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Mapping of QTL associated with root and related traits in DH population of rice (*Oryza sativa* L.)

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Abstract

Mapping of QTL associated with root and related traits was undertaken in a DH population of *indica* × *indica* cross (CT9993/IR62266). Totally 18 QTL, spread across chromosomes were detected at threshold LOD of 2.20. The alleles of IR62266 contributed to reduce plant height, total plant length, enhance tiller number, root number and root dry weight. As IR62266 is a short parent with shallow root and more tiller number, such an effect is explainable. A major QTL, explaining 20% of variation for plant height and flanked by RG109/ME10_14, was detected at LOD of 6.17. This genomic segment is close to the map position of *sd-1*, a major gene controlling semi-dwarfism. On the other hand, of the two QTL for root: shoot length ratio, one QTL on chromosome 1 (CDO345/RZ909) enhanced the ratio. This indicates that inferior parent (IR62266) can also contribute favorable alleles for root traits.

Key words: Rice, drought resistance, quantitative trait loci (QTL), roots.

Introduction

Rice (*Oryza sativa* L.) is among the most important staple food crops in the world, is grown on 148 million hectares in a wide range of environments. About 25% of the world's rice area is under rainfed lowlands and another 13% is in rainfed uplands [1]. Drought is a major constraint to the increased productivity of rice in the rainfed regions [2]. Among the several factors contributing to enhance tolerance/resistance to drought, root characters are believed to be a vital component of dehydration postponement mechanism since they contribute in regulation of plant growth, extraction of water and nutrients from deeper soil layers [3]. Plants with a large root system could thus maintain a high rate of water uptake from the soil when faced with a progressively depleting soil moisture profile and could therefore maintain higher leaf water potential [4]. Selection for better root system is difficult, time-consuming and destructive for plants. Therefore, improving root traits in crops is the least popular objective among plant breeders [5]. Application of molecular marker technology is seen as a way to overcome these barriers by using marker-assisted selection method [6&7]. Several plant characters including root morphological and plant physiological traits associated with drought tolerance in rice have been tagged to molecular markers [6-15].

Keeping this in view, the present study was undertaken to map QTL associated with root-related traits in *indica* × *indica* DH population of rice (*Oriza sativa* L.).

Material and methods

Plant material : A population of 127 doubled haploid lines (DHLs) derived from a cross between CT9993-5-10-1-M (abbreviated as CT9993) and IR62266-42-6-2 (abbreviated as IR62266) along with parents, and four checks *viz.*, Azucena, IR58821, IR64 and Moroberekan were used in this study. While CT9993 as a predominantly *indica* type, bred at CIAT by tissue culture, possesses a deep and thick root system, IR62266 is an *indica* type with short stature and a shallow root system. DHLs were developed at Centro International de Agriculture Tropical, Colombia and the International Rice Research Institute, Philippines.

DNA marker map : Molecular map of this population generated in 2001 [6]. For this, 153 AFLPS, 145 RFLPs and 17 microsatellites were used to construct a linkage map using MAPMAKER. To date the linkage map covers 1,788 cM in length with an average distance of 5.7 cM between adjacent markers. The overall frequency distribution of the (CT9993) allele for 315 markers and DHLs is close to the expected 50%.

Experimental details : The experiment was laid out in randomised complete block design with three replications. Seeds were sown in PVC pipes measuring

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Table 1. QTL linked to molecular markers identified by interval analysis in CT9993/IR62266 DH population of rice (LOD ≥ 2.2) under well-watered condition.

