Theor Appl Genet (2001) 103:75–83

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Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection

Received: 17 July 2000 / Accepted: 20 October 2000

Abstract Drought is one of the main abiotic constraints in rice. A deep root system contributes efficiently to maintaining the water status of the crop through a stress period. After identifying QTLs affecting root parameters in a doubled-haploid (DH) population of rice derived from the cross IR64/Azucena, we started a marker-assisted backcross program to transfer the Azucena allele at four QTLs for deeper roots (on chromosomes 1, 2, 7 and 9) from selected DH lines into IR64. We selected the backcross progenies strictly on the basis of their genotypes at the marker loci in the target regions up to the BC_3F_2. We assessed the proportion of alleles remaining from Azucena in the non-target areas of the BC_3F_2 plants, which was in the range expected for the backcross stage reached. Twenty nine selected BC_3F_3 near-isogenic lines (NILs) were developed and compared to IR64 for the target root traits and three non-target traits in replicated experiments. Of the three tested NILs carrying target 1, one had significantly improved root traits over IR64. Three of the seven NILs carrying target 7 alone, as well as three of the eigth NILs carrying both targets 1 and 7, showed significantly improved root mass at depth. Four of the six NILs carrying target 9 had significantly improved maximum root length. Five NILs carrying target 2 were phenotyped, but none had a root phenotype significantly different from that of IR64. A re-analysis of the initial data with the composite interval mapping technique revealed two linked QTLs with opposite effects in this area. Some NILs were taller than IR64 and all had a decreased tiller number because of a likely co-introgression of linked QTLs. The usefulness of NILs, the efficiency of marker-aided selection for QTLs and the relationship between root traits are discussed. The NILs with an improved root system will permit testing the importance of root depth for water-limited environments.

Keywords Drought tolerance · *Oryza sativa* · Root depth · Near-isogenic lines · Marker-aided selection · Introgression · QTLs

Communicated by H.C. Becker

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Introduction

Rice is the main food crop of the world with 85% of its production devoted to human consumption (IRRI 1997). Rice is a heavy consumer of water, needing some 5,000 liters of water to produce 1 kg of rice, and is less efficient in the way it uses water than eihter wheat or maize. Drought is an increasingly important problem limiting rice production in many areas of Asia. Drought naturally affects the rainfed rice ecosystems because the crop relies strictly on rainfall for its water supply. It also increasingly affects the irrigated rice ecosystem because of undependable irrigation water (IRRI 1995). Drought is the source of huge yield losses. For example, Widawsky and O'Toole (1990) evaluated at 3.0 million tons the annual drought losses from the 24 millions hectares of rice of Eastern India, which represented 22% of all losses from technical constraints. A deep and thick root system is generally considered as a favorable element allowing the crop to maintain its water status under stress conditions (Nguyen et al. 1997) when there is water at depth. However, little effort has gone into improving the genetic potential of rice for root traits because of the difficulties in measuring and manipulating them.

Studies using molecular markers to map the genes involved in the control of rice root morphology have started to accumulate (Champoux et al. 1995; Ray et al. 1996; Yadav et al. 1997; Price and Tomos 1997; Price et al. 2000; Zheng et al. 2000). Many quantitative trait loci (QTLs) affecting root morphology have been identified. By selecting for the desirable allele at markers closely linked to these QTLs in a backcross scheme, DNA markers offer the possibility of manipulating such traits more efficiently.

In rice, several authors have demonstrated the efficiency of marker-aided selection (MAS) for the successful transfer of major genes for blast resistance (Inukai et al. 1996; Hittalmani et al. 2000) and for bacterial blight resistance (Huang et al. 1997). MAS for QTLs has recently started to be applied to the genetic improvement of quantitative traits in several crops such as tomato (Lawson et al. 1997; Bernacchi et al. 1998), maize (Graham et al. 1997) and barley (Han et al. 1997; Toojinda et al. 1998). Useful guidelines have been provided for methodological choices (Visscher et al. 1996a; Hospital and Charcosset 1997), and overall breeding strategies have been proposed (Tanksley and Nelson 1995; Tuinsntra et al. 1997).

In this study, we report an effort to improve the rice root system by marker-aided transfer of several root QTLs and the development and evaluation of near-isogenic lines of IR64 carrying the QTLs. An additional objective of this study was to assess the possible effects of these introgressed segments on other important agronomic traits through pleiotropy or linkage drag.

