

Root QTL Pyramiding through Marker Assisted Selection for Enhanced Grain Yield Under Low Moisture Stress in Rice (*Oryza sativa* L)

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Abstract

Drought is the foremost limiting factor affecting rice production especially in the critical reproductive growth phase. Several mechanisms that resist/ tolerate or avoid drought stress have been elucidated and documented in many crops. In this study, Quantitative trait loci pyramiding (QTL) pyramiding for root morphological traits and an evaluation of the effects of QTLs in combination. Four QTLs present on chromosomes 1, 2, 7 and 9 in a near-isogenic line (NIL) population derived by backcrossing 4 doubled haploid lines of IR64/Azucena cross into IR64 were used to develop pyramids. These NILs were crossed pair-wise to arrive at combinations of two-QTL and three-QTL pyramids. Among the generated QTL pyramids, *qRT6-2 x qRT11-7* and *qRT6-2 x qRT19-1+7* were found to have higher yields than parent IR64 under moisture stress situation. QTLs present on different chromosomes showed maximum antagonistic effects in comparison with the different QTLs present on the same chromosome. The pyramids also show a relative increase of the NILs in terms of height, number of tillers and number of panicles, however, pyramids not show an increase in the dry matter accumulation which implies that the partitioning of the biomass is directed towards the below ground parts, leading to less sturdy plants that perform averagely under moisture stress situations.

Keywords: Rice; Pyramids; Root morphology; Drought; Stress situation

Introduction

Rice (*Oryza sativa* L), the staple cereal of the world's population is heavily dependent on availability of copious amounts of water for optimum productivity. With the diminishing water supplies in the rain fed regions that occupy predominant rice growing belts, frequent and intermittent drought that coincide with the critical reproductive growth periods often affect rice production. Severity of drought stress has led to a high yield decline in rice in recent years in drought-affected areas. This therefore necessitates the development of genotypes that survive on reduced water supply and which can adapt and cope with the climate changes [1-6].

Several physio-morphological characters contribute towards drought resistance or tolerance in plants, among which, root traits are known to impart resistance under conditions of increasing water deficits with substantial yields [5] several quantitative trait loci that affect root morphology have been identified in rice across mapping populations [7].

Pyramiding of genes controlling a trait in order to enhance the trait expression and also impart stability, is a widely followed approach in disease resistance breeding, where a crop is plagued by a spectrum of races/pathovars of a pathogen. The individual genes pyramided offer specific resistance against a race/pathovars. A combination thereof will therefore, ensure a wider disease resistance spectrum. Pyramiding is made more efficient through the use of molecular markers that are tightly linked to the gene of interest as is evident in the studies of [8] for blast resistance, for bacterial blight, for basmati quality traits, for fungal pathogen resistance and for heading date in rice [9-12].

Drought being a major abiotic factor affecting rice production especially in the critical reproductive growth phase. Novel approach, such as QTL pyramiding through marker assisted selection for root morphological traits and evaluation of the effects of root QTLs in combination is attempted to develop new drought tolerant rice genotypes.

Material and Methods

Plant material

One hundred and thirty five doubled haploid lines (DHLs) were derived from an IR64/ Azucena cross by Guiderdoni et al. in 1992 at IRRI. [13] identified the four target Quantitative trait loci pyramided (QTL) regions between the markers RZ19, RG690, RZ730 and RZ801 on chromosome # 1, RM29, RG171, RG157 and RZ318 on chromosome # 2, RM234, CDO418, RZ978, CDO38, and RM248 on chromosome # 7 and RZ228 and RZ12 on chromosome # 9. The markers were used by [1] to develop Near-Isogenic Lines (NILs) of IR64 with Azucena alleles at the target loci through backcross breeding. The selections were made strictly based on marker genotypes with the exception of RZ228 and RZ12 on chromosome # 9, which were replaced by RM201 and RM242 PCR-SSR markers after the selection in BC₂ generation [7] identified 25 new rice microsatellite markers to the QTL regions spanning 51.8 cm on chromosome 1, 88.7 cm on chromosome 2, 19.7cm on chromosome 7 and 15.1 cm on chromosome 9. These NILs were crossed pair-wise to develop two and three QTL combination pyramids and were evaluated in well-watered and low moisture stress used in this study (Table 1 and Figure 1).