Trait	QTL	Chr#*	Interval	LOD	R^2(%)	Effect
PHT	qphtl-l	1	RG109-ME10_14	6.17	20.0	-3.91
	qpht2-1	2	ME2_7-EMP2_7	3.37	11.5	-2.83
	qpht8-1	8	C1121-ME5_3	3.20	12.6	-3.18
	qpht8-2	8	ME5_7-EM15_10	2.45	8.5	-2.65
	qpht9-1	9	ME9_6-K985/RM242	2.65	9.9	-2.91
	qpht10-l	10	RG257-EMP2_9/ME5_16	2.91	10.4	-2.82
	qpht12-1	12	ME7_9-ME10_8	2.82	10.0	-2.88
NOT	qnotl-l	1	G359-RG140	3.69	12.5	1.60
	qnotl-2	1	R2417-RM212	2.58	9.6	1.48
	qnot3-1	3	RG409-RG224	2.98	10.2	1.74
	qnot4-1	4	ME7_7-EMP3_1c	2.72	9.9	1.74
TRN	qtrn4-1	4	RZ565-EMP3_10	2.86	10.1	6.79
RDW	qrdw4-1	4	ME6_10-RG449	2.20	7.6	0.52
TTL	qttl1-1	1	RG109-ME10_14	3.77	12.8	-4.43
	qttl2-1	2	RG437-ME10_18	2.25	8.1	-3.48
	qttl12-1	12	ME7_9-ME10-8	2.57	9.2	-3.92
R/S	qr/sl-l	1	CDO345-RZ909	2.22	7.8	0.05

Table 2. Phenotypic correlation among nine characters in 127 DHLs of rice derived from CT9993 × IR62266

	PHT	NOT	MRL	TRN	RTV	RDW	SDW	R/S
NOT	0.13							
MRL	0.28**	0.33**						
TRN	0.18	0.63**	0.53**					
RTV	0.31**	0.55**	0.55**	0.77**				
RDW	0.21*	0.57**	0.46**	0.76**	0.77**			
SDW	0.39**	0.68**	0.30**	0.46**	0.45**	0.46**		
R/S	-0.12	-0.04	0.21*	0.37**	0.41**	0.63**	-0.30**	
TTL	0.84**	0.27**	0.75**	0.42**	0.52**	0.40**	0.44**	0.04

1 meter in length and 18 cm in diameter, which were filled with a mixture of sandy-clay loam and FYM in 4:1 proportion. After germination, one seedling was allowed to grow in each pipe. Plants were watered daily throughout the experiment. Root sampling was done 70 days after sowing (DAS). Pipes were removed carefully and completely submerged in water over night to loose the soil. Later, the roots were cleaned thoroughly with water. Observation consisted of maximum root length (MRL) in cm, total root number (TRN), root volume (RTV) in cubic centimeters, root dry weight (RDW) in g, shoot dry weight (SDW) in g, plant height (PHT) in cm and number of tillers (NOT). Further, total plant length (PHT+MRL = TTL) in cm and root to shoot length ratio (R/S) were computed.

Statistical analysis and QTL mapping : The data of three replications was subjected to ANOVA to partition the variance. Interval analysis was performed to detect QTL using the computer package MAPMAKER/QTL [10]. A LOD threshold of 2.20 was adopted for analysis. The correlation coefficients [14] were computed between all the traits under study.

Results and discussion

Analysis of variance revealed highly significant differences among the DHLs for all the characters studied, which indicated ample variability.

Although mapping of QTL for nine characters were attempted, significant QTL were detected for only six traits. The low turnover may be due to the small population size and lack of significant differences for the genes carried by the two parents for the traits [16] or due to the environmental effects on the expression of traits [11]. Seven QTL associated with PHT were detected on chromosomes 1, 2, 8, 9, 10 and 12, explaining 82.9 % of total phenotypic variation. The substitution of IR62266 alleles decreased PHT at all loci (Table 1). A major QTL, explaining 20% of variation for PHT and flanked by RG109/ME10_14, was detected at LOD of 6.17. This genomic segment is close to the map position of *sd-1*, a major gene controlling semi-dwarfism on chromosome 1 (12). One QTL related to PHT was flanked by ME2_7/EMP2_7 on chromosome 2. This chromosomal segment was found to have two QTL affecting penetrated root thickness and root dry weight [7]. Significant and positive correlation between PHT and RDW were reported [16-18]. The different QTL identified in the same region for same population may be due to the positive correlation between these traits or due to pleiotropic effects [13]. Common QTL for PHT and TTL were found between RG109/ME10_14 on chromosome 1 and between ME7_9/ME10_8 on chromosome 12 (Fig. 1). High significant positive correlation was found between PHT and TTL [2, 18].