Material and methods

Target QTLs and markers analyzed

QTLs controlling rice root traits had been previously identified in the Azucena x IR64 doubled-haploid population using flanking-marker-regression analysis (Yadav et al. 1997). Four QTLs, on chromosomes 1, 2, 7 and 9 (designated as targets 1, 2, 7 and 9), were chosen as target regions for introgression in our marker-aided backcross scheme. A total of 17 restriction fragment length polymorphism (RFLP) and microsatellite markers linked to the target QTLs were used. These included RZ19, RG690, RZ730 and RZ801 for the target region of chromosome 1; RM29, RG171, RG157 and RZ318 for the target region of chromosome 2; RM234, CDO418, RZ978, CDO38 and RM248 for the target region of chromosome 7; and RZ228 and RZ12, replaced by RM201 and RM242 (microsatellite markers) after the BC_2 generation, for the target region of chromosome 9.

Development of the BC_3F_2 NILs

The marker-aided backcross program was conducted using IR64, an improved indica variety widely grown in South and Southeast Asia, as the recipient and recurrent parent, and four selected DH lines derived from the cross Azucena x IR64 as the donor lines. The selected DH lines (P0035, P0055, P0295 and P0475) carried Azucena alleles at the QTL locations and more than the average proportion of IR64 alleles in the rest of the genome (Table 1). Three of the four initial lines (P0055, P0295 and P0475) also carried Azucena alleles at other target areas, thus allowing selection of backcross progenies that simultaneously retained donor segments at several target regions.

The backcross procedure for introgression of the target QTLs is shown in Table 2. Briefly, the four donor lines were crossed with the recurrent parent and the F_1 plants were backcrossed on IR64 to produce the BC_1F_1 progenies. In BC_2F_1 and BC_3F_1, for each segment, 30 plants were genotyped with the appropriate markers and those BC plants heterozygous at the marker loci were selected as female parents for further backcrossing to IR64. A total of 32 BC_3F_1 plants heterozygous at the target markers were selfed to produce 312 BC_3F_2, which were genotyped to identify plants homozygous with the Azucena alleles at the target markers and possible recombinants within each target region. These BC_3F_2 plants were also genotyped with a total of 60 well-distributed microsatellite markers to estimate the proportion of the Azucena genome remaining in the non-target areas in each of the BC_3F_2's. A total of 22, 24, 30 and 21 markers, chosen to be polymorphic between IR64 and the parental DH line of target, were used for targets 1, 2, 7 and 9 respectively. Fifty eight BC_3F_3 lines were obtained that were near-isogenic to IR64 except in one or two of the introgressed QTL regions.

Table 1 Doubled-haploid lines selected for backcrossing

Donor		Primary target region			Information on QTLs at primary target[b]				
DH line	Azucena proportion[a] (%)	Chr.	Markers	Length of the interval (cM)	Trait	Interval	R^2 [c]	Effect	Presence of a secondary target in donor DH line
P0055	33.8	1	RZ19 - RG690 - RZ730-RZ801	61.4	MRL	RZ19-RG690	8.9	3.661	Yes (2)
					DRW	RG690-RZ730	7.5	0.028	
					TRW	RZ19-RG690	9.6	0.119	
P0035	38.9	2	RG437 - RG171 - RG157 - RZ318	99.2	MRL	RG171-RG157	9.9	3.812	No
P0295	44.9	7	RM234 - CDO418 - RZ978 - CDO38 - RG351 - RM248	42.4	MRL	CDO418-RZ978	17.7	4.896	Yes (1 and 2)
					DRW	CDO418-RZ978	14.7	0.034	
					TRW	CDO418-RZ978	4.8	0.080	
P0475	37.0	9	RZ228 - RM242 - RZ12 - RM201 - RG667	30.8	MRL	RZ12-RM201	8.8	3.582	Yes (7)
					DRW	RZ206-RZ422	5.6	0.022	

[a] Proportion of Azucena alleles in all genomes except the primary target region

[b] From Yadav et al. (1997) using regression on flanking markers as the method for QTL analysis. MRL = maximum root length; DRW = deep root weight (root weight below 30 cm); TRW = total root weight

[c] R^2 = percentage of phenotypic variability accounted for by the putative QTL. The sign of additive effects represents the effects of Azucena alleles

Table 2 Sequence of operations in the marker-aided selection (MAS) scheme for the introgression of QTLs for root depth using four DH lines derived from the cross IR64/Azucena as donors and IR64 as recurrent parent

Backcross generation	Season[a]	MAS for primary target	MAS for secondary target	Plants genotyped (no.)	Plants with desired genotypes (no.)	Plants selected for further backcrossing or selfing (no.)
F_1[b]	1996 DS	No	No			
BC_1F_1	1996 WS	No	No			
BC_2F_1	1997 DS	Yes	No	120	33	18
BC_3F_1	1997 WS	Yes	Yes	120	50	15
BC_3F_2	1998 DS	Yes	Yes	74	32	32

[a] DS = dry season, WS = wet season
[b] Hybridization DH line/IR64

Phenotyping of BC_3F_3 lines for root traits and other traits

Twenty nine of the 58 BC_3F_3 lines were chosen to represent different patterns of recombination at the markers within the target chromosomal segments in order to locate QTLs to the smallest possible genomic interval. They were evaluated for root traits in a replicated greenhouse experiment conducted during the 1999 dry season. The experimental design was an alpha-lattice replicated six times with five blocks per replication. The check, IR64, was included in all blocks.