Validation of pyramids

The generated pyramids were validated for the presence of the

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Sl. No.	Pyramids	Chro. Introgression on	QTL No	No. Plants
1	qRT11-7 X qRT18-1+7	7+ (1+7)	3	35
2	qRT24-9 X qRT11-7	9 + 7	2	38
3	qRT6-2 X qRT11-7	2+ 7	2	32
4	qRT11-7 X qRT19-1+7	7 + (1+7)	3	36
5	qRT20-1+7 X qRT18-1+7	(1+7) + (1+7)	4	37
6	qRT11-7 X qRT6-2	7 + 2	2	38
7	qRT6-2 X qRT19-1+7	2+ (1+7)	3	32
Total No. of plants				248

Table 1: List of two and three QTL pyramids generated for the study.

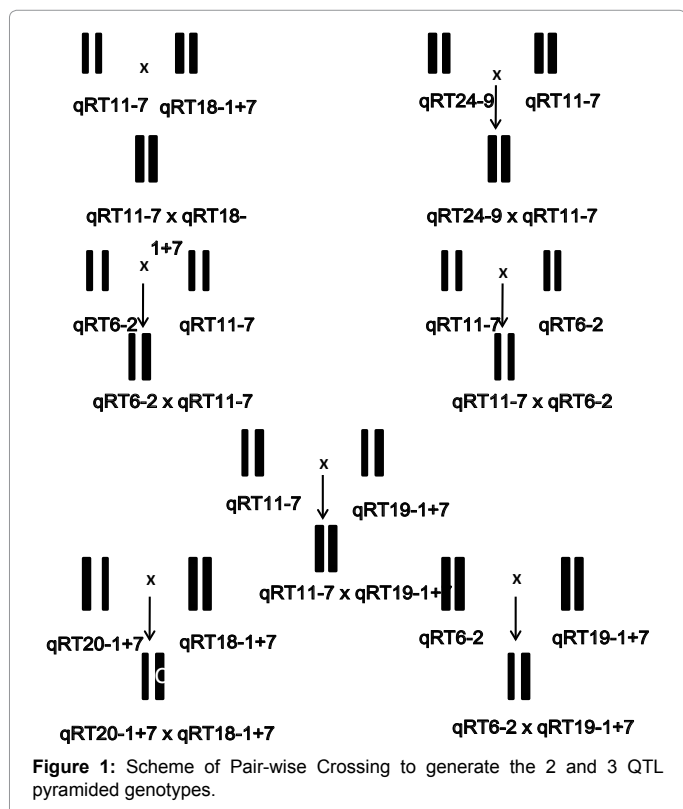


Figure 1: Scheme of Pair-wise Crossing to generate the 2 and 3 QTL pyramided genotypes.

QTLs through tightly linked and flanking SSR markers. Total DNA was extracted as per the procedure outlined by [14]. The PCR reaction mixture contained 50ng template DNA, 50 ng of each primer, 0.05 mM dNTPs, 1X PCR buffer (10mM TRIS, pH 8.4, 50 mM KCl, 1.8 mM MgCl₂ and 0.01 mg/ml gelatin) and 1U *Taq* DNA polymerase in a volume of 20 µl. Template DNA was initially denatured at 94°C for 5 min followed by 35 cycles of PCR amplification of 94°C denaturation for 30 seconds, primer annealing at 56°C for 1 minute, primer extension at 72°C for 2 minutes. The amplified product was electrophoretically resolved on a 3.5% agarose gel in 1X TBE buffer and visualized under UV light after staining with ethidium bromide. Pyramids selected strictly on the basis of presence of marker alleles were evaluated in the field (Figure 2).

