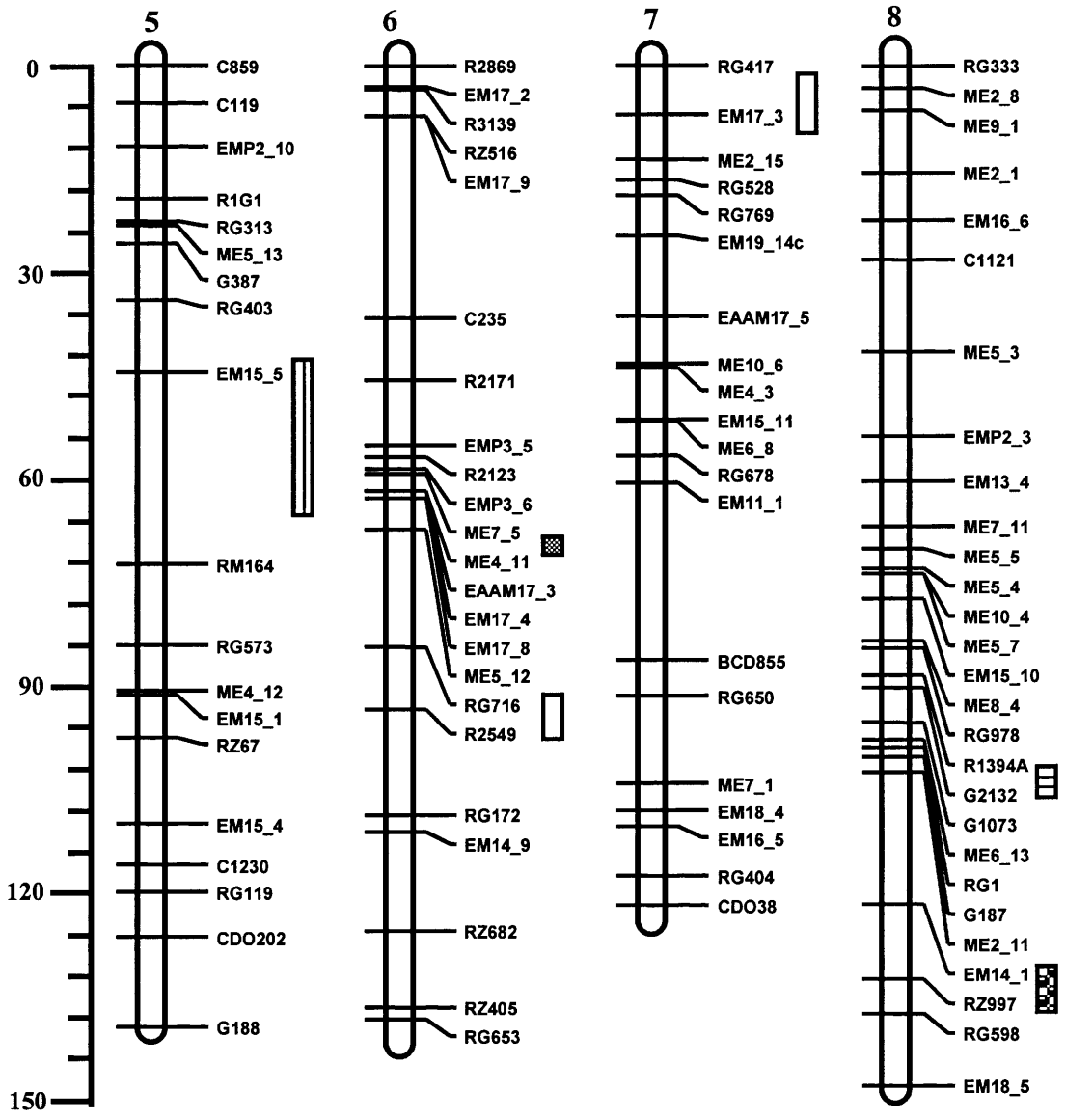

Fig. 1 (continued)

cause the marker RG472 was tightly linked to the marker RZ543 (Causse et al. 1994). The QTL (EM11_10-EM18_13) was also common to the QTL (RZ123-RG520) identified using the IR64/Azucena population (Zheng et al. 2000).