The techniques used to grow plants and measure roots were similar to those described in Yadav et al. (1997). Briefly, the plants were grown under aerobic conditions in well-drained plastic bags set into polyvinyl chloride cylinders 1-m long and 0.2-m in diameter, and filled with uniform sandy loam soil. The plants were watered three times a week and did not undergo any water stress.

At 43 days after sowing, the number of tillers per plant (TILg) was counted. The shoots were collected and the shoot dry weight (SDW) determined after oven-drying the samples at 65°C for 72 h. The soil column was cut into three sections of 30-cm length. The maximum root length (MRL) was then determined by searching the columns for the point reached by the longest nodal root. The roots from each section were then carefully washed. The number of roots (NBR) in the 0–30-cm layer was counted. Root thickness (THK) was measured on ten roots 2 cm below the tillering plateau using a micrometer. The roots were then oven-dried and weighed to determine the root dry weight in the three sections (RW0030, RW3060 and RW6090). The total root weight (TRW) was computed by summing the root dry weight of the three sections. The deep root weight (DRW) was obtained by adding the root dry weight of the 30 to 60- and 60 to 90-cm sections.

In the 1999 dry season, 43 of the BC_3F_3 NILs (those with sufficient seeds) and IR64 were phenotyped for plant height (HGT), the duration of sowing to maturity (DUR) and the number of tillers per plant (TILf) in a field-test conducted under irrigated conditions at IRRI. The design was an alpha-lattice with four replications and five blocks per replication. Each individual plot had three 3-m-long rows at 0.25-m spacing with 13 plants per row. The remaining 15 BC_3F_3 NILs were grown in the greenhouse during the 1999 dry season to increase seeds, and the BC_3F_4 lines were evaluated for the same traits in the 1999 wet season with the same design.

Analyses of variance were performed on all data using a mixed model (SAS Proc mixed). Contrasts were computed between the adjusted mean of each NIL and the adjusted mean of IR64 with a significance threshold set at 5%.

Results

Genotype of the BC_3F_2 plants

Table 3 shows the genotype of 29 selected BC_3F_2 plants for the tested markers and gives the proportion of Azucena alleles remaining in the non-target areas. The frequency of the Azucena alleles in the non-target regions ranged from 0.0% to 9.3%, with an average of 3.0% as expected from the mean proportion of the Azucena genome in the four donor lines and the generation reached. In the target QTL regions, BC_3F_2 plants introgressed with the same QTL differed in the size of the introgressed donor segment. This is because the original segments were fairly large and recombination had occurred within each target region during the process of MAS.

Relationships between genotype and phenotype in BC_3F_3 for the target root traits

Table 4 shows the results of the statistical test comparing each of the 29 BC_3F_3 NILs and the recurrent parent IR64 for the target root traits and other important agronomic traits.

Maximum root length

In the original DH population, QTLs for MRL were found in target 1, 2, 7 and 9 regions (Table 1).

In BC_3F_3 progenies, a significantly improved MRL was found in four of the six NILs for target 9. They outperformed IR64 by 12% to 27%. The MRL of the other two NILs carrying target 9 was also slightly higher than that of IR64. This confirmed the presence of a QTL for MRL on the target segment of chromosome 9.

All the other lines had an MRL similar to that of IR64, with one exception for line IR74405-720-12. Looking for an explanation of these unexpected results, we re-analyzed the initial data of Yadav et al. (1997) using the composite interval mapping technique (Zeng 1993, 1994). The results for QTLs on the target chromosomes are presented in Table 5.

The initial QTL for MRL on chromosome 1 was not detected with this method and its detection in the initial study might just have been a false positive.

