

Mapping QTL for Grain Yield under Moisture Stress Environments in Rice (*Oryza sativa* L.)

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Abstract

Polygenes (QTLs) for grain yield were mapped on rice chromosomes under two moisture stress environments by multiple interval mapping (MIM) method in a double haploid (DH) population derived from a cross between a deep-rooted *japonica* and a shallow-rooted *indica* genotype. In environment 1 (E1), the MIM detected a total of six QTLs for grain yield on chromosomes-two QTLs on chromosome 1 and four QTLs on chromosome 5 along with one additive x additive epistasis. But in environment 2 (E2), the MIM detected five QTLs for grain yield on two chromosomes-three QTLs on chromosome 1 and two QTLs on chromosome 7. One common QTL on chromosome 1 flanked by the markers RG109-ME1014 was detected in both the environments, although the other detected QTLs differed between environments. The magnitude of QTL effect, percent genetic variance and percent phenotypic variance explained by each QTL was also estimated in both environments. The common QTL explained about 26.05 and 13.93% of genetic variance in E1 and E2, respectively. Estimated broad sense heritability for grain yield was 48.01 in E1 and 25.27% in E2.

Keywords: drought, grain yield, polygenes, rice

Introduction

Rice is the world's largest staples food in many countries and provides the calorific needs of millions of people daily. It is the primary staple in the diet of the densely populated Asian countries as well as many African countries where overpopulation is becoming a critical issue. In spite of the successful introduction of resistance to biotic/abiotic stresses into a variety of rice cultivars that resulted in improved crop yield, benefits of the 'green revolution' will soon be exhausted due to the population pressure (Yamamoto *et al.*, 2009). Hence, research work is being continued to increase grain yield in rice. Grain yield is a quantitative trait controlled by polygenes and largely influenced by environment. It is actually a resultant trait of several component traits which maintain complex interrelationships and internal adjustments amongst themselves. Improving rice crop productivity by selection for the components of grain yield, optimal plant architecture has been the key focus of national and international rice breeding programs (Huang *et al.*, 2009; Zhang, 2007).

Grain yield in rice represents the multiplicative integration of three main components *i.e.* number of panicles per plant, number of grains per panicle and mean grain weight (Matsuoka, 2008; Sakamoto and Matsuoka, 2008; Xing and Zhang, 2010). In recent years, attempts have been made to detect and locate QTLs (Quantitative trait loci) or polygenes for grain yield and other important agronomical traits in rice. This has been possible because of the availability of molecular marker-based genetic maps

(Zhang, 2007). Rice is basically a water-loving plant and gives good yield under optimum soil moisture regime. But rice yield reduces drastically under soil moisture stress imposed by natural short dry-spells in rainfed upland rice or by withholding water supply under irrigated rice culture. It is of paramount importance to identify the QTLs that affect grain yield under moisture stress. Once the QTLs are detected and located on chromosomes, they may be further cloned in breeding varieties tolerant to moisture stress.

Identification of QTLs governing a quantitative trait paves the way for isolation of the gene(s) for positional cloning. QTLs are not necessarily genes themselves but stretches of genomic DNA that are closely linked to the genes that underlie the quantitative trait under study. QTLs could be molecularly identified to help map the regions of the genome that contain genes involved in specifying a quantitative trait. The QTLs thus identified for a trait through different QTL mapping methods provide information about the genetic architecture of the phenotypic trait and explain its variation.

Nowadays QTL mapping is one of the commonly used molecular marker technology for dissecting the complex nature of quantitative traits (Wang *et al.*, 1999). But the concept of detecting QTL for quantitative trait was first elucidated by Sax (1923) in beans by using pigment markers to analyze the genes affecting the seed size in F_2 progeny of crosses. Thoday (1960) proposed the idea of using two markers to bracket a region for detecting QTL and

was the first ever known statistical approach in mapping QTLs.

QTL mapping can be done by three different methods namely interval mapping (IM), composite interval mapping (CIM) and multiple interval mapping (MIM). QTL mapping by the MIM method was first proposed by Kao *et al.* (1999). It is more powerful than IM and CIM methods because the former uses multiple marker intervals simultaneously to construct multiple putative QTL in the method itself. In addition, MIM can readily search for and analyze epistatic QTL. It can also estimate the individual genotypic value of the genotypes, the heritability of the trait and the magnitude of genetic variation contributed by each QTL to the trait.