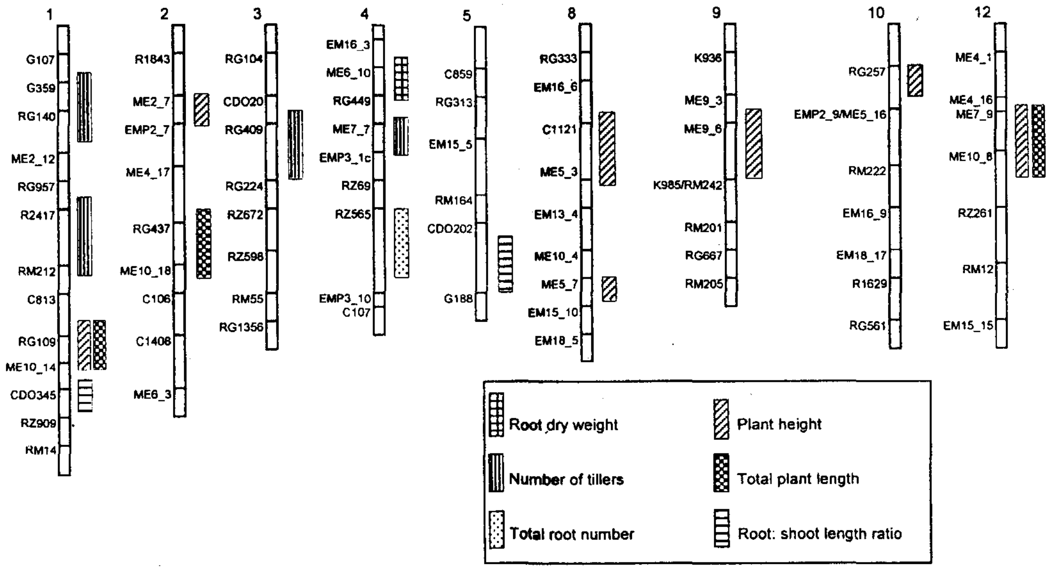

Fig. 1. QTL controlling root and shoot-related traits identified by interval analysis in CT9993/IR62266 DH population (LoD ≥ 2.20)

The QTL for RDW associated with ME6_10/RG449 on chromosome 4 reported here is close to two QTL for NOT associated with RG91/RG449 and RG449/RG788 interval in IR64/Azucena population [13]. The correlation observed between NOT and RDW also confirm such result. Three QTL affecting TTL with negative additive gene effects, two QTL located on chromosome 1 and 12 were congregated with PHT on the same regions. The QTL for TTL reported in this study on chromosome 2 was close to a QTL associated with panicle length in IR64/Azucena population [16]. Positive correlation between PHT and panicle length was found [16]. The locus G359/RG140 on chromosome 1 explained the highest phenotypic variation of 12.5% for NOT. This region also contained QTL for RDW. It is interesting to note that these two traits showed significant positive correlation with each other (r = 0.57**) (Table 2). On the other hand, of the two QTL for root: shoot length ratio, one QTL on chromosome 1 (CDO345/RZ909) enhanced the ratio. This indicates that inferior parent (IR62266) can also contribute favorable alleles for root traits.

Mean environment would be the most efficient condition for identification of QTL because of the reduced standard error of trait values. Also, the mean environment should allow the detection of QTL with too small effects to be detected in the individual environment. Environment seems to play a role in the ability to detect QTL for maximum root length [11]. Characterization of QTL for root-related traits in this study provides insight into the mode of drought resistance in rice. The most-practical application of the identified QTL 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.

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