Regarding root thickness, it is noteworthy that the QTL *brt4.1* and *prt4.1* (RG939-RG476) with the largest variation in BRT and PRT identified in this study was common to the QTL for PRT in the IR64/Azucena (Zheng et al. 2000) and the QTL for BRT in the CO39/Moroberekan (Champoux et al. 1995) populations, respectively (Fig. 2). The QTL *prt1.2* (RG957-RG345) identified in this study was common to the QTL in the RZ19-RZ730 region identified in these two populations (Champoux et al. 1995; Yadav et al. 1997; Zheng et al. 2000) because RG957 was tightly linked to the marker RZ19 (Causse et al. 1994). Previously, this QTL was identified as a consistent one across the IR64/Azucena and CO39/Moroberekan populations (Zheng et al. 2000). In addition, both EM14_1 and RZ66 were located between RG1 and RZ997 in this study and also in an earlier study by Causse et al. (1994). Therefore, the QTL *brt8.1* (RZ997-EM14_1) identified in this study was common to the QTL located in the RZ66 region in both the CO39/Moroberekan (Champoux et al. 1995) and IR64/Azucena (Yadav et al. 1997) populations. The QTL *prt9.2* (ME9_6-K985) was common to the QTL (Amy3ABC-RZ12) identified in the IR64/Azucena (Zheng et al. 2000), and the QTL (RZ12-RG570) identified in the CO39/Moroberekan (Champoux et al. 1995), populations because there was a distance of 11 cM between RG667 and K985 in this study and 7.4 cM between RG667 and RZ12 in the IR64/Azucena population (Zheng et al. 2000). We also observed that two QTLs for BRT with 2.0<LOD<2.8 were identified to be common to QTLs mapped in the CO39/Moroberekan population

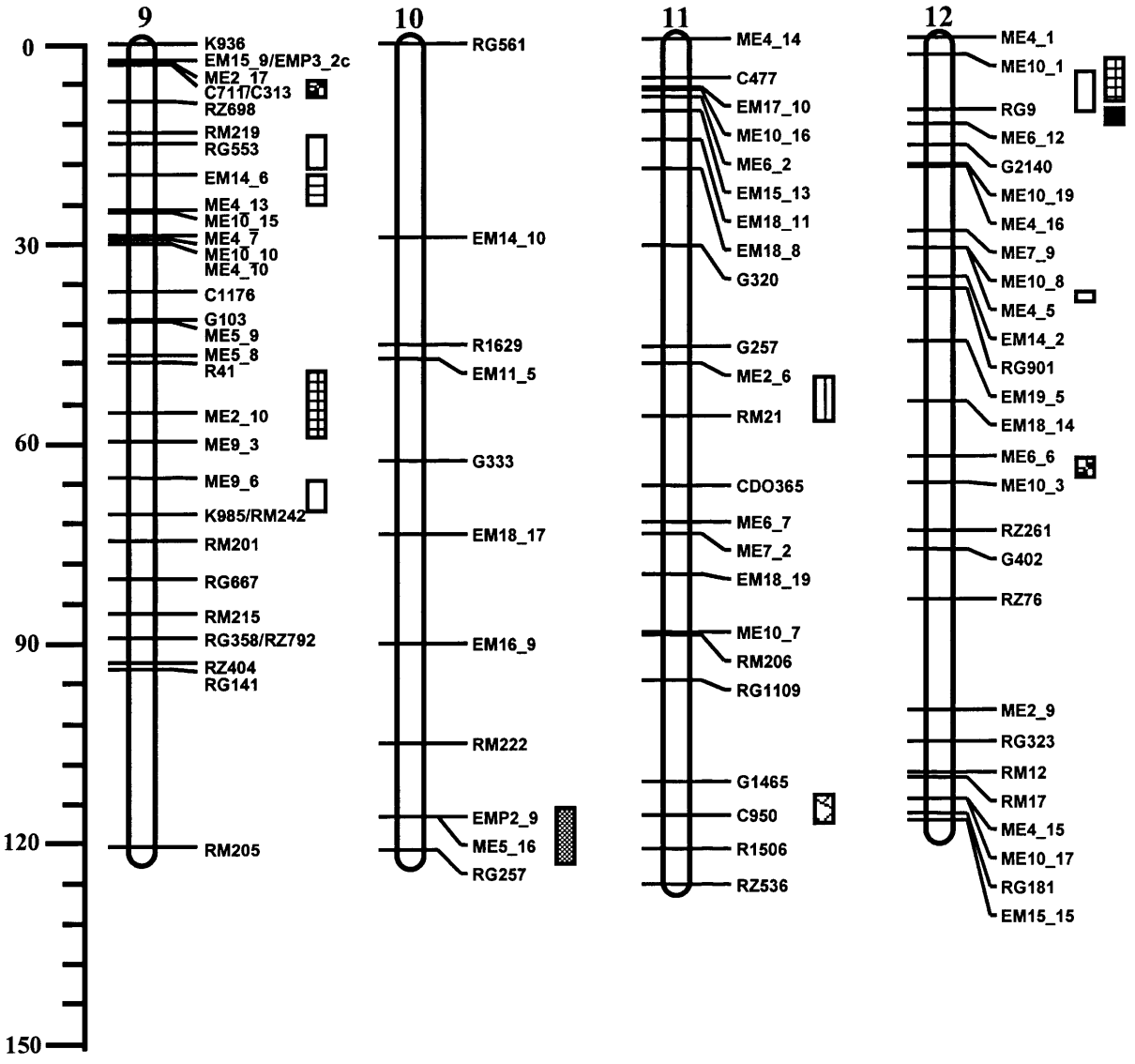

Fig. 1 (continued)