Phenotypic evaluation of pyramid combination plants

The experiment was conducted during the dry season at Main Research Station, Hebbal, Bangalore, and during the wet season at Zonal Agricultural Research Station, GKVK, in a replicated randomized complete block design. The parent genotypes, the recipient IR64, donor Azucena were evaluated along with the pyramids and checks: Buddha and Moroberekan under reproductive stage low moisture stress (50% field capacity between 65 to 80 DAS) and well-watered condition (100% field capacity) in 1m long, 8 inch diameter PVC pipes. The pipes were filled with sandy loam soils and the standard package of fertilizer dosage was followed. The entries were sown and upon germination, a single plant was maintained in each pipe. Regular irrigation schedule was followed until 65 days after sowing. Low moisture stress treatment was included by maintaining the pipes at 50% field capacity using controlled irrigation and a rain-out shelter was used to keep out rainwater. Well-watered treatment received the stipulated amount if irrigation so as to maintain 100% field capacity. The observations were recorded at harvest, plant height was measured from the base of the crown to the tip of the highest leaf, number of tillers, number of panicles were counted at harvest, panicle weight was recorded as an average of 5 panicles, panicle length was from an average of 5, plants measured from the collar to the tip, test weight was recorded on 100 randomly selected seeds, seed yield was recorded as the total yield produced by the plant, total biomass was the sum of both the shoot and root mass, maximum root length was recorded from the crown to the tip of the longest root, root number was recorded at 15 cm depth and root thickness was on an average of 5 roots at 15 cm depth measured with slide calipers.