Two adjacent QTLs with opposite effects located between RG171 and RG157, and between RZ318 and PalI, were detected for target 2. Four of the five NILs carried

Table 3 Genotype of parental lines and derived NILs at the four target areas and proportion of Azucena alleles in the rest of the genome. 1 = IR64 allele; 3= Azucena allele; 2 = heterozygous; 3/2 = undetermined between 3 and 2; 0 = not tested

Line	Target	Prop Azu. [a]	RZ19	RG690	RZ730	RZ801	RM29	RG171	RG157	RZ318	RM234	CDO418	RZ978	CDO38	RM248	RM242	RM201
Chromosome			1	1	1	1	2	2	2	2	7	7	7	7	7	9	9
Dist. between mks. (cM)			0.0	7.8	13.4	41.8	0.0	1.5	24.9	37.7	0.0	11.8	11.4	5.2	12.6	0.0	5.8
IR64		0.0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P0055	1	33.8	3	3	3	1	3	3	3	3	1	1	1	1	1	1	1
P0035	2	38.9	1	1	1	1	3	3	3	3	1	1	1	1	1	1	1
P0295	7	44.9	3	3	3	3	3	3	3	1	3	3	3	3	3	1	1
P0475	9	37.0	1	1	1	1	1	1	1	1	3	3	3	3	3	3	3
IR74392-108-6	1	0.0	3	2	1	1	1	1	1	1	1	1	1	1	1	1	1
IR74392-118-4	1	1.5	2	3	3	1	3	1	1	1	1	1	1	1	1	1	1
IR74392-135-1	1	1.5	3	3	3	1	1	1	2	1	1	1	1	1	1	1	1
IR74392-201-14	2	1.6	1	1	1	1	3	3	3	2	1	1	1	1	1	1	1
IR74399-204-10	2	0.0	1	1	1	1	1	2	1	3	1	1	1	1	1	1	1
IR74401-215-5	2	0.0	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
IR74401-215-18	2	2.2	1	1	1	1	1	1	3	3	1	1	1	1	1	1	1
IR74401-216-7	2	2.1	1	1	1	1	3	3	3	3	1	1	1	1	1	1	1
IR74405-711-1	7	0.0	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1
IR74405-720-7	7	1.9	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1
IR74405-720-12	7	0.0	1	1	1	1	1	1	1	1	1	1	3	1	1	1	1
IR74409-730-8	7	2.3	1	1	1	1	1	1	1	1	1	3	3	1	3	1	1
IR74409-730-9	7	1.6	1	1	1	1	1	1	1	1	1	3	3	1	3	1	1
IR74409-730-10	7	1.6	1	1	1	1	1	1	1	1	1	3	3	1	3	1	1
IR74409-734-4	7	3.9	1	1	1	1	1	1	1	1	1	3	2	1	3	1	1
IR74409-735-2	1+7	6.0	3/2	3	2	2	1	1	1	1	1	3	3	1	3	1	1
IR74409-735-12	1+7	4.5	2	2	2	2	1	1	1	1	1	3	3	1	2	1	1
IR74409-736-11	1+7	8.0	3	3	1	3	1	0	1	1	1	2	3	0	1	1	1
IR74409-737-5	1+7	9.3	2	2	3	3	1	2	1	1	1	2	1	2	3	1	1
IR74409-737-12	1+7	7.7	3	1	1	2	1	1	1	1	1	3	3	3	3	1	1
IR74409-738-11	1+7	4.6	2	2	3/2	3	1	1	1	1	1	3	3	1	3	1	1
IR74409-739-4	1+7	8.0	1	0	3	3	1	1	1	1	1	1	1	2	1	1	1
IR74409-739-7	1+7	8.0	2	2	3	3	1	1	1	1	1	1	2	0	1	1	1
IR74418-910-2	9	2.8	1	1	1	1	1	1	1	1	1	1	0	0	1	3	3
IR74418-910-3	9	3.5	1	1	1	1	1	1	1	1	1	1	2	0	1	3	3
IR74418-910-12	9	0.9	1	1	1	1	1	1	1	1	1	1	1	0	1	2	3
IR74418-913-7	9	2.6	1	1	1	1	1	1	1	1	1	1	2	0	1	3	3
IR74419-921-1	9	0.0	1	1	1	1	1	1	1	1	1	1	0	0	1	3	3
IR74419-921-8	9	0.0	1	1	1	1	1	1	1	1	1	1	0	0	1	3	3

* Proportion of Azucena alleles in the non-target areas

Table 4 Root characteristics and other important agronomic traits of the NILs of IR64 evaluated at IRRI, The Philippines, in 1999. MRL = maximum root length; NBR = number of roots; THK = root thickness; SDW = shoot dry weight; RW0030 = root mass in the 0 to 30-cm layer; RW3060 = root dry weight in the 30 to 60-cm layer; RW6090 = root weight in the 60 to 90-cm layer; TRW = total root weight; DRW = root weight below 30 cm; DUR = duration (field experiment); HGTf = plant height (field experiment); HMG = homogeneity for plant height (H= homogeneous, S = segregating); TILg = number of tillers (greenhouse experiment); TILf = number of tillers (field experiment)