(Champoux et al. 1995). One QTL (ME10_18-C106) located between RG437 and RG171 on chromosome 2 was common to the QTL located in the RG437-RG171-RG157 region, and the second QTL (C235-EM17_9) on chromosome 6 was common to the QTL located in the WAXY-RZ516 region in the CO39/Moroberekan population.

Comparison of QTLs for OA and other physiological response to drought

The *oa8.1* QTL identified in the present study was located in the same genomic region as the OA QTL detected by Lilley et al. (1996). Comparative mapping indicates that this genomic region is homoeologous with a segment of wheat chromosome 7 S where a single locus putatively associated with OA was identified (Fig. 3) (Morgan and Tan 1996). In barley, Teulat et al. (1998) identified several QTLs for osmotic adjustment-related traits, and the osmotic potential QTL identified on barley chromosome 1 was homoeologous with the OA QTL region of rice chromosome 8. These results suggest that this genomic region contains a gene or cluster of genes that may confer drought adaptation in many cereals, at least in barley, rice and wheat.

The *oa3.1* QTL was located on chromosome 3 between EM17_1 and C63 (Fig. 1). Another two flanking RFLP markers for this QTL are RZ313 and RG369. In this region, a QTL for stomatal behavior was detected in a rice F_2 mapping population (Price et al. 1997). Based on the syntenic relationship between maize and rice (Ahn and Tanksley 1993), this corresponds to the UMC11 region of maize chromosome 1 (Fig. 4). This region was also associated with various physiological and agronomic traits in maize, i.e., stomatal conductance, xylem ABA concentration and RPF under drought and other stress conditions (Lebreton et al. 1995), leaf ABA

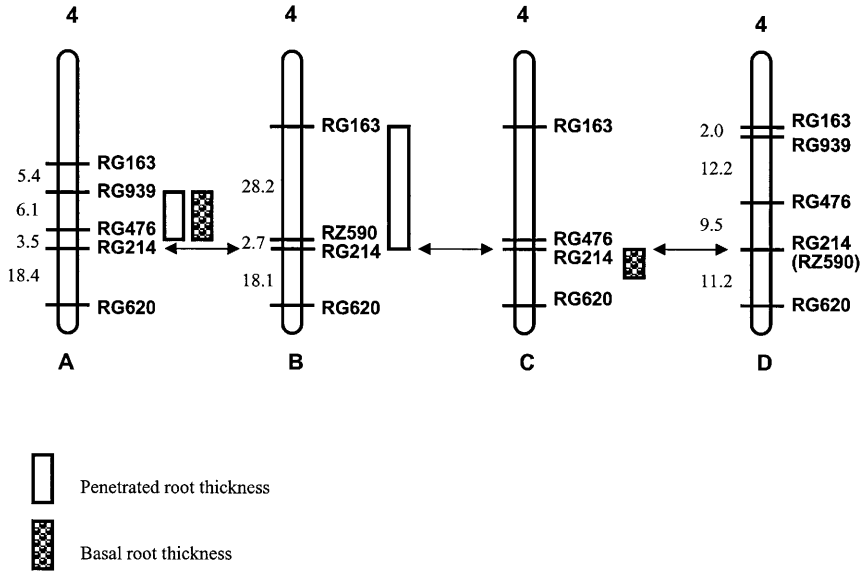

Fig. 2 A genomic region of rice chromosome 4 showing the common QTLs with the largest variation for root thickness across rice genetic backgrounds. *Map A* was from Fig. 1 of this study, *B* was redrawn from Zheng et al. (2000), *C* was from Champoux et al. (1995) and *D* was from Causse et al. (1994). Map *D* showed all the anchor RFLP markers in this chromosomal region