Since grain yield is a polygenic trait, the variation in its phenotypic expression under different environments is putatively controlled by some environment-specific QTLs, though not necessarily entirely different set of QTLs, in different environments. In other words, it is expected that a few environment-specific QTLs could be detected for grain yield in each environment. The present study was therefore undertaken to detect QTLs for grain yield in rice under two different moisture stress environments using multiple interval mapping method.

Materials and methods

Plant material

The plant material used for mapping of QTL for grain yield consisted of a population of 154 double haploid (DH) lines derived from a cross between a deep-rooted upland adapted *japonica* rice genotype, (CT9993-5-10-1-M) and a shallow rooted lowland adapted *indica* rice genotype having moderate drought tolerance, (IR62266-42-6-2).

Evaluation for grain yield

The lines were grown in 3 m×3 m plots in randomized block design with three replications for two years. Two weeks after transplanting, the fields were drained out and not irrigated again for the rest of the season leading to drought stress. Mean data for grain yield (g/sq. m) in each line under moisture stress condition were recorded in both the years (E1 and E2) from 99 lines out of 154 lines and these data obtained from IRRI were used for the present analysis.

Linkage map

The genetic linkage map of rice consisting of 280 marker loci including 134 restriction fragment length polymorphisms, 131 amplified fragment length polymorphisms and 15 simple sequence repeats was previously developed from an initial population of 154 DH lines (Zhang *et al.*, 2001). The linkage map covered a map distance of 1602 centimorgans (cM) on the basis of Kosambi function with an average distance of 5.7cM between adjacent markers.

Linkage was declared at LOD (logarithm of the odds) score greater than or equal to 2.0.

QTL analysis

QTL for grain yield were detected and mapped on rice chromosomes by multiple interval mapping (MIM) analysis using WinQTL Cartographer/version 2.5.006 (Wang *et al.*, 2010) with threshold LOD score of 2.0.

Results and discussion

The number of QTLs for grain yield in rice detected by multiple interval mapping method in environments E1 and E2 are presented along with the chromosome number, flanking markers and peak LOD scores (Tab. 1).

LOD is a statistical term and stands for likelihood of odds ratio. It is the base-10 log (log10) of the likelihood of the mostly likely QTL model divided by the likelihood of the no-QTL (null hypothesis) model. In the present study, LOD score 2 was the threshold level indicating that the detected QTL was 100 times more likely than no-QTL at the specific region of the genome.

In E1 environment, multiple interval mapping analysis for grain yield detected a total of six QTLs *i.e.* QTLs 1 and 2 on chromosome 1 and remaining QTLs (3 through 6) on chromosome 5 with LOD score ranging from 3.3 to 6.0 (Fig. 1). The QTLs 4, 5 and 6 were adjacent to each other on chromosome 5 and hence this could be a potentially significant region for further study on fine mapping. Martinez and Thome (2001) mapped QTLs for grain yield on rice chromosome 1, 3, 5, 6, 9, 11 and 12 from the study of BC₂F₂ population of a rice cross. The QTL for total spikelet number, a component of grain yield, under moisture stress was mapped on chromosome 9 (Lanceras *et al.*, 2004). The QTL for shoot biomass under stress was mapped on rice chromosome 2 (Kamoshita *et al.*, 2002).

Kaladhar *et al.* (2008) detected nine QTLs for grain yield per plant in rice. Among them, five major QTLs were located on chromosome 3, 4, 8 and 11. The QTL on

Tab.1. QTLs for grain yield detected under two water stress environments (E1 and E2) by multiple interval mapping (MIM) model in rice

Env.	Chromosome	No. of QTLs	Flanking markers	LOD
E1	1	1	RG109-ME1014	6.0
		2	CDO345-RZ909	5.8
	5	3	EMP210 - R1G1	3.7
		4	G387 - RG403	3.3
		5	RG403 - EM155	4.9
		6	EM155 - RM164	4.7
E2	1	1	C45 - EM810	2.0
		2	ME418 - EM1111	2.1
	7	3	RG109 - ME1014	1.6
		4	EM173 - ME215	6.0
		5	RG769 - EM1914	1.8