Line	Target	MRL	NBR	THK	SDW	RW0030	RW3060	RW6090	TRW	DRW	DUR	HGTf	HMG	TILg	TILf
IR64		69.1	133	1.19	11.25	1.217	0.159	0.015	1.392	0.175	116.8	107.4	H	34.0	26.3
IR74392-108-6	1	75.9	141	1.21	12.13	1.553*	0.129	0.017	1.699~*	0.146	119.0*	107.7	H	34.1	21.9**
IR74392-118-4	1	73.5	130	1.22	12.04	1.367	0.245*	0.034**	1.645	0.279*	118.0	150.2**	H	29.9	23.4*
IR74392-135-1	1	70.7	162*	1.21	12.46	1.436	0.212	0.021	1.669~*	0.233	117.1	112.0*	H	38.8	27.4
IR74392-201-14	2	72.1	123	1.20	11.08	1.074	0.136	0.018	1.230	0.154	114.1**	110.8	H	24.9**	19.4**
IR74399-204-10	2	65.0	123	1.19	9.66	1.056	0.108	0.011	1.173	0.117	115.1	105.3	H	21.2**	20.0**
IR74401-215-5	2	70.4	112	1.20	9.10	1.203	0.116	0.010	1.333	0.127	116.1	101.6**	H	25.7**	23.3*
IR74401-215-18	2	73.2	128	1.15	11.43	1.196	0.203	0.024	1.424	0.228	nd	nd	nd	31.4	nd
IR74401-216-7	2	72.6	131	1.18	12.22	1.464	0.176	0.018	1.656	0.194	115.4	100.8*	H	30.6	23.6*
IR74405-711-1	7	64.3	127	1.20	12.18	1.394	0.100	0.017	1.510	0.116	111.9**	101.9*	H	36.8	24.6
IR74405-720-7	7	70.8	135	1.20	14.21**	1.316	0.248*	0.029*	1.593	0.278*	115.0*	122.7**	S	32.3	25.1
IR74405-720-12	7	78.5*	125	1.18	14.45**	1.312	0.239*	0.021	1.572	0.260*	120.0**	119.5**	S	33.4	21.0**
IR74409-730-8	7	67.9	121	1.22	8.21**	0.888*	0.091	0.009	0.988*	0.100	118.0	99.0**	H	24.9**	20.9**
IR74409-730-9	7	65.5	126	1.19	8.93*	1.187	0.095	0.007	1.288	0.103	115.1	94.9**	H	23.3**	20.3**
IR74409-730-10	7	68.2	161*	1.13	11.89	1.631**	0.245*	0.020	1.897**	0.265*	117.6	137.1**	H	40.6**	24.5
IR74409-734-4	7	65.6	124	1.21	10.45	1.387	0.079	0.002	1.471	0.082*	118.0	101.9*	H	30.9	24.7
IR74409-735-2	1+7	66.2	116	1.23	12.04	1.191	0.202	0.019	1.411	0.222	115.9	120.9**	S	32.1	21.4**
IR74409-735-12	1+7	71.6	117	1.29**	10.85	1.180	0.171	0.014	1.365	0.185	113.9**	124.3**	S	28.1*	20.9**
IR74409-736-11	1+7	74.9	117	1.24	13.40	1.543*	0.233	0.030*	1.807**	0.263*	118.5*	148.4**	H	30.2	23.9
IR74409-737-5	1+7	75.6	123	1.23	12.07	1.310	0.286**	0.023	1.616	0.308**	120.9**	147.4**	H	34.7	20.2**
IR74409-737-12	1+7	69.7	148	1.22	11.41	1.385	0.270**	0.032*	1.685~*	0.302**	117.7	123.8**	S	37.5	23.0*
IR74409-738-11	1+7	66.8	119	1.17	10.85	1.205	0.143	0.007	1.353	0.150	118.1	142.1**	H	27.8*	22.2**
IR74409-739-4	1+7	67.9	124	1.21	10.11	1.332	0.126	0.009	1.467	0.135	126.5**	165.8**	H	32.0	23.8
IR74409-739-7	1+7	68.5	130	1.23	10.40	1.473	0.102	0.005	1.581	0.106	120.1**	155.6**	H	30.8	24.9
IR74418-910-2	9	88.7**	139	1.17	10.49	1.358	0.196	0.029*	1.585	0.225	115.8	109.0	H	27.4*	23.3*
IR74418-910-3	9	71.5	114	1.17	8.18**	1.088	0.171	0.017	1.271	0.187	116.1	107.0	H	26.6**	22.0**
IR74418-910-12	9	72.8	135	1.15	10.30	1.068	0.121	0.022	1.212	0.143	116.4	109.8	H	27.9*	23.3*
IR74418-913-7	9	79.7*	105*	1.12*	8.14*	1.011	0.131	0.016	1.160	0.148	120.0*	105.8	H	28.3*	20.6**
IR74419-921-1	9	77.9*	127	1.15	9.74	1.277	0.128	0.025	1.430	0.153	118.0	107.4	H	27.7*	21.6**
IR74419-921-8	9	88.1**	126	1.17	7.52**	1.117	0.163	0.023	1.306	0.187	116.1	105.2	H	24.5**	21.7**
Genotype effect		**	ns	ns	**	**	**	*	**	**	**	**		**	**
CV		13.9	20.4	6.0	22.6	24.0	54.3	84.4	24.1	54.2	1.2	3.3		19.7	9.2
h^2		0.48	–	–	0.64	0.44	0.56	0.40	0.48	0.56	0.95	0.99		0.74	0.68