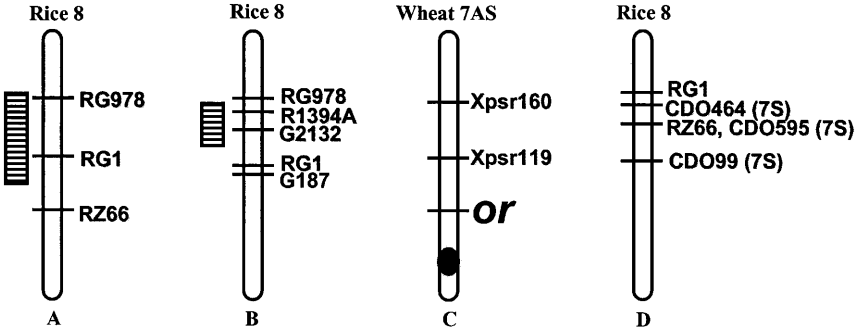

Fig. 3 A conserved genomic region for osmotic adjustment between rice and wheat. Chromosome numbers are indicated above each chromosome (for clarity, only partial chromosomes were drawn). *Vertical bars* beside rice chromosome 8 (*A and B*) indicate the positions of QTLs for osmotic adjustment as detected in the CO39/Moroberekan recombinant inbred and the CT9993/IR62266 doubled-haploid populations. The *or* on wheat chromosome 7AS represents the osmoregulation gene (*C*). Markers CDO464, CDO595 and CDO99 followed by 7 S in the parentheses on rice chromosome 8 indicate that they are also mapped on the short arm of wheat chromosome 7. Map *A* is redrawn from Lilley et al. (1996), B is from Fig. 1 of this study, *C* is from Morgan and Tan (1996), and *D* is from Ahn et al. (1993) and Causse et al. (1994).

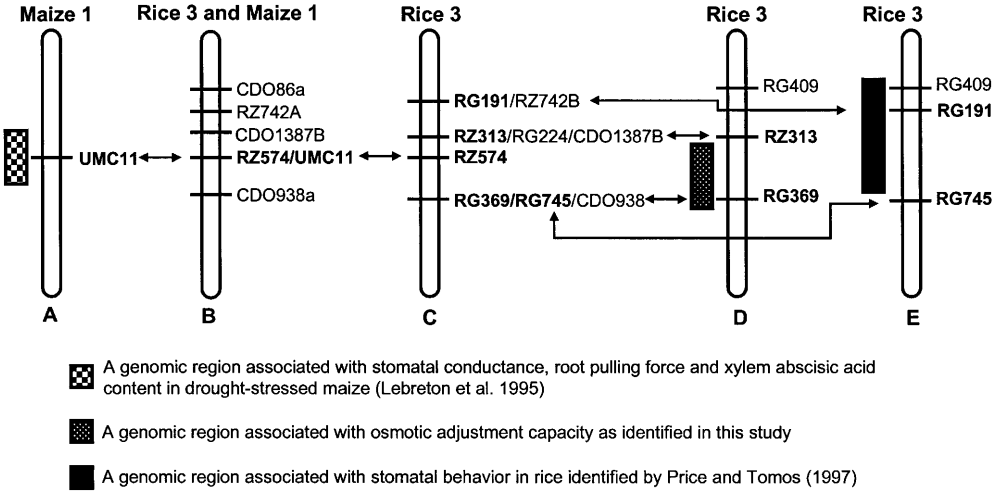

Fig. 4 A genomic region for drought response conserved between rice and maize. The partial map *A* was redrawn from Lebreton et al. (1995), *B* was from Ahn and Tanksley (1993) and Rice3-maize at http://genome.cornell.edu, *C* was from Causse et al. (1994), *D* was from Fig. 1 of this study and *E* was from Price et al. (1997). The maps *B* and *C* were used as a bridge to infer the conserved region between rice and maize

concentration (Tuberosa et al. 1998), anthesis-silking interval (Ribaut et al. 1996), enzymatic activity involved in carbohydrate metabolism (Prioul et al. 1997) and yield components (Agrama et al. 1996; Ribaut et al. 1996, 1997; Austin and Lee 1998). These results suggest that, during cereal evolution, genes in this genomic region in maize and rice have been conserved to respond to drought conditions.

The accumulation of osmolytes and ABA are two common responses of plants to drought stress. To determine whether QTLs for OA and ABA are located in similar genomic regions across genetic backgrounds in rice, results from this study were compared with those of Quarrie et al. (1997) using the linkage maps established by Causse et al. (1994), Cho et al. (1998) and Harushima et al. (1998) as bridges. Two QTLs for OA and ABA in these two populations were located in similar genomic regions: *oa2.1* vs AFLP9 on chromosome 2, and *oa9.1* vs R1751 on chromosome 9. In addition, an ABA QTL was mapped on the long arm of wheat chromosome 5 A (Quarrie et al. 1994), which was homoeologous to rice chromosome 9 (Ahn et al. 1993). The RFLP markers (psr575, psr426 and psr145) linked to the ABA QTL in wheat (Quarrie et al. 1994) cosegregated with CDO388 (Gill and Raupp 1997) and was mapped in the *oa9.1* region. Therefore, the ABA QTL detected in wheat was located in a similar genomic region where an ABA QTL (R1751) and OA QTL *oa9.1* were mapped in rice. Again, a conserved genomic region for drought response was found between wheat and rice.