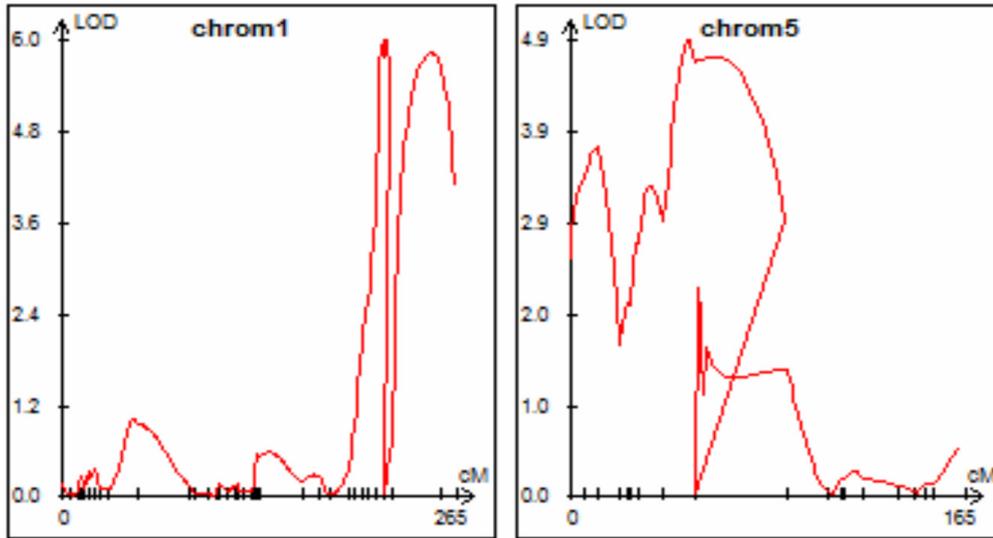


Fig 1. Six QTLs and one epistasis detected by MIM for grain yield on rice chromosome 1 and 5 under E1

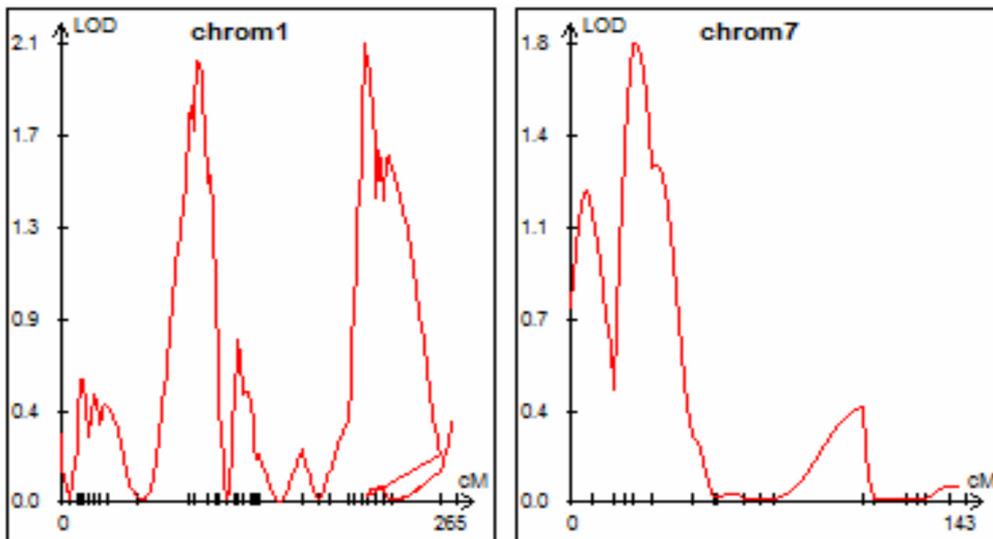


Fig 2. Five QTLs detected by MIM for grain yield on rice chromosome 1 and 7 under E2



Fig 3. Location of common QTL (red band in axis) for grain yield on rice chromosome 1 (partly shown) detected by MIM across two environments

chromosome 8 flanked by the markers RM38 and RM223 explained the highest phenotypic variance (54%) for grain yield. Using interval mapping and regression analysis, Li *et al.* (1997) detected four QTLs for grain weight per plant on chromosome 3, 4, 5 and 8. Collectively, these four QTLs accounted for 22.7% of the genotypic variance for grain yield.