** Significantly different from IR64 at the 1% threshold, * at the 5% threshold, ~* the at 10% threshold

[a] CV = coefficient of variation

[b] h^2 = broad-sense heritability

[c] nd = missing data

Table 5 QTLs for root morphology, plant height and number of tillers located on chromosomes 1, 2, 7 and 9 identified using composite interval mapping

Trait[a]	Chr.	Interval	Marker-QTL distance[b]	QTL position (cM)	LOD score	R^2 [c]	Additive effect
MRL	2	RG171-RG157	14.0	103.0	6.3	16.6	–6.160
MRL	2	RZ318-Pal1	0.0	146.8	3.4	6.8	+4.565
MRL	7	CDO418-RZ978	0.0	128.4	7.4	15.7	–5.078
MRL	9	RZ12-RM201	0.0	101.3	4.2	8.0	–3.561
DRW	1	RG690-RZ730	10.0	185.9	4.7	11.9	–0.035
DRW	7	RG773-RZ488	8.0	8.0	3.4	10.0	–0.035
DRW	7	CDO418-RZ978	0.0	128.4	7.0	17.2	–0.039
TRW	1	RZ730-RZ801	12.0	202.2	5.1	18.2	–0.158
TRW	7	RZ978-CDO38	6.0	144.1	2.8[b]	5.8	–0.089
TRW	9	RZ206-RZ422	4.0	34.0	3.6	8.5	–0.112
HGTg	1	RZ730-RZ801	40.0	230.2	4.3	16.6	–3.810
HGTg	2	RG171-RG157	2.0	91.0	2.2[d]	5.2	–2.302
NBTg	2	RG157-RZ318	28.0	138.0	3.6	14.7	+1.035

[a] MRL = maximum root length; DRW = deep root weight (root weight below 30 cm); TRW = total root weight; THK = root thickness; HGTg= plant height under greenhouse conditions; NBTg = number of tillers under greenhouse conditions

[b] Marker-QTL distance = distance in cM from the left marker of the interval; QTL position = distance in cM from the top arm of the chromosome

[c] R^2 = percentage of phenotypic variability accounted for by the putative QTL. The sign of additive effects represents the effects of IR64 alleles

[d] QTLs did not reach the 0.05 genome-wide threshold (LOD>3.3)

Azucena alleles at both QTL regions. IR74399-204-10 was the only one of the five NILs carrying target 2 in which a recombination had occurred between the two QTLs, and this line indeed had a slightly reduced MRL as would be expected from its genotype. Thus, the repulsion-phase linkage between the two QTLs appeared to be largely responsible for the failure to observe an improvement over IR64. No plant with the opposite allelic combination was found in our sample or among the non-evaluated lines. However, it would be interesting to select such a recombinant from the selfed progeny of IR74392-201-14, which is homozygous for the Azucena allele at RG171 and heterozygous at RZ318, and evaluate it to check whether our hypothesis is correct.

The QTL on chromosome 7 was detected with both methods and in both cases explained a non-negligible proportion of the variability. A QTL at the same position was also reported in the studies of Zheng et al. (2000) with the same population and Champoux et al. (1995) with a different one. However, only one of the lines showed an improved MRL in comparison with IR64. A localization of the QTL outside our selected target region is unlikely. All QTL analysis techniques gave the same location and, because of the high MRL value of P0295, the parent DH line, we can reasonably assume that the QTL was present in the donor line. One possibility is that it was lost during repeated backcrossings. If this is the case, the QTL might be located in a small region near RM234 because this is the only region that had returned to IR64 in all the above-mentioned NILs, which is consistent with the fact that the QTL mapped quite close to this end of our target block. Another explanation could be that this QTL was involved in gene interactions and that the replacement of Azucena alleles by the recurrent parent alleles at the other marker had a negative effect on root depth. The results of Yadav et al. (1997) showed epistatic interactions between CDO418 and markers located on chromosome 3 for maximum root length.