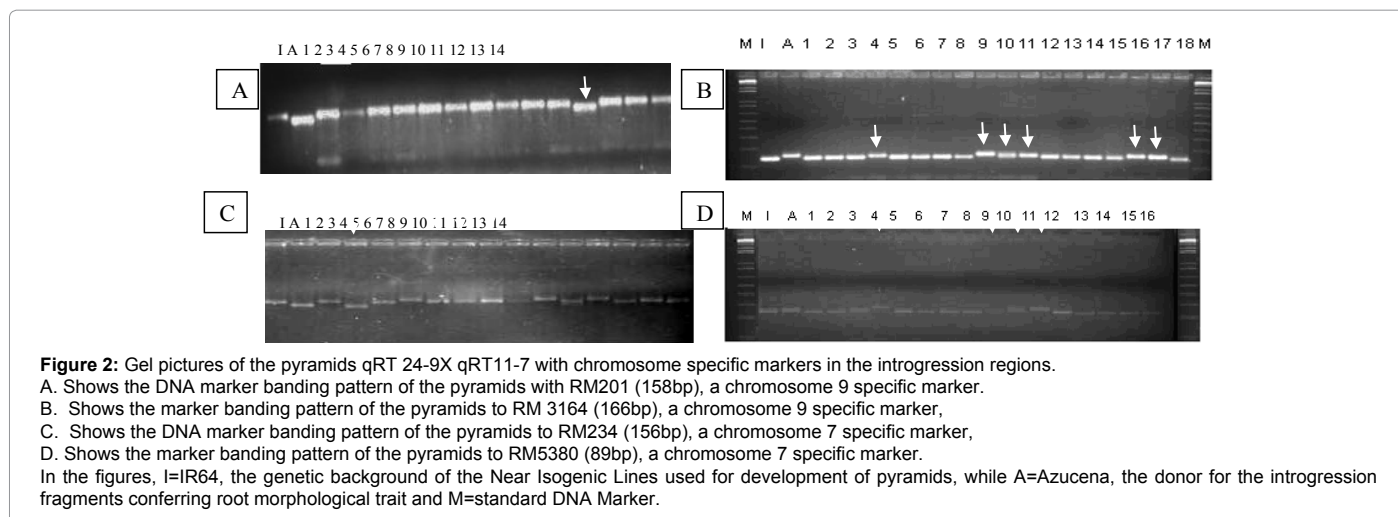


Figure 2: Gel pictures of the pyramids qRT 24-9X qRT11-7 with chromosome specific markers in the introgression regions. A. Shows the DNA marker banding pattern of the pyramids with RM201 (158bp), a chromosome 9 specific marker. B. Shows the marker banding pattern of the pyramids to RM 3164 (166bp), a chromosome 9 specific marker, C. Shows the DNA marker banding pattern of the pyramids to RM234 (156bp), a chromosome 7 specific marker, D. Shows the marker banding pattern of the pyramids to RM5380 (89bp), a chromosome 7 specific marker. In the figures, I=IR64, the genetic background of the Near Isogenic Lines used for development of pyramids, while A=Azucena, the donor for the introgression fragments conferring root morphological trait and M=standard DNA Marker.

Statistical Analysis

The data that was generated was subject to a series of statistical analyses to elucidate the relative effects of the presence of various QTLs in the rice genotypes. The data was analyzed to individual and combined ANOVA over two moisture regimes based on RCBD for different experiments to assess the variability that is present in the various rice NILs and in the generated pyramids. The data was pooled upon testing for homogeneity of variances. The mean difference in the performance of the entries under low moisture stress in comparison with the well-watered conditions was arrived at using the formula

$$\text{Mean \% performance} = \left[\frac{\text{LMS-WW}}{\text{LMS}} \right] * 100$$

The significance of the difference was tested using the t-test. All statistical analyses utilized the SPAR 2 software analysis package.

Results and Discussion

The mean performance of the QTL-NILs, the generated pyramids and the checks are presented in Table 2. Most genotypes showed significant increases plant height over IR64 but not over Azucena and the checks (Moroberekan and Budda) under low moisture stress. Under well-watered conditions, the QTL-NILs and the pyramids are significantly shorter than the lesser parent IR64. The number of tillers and panicles are comparable with both Azucena and Budda but are significantly lower than both IR64 and Moroberekan under two