On the other hand, in E2 environment multiple interval mapping detected five QTLs for grain yield *i.e.* QTLs 1, 2 and 3 on chromosome 1 and QTLs 4 and 5 on chromosome 7 (Fig. 2). QTLs 2 and 3 were adjacent to each other. The comparison of detected QTLs in E1 and E2 revealed a common QTL flanked by the markers RG109-ME1014 over both environments indicating that this QTL expressed in both the moisture stress environments (Fig. 3). Besides the common QTL, a new set of QTLs specific for each environment was detected for grain yield. It was in accordance with the concept of developmental genetics. In fact, development is sequential and hierarchical in nature that generates integrative networks of relationships within and between levels of organization (Pathak and Zhu, 2007). Because of its sequential and hierarchical nature, development is epigenetic and involves cascades of interactions among directly and indirectly acting controlling factors and these interactions may vary during ontogeny

Tab. 2. Estimates of QTL effects and percent variance explained by each QTL in MIM model for grain yield in rice under two water stress environments (E1 and E2)

Env.	Chromosome	QTL No.	Flanking markers	QTL effect	% Genotypic variance	% Phenotypic variance	Gene action
E1	1	1	RG109-ME1014	27.96	26.05	12.51	Additive
		2	CDO345-RZ909	-28.82	30.59	14.69	Additive
	5	3	EMP210 - RIG1	13.47	10.83	5.19	Additive
		4	G387 - RG403	-13.70	7.05	3.38	Additive
		5	RG403 - EM155	12.55	10.70	5.14	Additive
		6	EM155 - RM164	10.89	8.78	4.22	Additive
(Epistasis)	2x4	---	22.12	6.00	2.88	Additive x Additive	
E2	1	1	C45 - EM810	14.39	44.41	11.23	Additive
		2	ME418 - EM1111	-13.72	6.76	1.71	Additive
	7	3	RG109 - ME1014	-9.60	13.93	3.52	Additive
		4	EM173 - ME215	2.78	0.21	0.05	Additive
		5	RG769 - EM1914	4.69	34.69	8.76	Additive

Tab. 3. Heritability estimates for grain yield in rice under two water stress environments (E1 and E2)

Env.	Genotypic variance	Phenotypic variance	Heritability (%)
E1	323.40	673.60	48.01
E2	157.60	623.60	25.27

(Atchley, 1990). So, the detection of a new set of QTLs for grain yield in each environment was highly expected in the present study.

While studying the genetic basis of heterosis in rice, Xiao *et al.* (1995) detected two significant QTLs for grain yield on chromosome 8 (between markers RZ562-RG333; LOD 2.49) and 11 (RZ 638-CDO127; LOD 2.64) explaining 6.3 and 6.8% genetic variance. Hittalmani *et al.* (2003) detected three QTLs for grain yield in rice across environments on chromosome 4, 9 and 10 flanked by the markers RG91-RG449, RZ12-RM201 and RG257-RG241 respectively with LOD varying from 2.10 to 3.62.

One additive x additive epistasis (AA) was detected between QTLs 2 and 4 in addition to six QTLs in E1 (Tab. 2). The estimates of QTL effects, the extent of genetic variance and the phenotypic variance explained by each QTL in E1 and E2 are presented (Tab. 2). In E1 the QTLs 2 and 4, although located on separate chromosomes, recorded negative effect whereas the remaining four QTLs showed positive effect for grain yield. In E2 the adjacent QTLs 2 and 3 had negative effect but the other three QTLs had positive effect on grain yield.

Xing and Zhang (2010) reviewed the genetic bases of rice yield over last 10 years and recorded that ten QTLs were detected in $F_{2,3}$, five in VF_2 , four in RIL and seven in IF_2 populations for grain yield in rice. These QTLs are distributed in two distinct locations and thus regarded distinct QTLs for grain yield. Only 5 of the 20 distinct QTLs were recovered in two or more populations. Five QTLs have relatively large effects *i.e.* individually explaining >10% of the variation in grain yield.

Comparison of percent genetic variance explained by QTLs revealed that the QTL flanked by markers RG109-ME1014 could explain the highest genetic variance (26.05%) in E1. But the same QTL in E2 environment explained much lesser genetic variance (13.93%). On the other hand, the QTLs 1 and 5 in E2 explained substantial genetic variance for grain yield (44.41 and 34.69%, respectively).

The estimate of broad sense heritability is of great importance in QTL mapping. Heritability for grain yield was calculated by multiple interval mapping in E1 and E2 and are presented (Tab. 3). The heritability of grain yield was higher in E1 (48.01%) than E2 (25.27%). This indicated that the combined effects of all the detected QTLs could explain only 48.01 and 25.27% of the phenotypic variance in grain yield in E1 and E2, respectively.