Deep root weight

QTLs for DRW were located in both target-1 and target-7 regions in the initial population. These QTLs were also identified by composite interval mapping. One NIL carrying target 1 and three NILs carrying target 7 showed improved DRW over IR64. These results confirm the presence of a DRW QTL in the target-1 region near RZ730 and in the target-7 region near RZ978. Three lines carrying both targets 1 and 7 had a DRW higher than that of IR64. Two of them also had the highest phenotypic gain, outperforming IR64 by up to 75%. This provides further evidence for the existence of QTLs for DRW on targets 1 and 7, but their complicated genotypes in the target regions make it difficult to associate a QTL with a specific interval. One line showed a root weight significantly lower than IR64. This may be due to a partial loss of roots from one of the replications during root washing, although great care was taken for this operation.

Total root weight

QTLs for TRW existed in similar positions as QTLs for DRW through both methods. All three NILs carrying target 1 showed improved TRW. Two of them were different from IR64 at $P < 0.10$, thus confirming the effect of

the QTL in the target-1 region. A relatively weaker QTL for TRW existed in the target-7 region, and this complicates the interpretation of phenotyping results for lines carrying both targets 1 and 7. One line carrying target 7 and one carrying targets 1 and 7 had a TRW significantly higher than that of IR64. For the target-7 region, the existing markers could not explain the phenotype because two lines with the same genotype (IR74409-730-8 and IR74409-730-10) had different phenotypes.

Relationships between phenotype and genotype for non-target agronomic traits

Duration

The duration of the lines was modified significantly in a few cases, but, with the exception of IR74409-739-4, was in the narrow range of plus or minus 3 days. These modifications were inconsistent with the marker genotype in any of the target regions. This indicated that this variation among NILs was due to random introgression of donor genes in the non-target regions.

Plant height

Plant height showed important variation in the groups of NILs carrying target 1 and/or target 7. The variation for height observed was largely due to the segregation of the semi-dwarf gene *sd-1* located between RZ730 and RZ801 (Huang et al. 1996). The IR64 allele contributed to semi-dwarfism and the Azucena allele contributed to tallness. For the lines carrying both targets 1 and 7 that were homozygous for Azucena alleles at RZ801, plant height in the field ranged from 142.1 to 165.8 cm. When it was heterozygous, the mean height varied from 120.2 to 124.3 cm and the line was segregating for this trait as expected. Two recombinant lines carrying target 1 with the same genotype but different phenotypes (IR74392-118-4 and IR74392-135-1) could assist in the fine of mapping *sd-1* if markers were to be inserted between RZ730 and RZ801.

The lines carrying target 7 alone showed variations in plant height indicating that *sd-1* was not the only gene involved in the control of plant height. Examining the genotype of these lines suggests the presence of a QTL between CDO418 and RZ978 on chromosome 7. Although no such QTL was detected in a previous study with this population (Courtois et al. 1995), a QTL was found in this area in other rice populations (Huang et al. 1996).

Number of tillers

For the five groups of NILs, TILf (field experiment) was significantly lower than, or similar to, that of IR64. For a given line, TILg (greenhouse experiment) was higher on average than TILf, but was still reduced in comparison with IR64 with only one exception (IR74409-730-10) with a significantly increased tiller number. All NILs with a significantly reduced TILg also showed a reduced TILf. NILs carrying target 1, although similar to IR64 in the greenhouse experiment, had a slightly reduced TILf. The extent of the differences might not have been fully expressed in TILg, measured 43 days after sowing, whereas TILf was recorded at maturity. These results suggest the existence of QTLs for tillering associated with the four root QTLs. As a matter of fact, Yan et al. (1998), working with the same population as we were, found QTLs for tiller number at our target areas of chromosomes 1, 2 and 7, although not on chromosome 9. Similarly, they showed that some QTLs for tiller number identified at the final growth stage were undetectable at the early stage and reciprocally.

Discussion

In the present study, we have shown that it was possible to transfer QTLs for root depth in rice using marker-aided selection without phenotypic selection, and recorded significant improvement in the root system of several of the tested lines. This is clearly a step that would have been very difficult to achieve before the advent of DNA markers. The NILs of IR64 developed in this study offer a unique opportunity to answer the long-standing question on which traits in rice really confer a yield advantage under water-limited conditions without interference from differences in other drought-related traits (Lafitte 1999; Price and Courtois 1999). The lines with an improved root system are now being tested under field conditions to see whether their improvement provides an advantage under water-limited conditions and to check to what extent their expression is affected by environmental conditions.