Entries		PHT	NOT	NOP	PWT	PL	TWT	SWT/PLT	TBM	MRL	RTN	RTT
QTL-NILs	LMS	46.76	7.34	4.26	5.29	12.81	1.54	4.52	25.48	16.87	49.57	0.75
	WW	61.02	9.25	7.23	8.24	14.24	2.13	7.87	47.94	14.72	59.78	0.63
	Performance	-30.51**	-26.01*	-69.54**	-55.64**	-11.2	-38.56**	-74.14**	-88.16**	12.74*	-20.60*	16.79
qRT11-7 x qRT18-1+7	LMS	50.91	7.07	4.97	5.26	13.49	1.28	4.14	34.35	15.89	37.24	0.75
	WW	55.24	8.59	7.1	7.45	14.83	2.06	6.97	62.11	12.04	59.62	0.62
	Performance	-8.5	-21.6	-43.05**	-41.71**	-9.98	-60.84**	-68.33*	-80.84**	24.19**	-60.09*	16.93
qRT24-9 x qRT11-7	LMS	45.89	7.46	4.15	6.84	13.75	1.3	4.65	29.6	17.34	36.62	0.75
	WW	55.49	9.2	6.92	8.94	15.11	2.07	7.49	53.47	14.19	66.82	0.63
	Performance	-20.92*	-23.42*	-66.53**	-30.83*	-9.83	-59.55**	-61.20**	-80.63**	18.17**	-82.46*	16.98
qRT6-2 x qRT11-7	LMS	54.95	7.66	5.3	6.3	12.95	2.14	4.61	25.95	14.01	44.47	0.79
	WW	57.31	9.13	9.1	8.36	14.19	2.96	7.75	49.72	11.82	69.56	0.62
	Performance	-4.29	-19.18*	-71.78**	-32.70**	-9.57	-38.16	-68.27**	-91.60**	15.61*	-56.42**	22.23
qRT11-7 x qRT19-1+7	LMS	43.77	7	4.35	7.07	12.38	1.38	5.17	26.47	15.9	37.7	0.73
	WW	45.99	9.28	7.91	9.39	13.64	2.09	8.18	52.52	13.24	51.78	0.58
	Performance	-5.06	-32.66*	-81.90**	-32.81*	-10.15	-52	-58.10**	-98.44**	16.71*	-37.34**	20.95
qRT20-1+7 x qRT18-1+7	LMS	45.83	6.58	3.59	6.38	12.2	1.32	5.11	26.43	12.21	34.96	0.73
	WW	47.32	9.1	7.27	9.01	13.92	2.02	7.95	49.97	10.21	53.69	0.59
	Performance	-3.27	-38.35*	-102.51**	-41.13**	-14.08	-53.70**	-55.50**	-89.09**	16.43*	-53.57**	19.32
qRT11-7 x qRT6-2	LMS	37.48	4.64	3.46	5.18	12.19	1.46	5.02	24.07	11.01	44.91	0.76
	WW	43.74	7.3	6.86	7.96	13.48	2.15	8.35	46.91	8.54	56.67	0.64
	Performance	-16.71*	-57.45*	-98.10**	-53.63*	-10.54	-46.86**	-66.31**	-94.90**	22.44*	-26.19**	15.64
qRT6-2 x qRT19-1+7	LMS	36.03	5.36	3.52	5.08	12.19	1.72	5.03	22.83	10.56	39.03	0.79
	WW	40.52	7.23	6.22	7.53	13.49	2.39	8.3	46.53	8.31	60.72	0.62
	Performance	-12.48	-35.08	-77.04**	-48.23*	-10.73	-39.23**	-65.14**	-103.79**	21.34*	-55.60**	20.54
IR64	LMS	47.07	12.5	5.33	5.28	12.94	1.38	5.45	28.98	9.81	66	0.79
Azucena		61.94	4.5	2.67	3.34	12.6	1.23	4.12	22.22	28.78	27.67	0.8
Budda		57.26	4.33	3.17	6.64	15.08	1.71	4	25.59	29.36	38.77	0.85
Moroberekan		68.4	7.17	4.83	11.33	11.97	3.03	8.95	36.24	32.16	30.9	0.76
IR64	WW	60.54	15.67	8	9.38	15.1	2	8.8	49.38	10.13	90.5	0.66
Azucena		71.5	7.33	5.5	5.96	14.29	1.79	7.35	38.7	24.94	54.5	0.68
Budda		84.2	6.5	6.33	9.38	16.16	2.26	7.28	42.86	28.46	62.43	0.67
Moroberekan		86.13	10	9.33	18.87	13.71	3.82	8.36	53.85	28.79	59.9	0.71

*: Significance at 5%. **: Significance at 1%.

LMS: low moisture stress (50% FC); WW: well watered condition (100% FC)

PHT: Plant height (cm); NOT: Number of tillers per plant

NOP: Number of panicles per plant; PWT: Panicle weight (g)

TWT: Test weight (g); SWT/PLT: Seed yield per plant (g)

MRL: Maximum root length at maturity (cm); RTN: Total root number at the crown.

TBM: Biomass accumulated at maturity (g); RTT: Root thickness (mm).

Table 2: Mean performance of the QTL-NILs and the pyramids under low moisture stress in comparison with well watered conditions.

moisture regimes. While the panicle length and panicle weight of the pyramids is higher than Azucena and is comparable with IR64, the test weight is significantly higher than IR64 in the pyramids qRT6-2 × qRT11-7 and qRT6-2 × qRT19-1+7. The root length under low moisture stress was significantly higher than IR64 but was less than the check varieties, while under well-watered conditions; the root length was lesser than IR64. The root number was significantly lower than IR64 under two moisture regimes, while the roots were comparatively thicker than IR64 under different moisture regimes (Table 2).