Discussion

Two rice populations, derived from the cross CO39/Moroberekan and IR64/Azucena, were previously used to detect QTLs for root penetration ability, OA and root morphology (Champoux et al. 1995; Lilley et al. 1996; Ray et al. 1996; Yadav et al. 1997; Zheng et al. 2000). These studies were focused either on root traits or OA. In order to better understand the mechanisms of drought tolerance via OA and drought avoidance via a deep root system in rice, a molecular dissection of QTLs for both OA and root traits in one genetic background is important. In the present study, a DHL population from CT9993/IR62266 was used for mapping QTLs for OA and root traits. The parents were well-studied at morphological and physiological levels and performed differently. The DHL population segregated both under field conditions and controlled greenhouse conditions for physiological and morphological traits and plant production (Blum et al. 1999). These contrasting features in the two parents and the DHL mapping population make this germplasm ideal for the study of drought resistance in rice.

On molecular mapping of drought resistance, the present study differs from others. The population type (DHLs) and size (154 lines) used were more appropriate than the F_2 type (Lebreton et al. 1995; Price and Tomos 1997) or the small size employed by others (Lilley et al. 1996; Quarrie et al. 1997). For mapping QTLs conferring complex traits, accurate phenotyping requires replicated experiments, which F_2 populations can not provide. In addition, in this study, proper protocols were used to evaluate OA capacity and plants were stressed in a more-natural way (Babu et al. 1999; Zhang et al. 1999). Furthermore, the comparative QTL mapping was conducted within and across species. The QTLs for root penetration ability and root thickness were compared across rice genetic backgrounds. The consistent QTLs identified in this study could be useful in marker-assisted selection for thick roots with high root-penetration ability in rice drought resistance breeding programs.

Osmotic adjustment is the only putative drought-resistant trait that has been associated with sustained yield in crop plants under drought (Zhang et al. 1999). However, due to the complicated protocols involved in phenotyping this trait, only 52 lines were used to map the QTL for OA in rice (Lilley et al. 1996). Based on the information from their conventional genetic studies, Morgan and Tan (1996) selected 14 lines and then mapped an OA gene on the short arm of chromosome 7 A in wheat. For the first time, using a larger mapping population of 154 DHLs, we mapped QTLs for OA and compared genomic regions associated with various physiological responses to drought in several grass species. These genomic regions conferring drought adaptation across the grass species identified in this study might be directly applied across species for the improvement of drought resistance in cereal crops.

Root thickness, RPI, RPF and OA capacity apparently contributed to increased drought resistance. Champoux et al. (1995) found that the lines with the most extensive roots had increased drought avoidance over the lines with the least extensive roots. Furthermore, they found that about 30 putative QTLs for root characters from the greenhouse experiments, located in 12 genomic regions, were identified to be associated with field drought avoidance (Champoux et al. 1995). In our study, we observed that nine QTLs, including *prt1.2*, *trdw4.1*, *rpi4.1*, *rpi4.2*, *prt4.1*, *brt4.1*, *prdw4.1*, *rpf4.1* and *oa8.1*, overlapped with these QTLs located on four genomic regions of chromosomes 1, 4 and 8 associated with drought avoidance identified in the CO39/Moroberekan population (Champoux et al. 1995).

Characterization of QTLs for drought resistance in this study provides insight into an understanding of the mode of drought resistance in rice. The most-practical application of the identified QTLs for drought resistance components is to perform marker-assisted selection aimed at the efficient pyramiding of favorable QTL alleles to improve drought resistance in rice.

Research results from rice can easily be used to explore particular drought resistance mechanisms in other cereals because of the high level of synteny and colinearity (Devos and Gale 1997). Therefore, discovery of genomic regions associated with drought resistance across genetic backgrounds or species may have an impact on map-based cloning of the genes controlling the resis-

tance to drought in plants. Currently, fine QTL-mapping of root traits and OA and the development of near-isogenic lines carrying various QTLs of interest for a cause-and-effect study are under way in our laboratory. This research will lead to the development of marker-assisted selection in breeding for drought resistance in rice.

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