Several important issues regarding the success and efficiency of MAS for QTLs merit further discussion. The number of lines with a significantly improved phenotype was not very high. There are several possible sets of reasons for this. The first set relates to the quality of the initial QTL analysis. We have seen that a shift in the analysis methods from regression on flanking markers to composite interval-mapping on the same raw data led to different conclusions on the number of QTLs and the direction of their effects, notably for the target segment on chromosome 2. The composite interval mapping method was actually designed to improve the quality of QTL analysis in situations where several QTLs are present on the same chromosome region (Zeng 1993, 1994). The presence of non-allelic interactions between donor alleles that may be disrupted by the recurrent parent alleles during the backcross process is another likely possibility. Non-allelic interactions were detected by Yadav et al. (1997) through a simple two-way analysis of variance. Recent software taking epistasis into account in the framework of composite interval mapping (Wang et al.

1999) would allow further improvement of the precision in QTL analysis. Another problem is the uncertainty of the QTL position, notably for those with a small effect. Some studies have shown that the confidence interval for QTL location, when it can be determined, is huge by current QTL analysis techniques, sometimes up to 30 cM for small populations (Hyne et al. 1995; Visscher et al. 1996b). Han et al. (1997) described such a situation where the target region transferred might not have contained the desired QTL. We chose initial DH lines with long segments of Azucena to limit this risk, but appropriate mapping methodologies are certainly crucial for the success and efficiency of MAS for QTLs.

The second possibility is that the target QTL can be lost during successive backcrosses through double-crossovers between markers. Some of the intervals between markers followed in this population were long enough to consider this a possibility (e.g. the 41.8-cM interval between RZ730 and RZ801 on chromosome 1). A more-saturated map would limit this risk, but, besides the cost of adding more markers, some areas seem to be difficult to saturate. As an example, the addition of 115 microsatellites to our DH population allowed the selection of better markers only for target 9. If double-crossovers occur, they are impossible to detect in a case such as this one where we must rely strictly on the genotype to choose plants for several successive rounds of backcrossing because of the destructiveness of the phenotyping method. The situation where a simple phenotyping technique can be applied on a plant by plant basis, and combined with marker-aided selection either in a two-stage selection scheme or in an index, should result in better efficiency as shown by Han et al. (1997) in barley.

One more possible reason is that the QTLs we targeted were actually of intermediate effect, explaining 5.6% to 17.7% of the variability. Weak effects are more difficult to assess and additional replications of the phenotyping might give clearer conclusions. In contrast, most other reported MAS studies worked on major genes or QTLs with larger effects.

With the material employed it was possible to look at the genetic relationships among root traits, and between root traits and other important agronomic characters. Considering MRL and DRW, the four NILs carrying target 9 with a significantly improved MRL were not significantly different from IR64 in terms of DRW. Only one of the NILs with a significantly increased or decreased DRW showed a significant change in MRL. So, we can conclude that the strong positive correlation between MRL and DRW observed by Yadav et al. (1997) was not due to pleiotropy but to linkage, which opens a new opportunity for independent genetic improvement of the two traits in breeding for drought-tolerant rice varieties. Yoshida and Hasegawa (1982) mentioned links between root number and tillering. This trend was also found in our study. Tillering was systematically reduced, indicating important linkage drag although this was independent of other root phenotypes. We observed a consistent association between plant height and root mass in depth: the varieties with the deeper root mass were all tall varieties although the reverse was not true. This relationship had been studied by comparing dwarf and wild mutants in rice and sorghum (Yoshida and Hasegawa 1982) and wheat (Clarke and McCaig 1993; Miralles et al. 1997) with the conclusion that not all height-reducing genes affected root distribution. Several papers have proposed that *sd-1*, controlling semi-dwarfism in rice, is pleiotropically associated with higher tiller number, root number and shallower roots (Xia et al. 1991). But in our experiments, *sd-1* is not consistently associated with TIL, NBR, MRL or DRW. Therefore, genes other than *sd-1* influence these traits.

The NILs with an improved root system represent valuable material that can now be used for QTL pyramiding and further studies for fine mapping.

Acknowledgements The authors thank S. Carandang, M. Amante, T. Borabo and R. Lanceras for their help in the greenhouse experiment. They also thank S. McCouch from Cornell University, USA, for providing the microsatellite pattern of the parental DH lines. This work was partly funded by the German Federal Ministry for Economic Cooperation and Development (BMZ) in the framework of the perennial upland rice project, and by a grant from the Rockefeller Foundation to IRRI.

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