Conclusions

Genetic mapping of polygenes for grain yield in rice under two different moisture stress environments was performed by multiple interval mapping (MIM) method using DH population derived from a cross involving a deep-rooted upland adapted *japonica* genotype and a shallow rooted lowland adapted *indica* genotype. In E1 environment, the MIM detected 6 QTLs for grain yield, of which two QTLs were mapped on chromosome 1 and the rest four mapped on chromosome 5. It also detected one additive x additive gene interaction between QTLs 2 and 4. But in E2 environment, the MIM detected 5 QTLs for grain yield of which three QTLs on chromosome 1 and two QTLs on chromosome 7. Only one QTL on chromosome 1 flanked by the markers RG109-ME1014 was found to be common between the two sets of QTLs detected over two environments. The common QTL explained 26.05 and 13.93% of genetic variance of grain yield in E1 and E2, respectively. MIM estimated the broad sense heritability of grain yield as 48.01% in E1 and 25.27% in E2 environments.

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References

- Atchley WR (1990). Heterochrony and morphological change: a quantitative genetic perspective. *Sem Develop Biol* 1:289-297.
- Hittalmani S, Huang N, Courtois B, Venuprasad R, Sashidhar HE, Zhuang JY, Zheng KL, Liu GF, Wang GC, Sidhu JS, Srivantaneeyakul S, Singh VP, Bagali PG, Prasanna HC, McLaren G, Khush GS (2003). Identification of QTL for growth and grain yield related traits in rice across nine locations of Asia. *Theor Appl Genet* 107:679-690.
- Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J, Fu X (2009). Natural variation at the *DEP1* locus enhances grain yield in rice. *Nature Genetics* 41(4):494-497.
- Kaladhar K, Swamy BPM, Babu AP, Reddy CS, Sarala N (2008). Mapping quantitative trait loci for yield traits in BC₂F₂ population derived from 'Swarna' x *O. nivara* cross. *Rice Genet Newslett* 24:34-36.
- Kamoshita A, Zhang J, Siopongo J, Sarkarung S, Nguyen HT, Wade LJ (2002). Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under anaerobic conditions. *Crop Sci* 42(1):255-265.
- Kao CH, Zeng Z, Teasdale K (1999). Multiple interval mapping for quantitative trait loci. *Genetics* 152:1203-1216.
- Lanceras JC, Pantuwan G, Jongdee B, Toojinda T (2004). Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiol* 135:384-399.
- Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997). Epistasis for three grain yield components in rice (*Oryza sativa* L.). *Genetics* 145:453-465.
- Martinez CP, Thome J (2001). Progress in the genetic improvement supported by molecular markers. *Ann Rept, CIAT Rice Project*.
- Pathak PK, Zhu J (2007). QTLs for response to low temperature stress during seedling growth in rice. *Indian J Genet* 67(4):337-346.
- Sakamoto T, Matsuoka M (2008). Identifying and exploiting grain yield genes in rice. *Curr Opin Plant Biol* 11:209-214.
- Sax K (1923). The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* 8:552-560.
- Thoday JM (1960). Location of polygenes. *Nature* 191:368-370.
- Wang D, Zhu J, Li Z, Paterson AH (1999). User Manual for QTL Mapper v1.1. A computer software for mapping quantitative trait loci with main effects, epistatic effects and QTL x Environment interactions. Zhejiang University, China.
- Wang S, Basten CJ, Zeng ZB (2010). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC, USA (Available at <http://statgen.ncsu.edu/qtcart/WQTLCart.htm>).
- Xiao J, Li J, Yuan L, Tanksley SD (1995). Dominance is the major genetic basis of heterosis in rice revealed by QTL analysis using molecular markers. *Genetics* 140:745-754.
- Xing Y, Zhang Q (2010). Genetic and molecular bases of rice yield. *Ann Rev Plant Biol* 61:421-442.
- Yamamoto T, Yonemaru J, Yano M (2009). Towards the understanding of complex traits in rice: Substantially or superficially? *DNA Res* 16:141-154.
- Zhang Q (2007). Strategies for developing green super rice. *Proc Natl Acad Sci USA* 104:16402-16409.
- Zhang J, Zheng HG, Aartig A, Pantuwan G, Nguyen TT, Tripathy JN, Sarial AK, Robin S, Babu RC, Nguyen BD (2001). Locating genomic regions associated with components of drought resistance in rice: comparative mapping within and across species. *Theor Appl Genet* 103:19-29.