The results of analysis of variance for the growth, yield and yield contributing characters and three root morphology characters were studied under well-watered (WW) and low moisture stress (LMS) condition revealed highly significant differences among the QTL-NILs (data not shown). This reveals that large amount of genetic difference is present in these QTL-introgressed lines for all other traits, thus indicating the scope for selection and to forward the best among them for further study. The mean performance of the genotypes differed for all the traits under these contrasting moisture regimes of low moisture stress (LMS) and well-watered (WW) conditions indicating an adaptive response of drought tolerance [15,16]. A significant reduction in plant height, number of tillers and panicles per plant, panicle length, panicle weight, test weight, seed yield per plant and total biomass were observed under low moisture stress condition in different growth environments.

Considering root traits, a significant increase in root length and thickness, while a significant reduction in the number of roots was reported during dry season 2004, at MRS, Hebbal. A non-significant increase in root length and thickness, with a significant increase in root number was reported during wet season 2005 at ZARS, GKVK. This difference in the performance of the genotypes is ascribable to the seasonal difference [7,17]. The pyramid qRT11-7 × qRT18-1+7 recorded significant variance for all traits except for seed yield per plant during dry season at MRS, Hebbal, while during wet season at ZARS, GKVK, significant variance was recorded only for panicle weight, panicle length, seed yield per plant, total biomass, root length and number under both the moisture regimes. Significant variance was recorded for plant height, number of panicles per plant under well-watered conditions, and for test weight and root thickness under low moisture stress conditions. Similar results were also obtained in qRT20-1+7 × qRT18-1+7, while in the pyramid qRT11-7 × qRT19-1+7, contrasting results were obtained for number of chaffy seeds per panicle, total biomass and root length. This indicates that though these crosses involve the same QTL regions being introgressed, there exists differences within the inherited fragments and hence a difference in the phenotypic expression patterns. Between the pyramids qRT6-2 × qRT11-7 and qRT11-7 × qRT6-2, similar results between both of them were obtained in both the growth environments. qRT24-9 × qRT11-7 significant variance for all traits except for panicle length and root thickness.

The mean performance of the genotypes differed for all the traits under these contrasting moisture regimes of low moisture stress (LMS) and well-watered (WW) conditions. A significant reduction in plant height, number of tillers and panicles per plant, panicle length, panicle weight, test weight, seed yield per plant, total biomass and root number, with a significant increase in root length and thickness were observed under low moisture stress condition in all the three growth environments. The initial near isogenic line population that was used for the crossing programme recorded a low number of lines that showed an increase in the root morphology. This was attributed to non-allelic interactions between the donor alleles that

may be disrupted by the recurrent parent alleles [1]. Initial mapping procedures indicated antagonistic effects between the QTLs so large so as to inhibit QTL detection [13,18]. They also stated that the QTLs present on different chromosomes showed maximum antagonistic effects in comparison with the different QTLs present on the same chromosome. The pyramids also show a relative increase of the NILs in terms of height, number of tillers and number of panicles, however, did not show an increase in the dry matter accumulation which implies that the partitioning of the biomass is directed towards the below ground parts, leading to less sturdy plants that perform averagely under stress situations. The increases in the root morphology performances in the present study could be attributed to the disruption of such deleterious interaction effects through recombination.

Conclusion

In the present study, it was demonstrated that it is possible to combine QTLs in a common genetic background using co-dominant markers that reside in the QTL region. The combination of QTLs for root morphology had both additive as well as interactive effect epistatic effect on grain yield. The combinations of more than one QTL present in a single genotype that performed superior in both stress and well-watered condition is being tested on large scale and in natural water deficit situation.

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