



Research Article

ROOT QTL TRAITS AND WATER USE EFFICIENCY PYRAMIDING THROUGH MARKER ASSISTED SELECTION LEADS TOWARD SUPERIOR GENOTYPES FOR DROUGHT TOLERANCE IN RICE (*Oryza sativa* L.)

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Abstract- Twenty two NILs and 94 pyramided lines were evaluated for shoot, root traits and water use efficiency (WUE) under two water regimes. Significant differences for different pyramid genotypes were observed under low moisture stress (LMS) condition. Higher yield was recorded in qRT 2+7-14-4 genotype containing root length QTL on chromosome 2 and 7, under well watered condition; under stress, yield was high in genotype qRT 2+9- 19-3 having QTL on chromosome 2 and 9. A set of 50 root pyramid genotypes were analyzed for carbon-13 isotope discrimination, a surrogate trait for WUE. Highest WUE among QTL introgressed lines was observed for qRT 1+7-16-4 genotype for root QTL on Chr. 1 and 7. Root phenology using polypropylene pipes revealed significant differences among genotypes for root length, root number, root volume, root fresh weight and root dry weight. Maximum root length and root number were for qRT 1+7-18-4 and qRT 1+7-19 genotypes containing QTL on chromosome 1 and 7 respectively. Root volume was highest in QTL introgressed genotype qRT 2+7-10-4. The best performed lines were forwarded for subsequent testing towards developing drought tolerant genotypes.

Keywords- Near isogenic lines (NILs) – root QTL– pyramiding– Drought–Carbon 13 isotope discrimination– Rice.

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Introduction

Rice (*Oryza sativa* L.) is the most sought after food by most Asians both for nutrition and as energy food. It is eaten as the main food in most of the South Asian and South East Asian countries [1]. It is a sustainable food for most Asians [2]. Asia produces and consumes about 90 per cent of the rice grown in their countries [3]. About 65 % Indian population eats rice as main food [4,5]. Rice is cultivated in a diverse range of environments such as irrigated, rain fed upland, rainfed lowland, and flooded conditions. It accounts for more than 65% of caloric intake, providing 23% of global human per capita energy and 16% of per capita protein [6,7]. The population is growing, but the rice production is plateaued, and there is a challenge to produce more than 35 percent of extra rice to meet the growing population in the next 20 years [1]. This increasing demand for rice will have to be met by limited resources like arable land, water, and existing rice with higher yield potential and greater yield stability. Developing drought tolerant genotypes that can be grown well on marginal soils with limited water supply accompanied with maximum productivity is a first and foremost objective in breeding for abiotic stresses [8]. Root penetration in soil layers is identified as an important drought tolerance trait in rice improvement [9,10]. Incorporation of root-based selection criteria such as root penetration ability, rooting depth, root length density and root mass at deep soil layer, in the plant-breeding program is more difficult. Quantitative measurement of above root parameters is time-consuming and highly laborious due to the lack of reliable and two efficient screening techniques [11]. The plant part that frequently suffers when water becomes scarce is the root. Hence, the root is one important part that will contribute to plant growth and yield under stress condition. Increased root growth, increased number of roots and its ability to go down and extract water during a water deficit situation is

important for genotype survivability. Root traits are complex quantitative characters, which are governed by polygenes. However, the progress in breeding for drought tolerance is slow due to the genetic complexity of the trait under field conditions. The exact number of QTLs controlling a trait can be identified and their presence can be detected using the contemporary molecular tools. NILs have been extensively used by plant breeders for pyramiding genes/QTLs [12,13] and developed cultivars for various agronomically important traits. The interaction and epistatic effect of QTL information is more important. The choice of combining QTL of least interactive type and making the trait more stable in the long run is an important aspect of gene/QTL pyramiding [14].

Several researchers have attempted to correlate root parameters with easily quantifiable shoot characters, but yielded limited success [15]. Several studies showed positive correlation between plant height and deep rooting system [16], whereas there were reports of obtaining recombinant plants with short height and deep root system, indicating doubt in the consistency on this correlation. The correlation between shoot and root traits have given significant results [17], whereas no correlation has so far been reported between root penetration ability and any shoot character. These studies obviously point out the need for effective and reliable selection criteria for root traits controlling drought tolerance. To achieve this, we need to use molecular markers associated with root traits related to drought tolerance in breeding program to develop rice varieties with greater adaptability to moisture stressed condition [18].

Rice is a major water consumer among agriculturally important crops and it is also a model cereal food crop for genomic studies. Recent predictions of climate changes revealed an increase in water scarcity in the near future [19] leading to frequent drought spells [20]. The water is receding and rice requires large

quantities of water. Hence there is a need to develop water use efficient genotypes that produce more with lesser irrigation water [21,22]. Hence utilization of a surrogate trait like carbon-13 ($\Delta^{13}C$) discrimination has potentially helped in identifying the water use efficient genotypes indirectly and use it for selection for breeding purposes [23]. The water use efficiency (WUE) is a multigenic controlled trait and is highly dynamic in different environmental conditions. The inverse relationship between $\Delta^{13}C$ and WUE reveals WUE of a given genotype [23] in water conservation. Our effort in this study is to quantify the various phenological traits of shoots and roots under stress and non-stress conditions using root-QTL pyramided genotypes and estimate their water use efficiency by $\Delta^{13}C$ isotope discrimination and finally to identify plants that perform well in low moisture stress and deploy them in breeding programs.

Materials and Methods

Experimental population, planting

A set of 22 near-isogenic lines (NILs) of IR64 (*Indica*, High yielding) with QTL introgressions from Azucena (*Japonica*, Drought tolerant) controlling the root length were used for the study. These NILs were developed by backcrossing selected doubled haploid (DH) lines to a recurrent parent IR64 [24] at the International Rice Research Institute (IRRI). The backcrossed progenies were selected for target loci strictly based on linked SSR markers. The NILs developed

were grouped into five groups depending on the introgressed fragment from Azucena. They are qRT1- qRT3, having introgression on chromosome 1, qRT4-qRT8 having introgression on chromosome 2, qRT9- qRT15 having introgression on chromosome 7, qRT16- qRT23 having introgressions both on chromosomes 1 and 7 while qRT24 - qRT29 had introgression on chromosome 9. These NILs were used to develop pyramided lines for two and three QTLs combination. The segregating progenies were selected purely based on tightly linked SSR markers. Finally, we selected 94 QTL pyramids having combination on chromosomes 1, 2, 7 and 9. These pyramids were evaluated along with the parents: IR64, Azucena and checks: Buddha and Rasi were studied [Table-1]. The experiment was carried out at 'K1' block, GKVK, Bangalore, India representing the eastern dry zone, which is located at the latitude of 12° 58' North; longitude 77° 35' East and altitude of 930 meters above mean sea level (MSL). The experiment was laid out in augmented I design with 4 blocks where 4 checks (IR64, Azucena, Rasi and Buddha) were replicated once in each block. These lines were evaluated under both well watered (WW) and low moisture stress (LMS) condition. When the plants were 60 days old (Vegetative stage) complete moisture stress was induced to the stress plots by withholding water, while the WW plots were irrigated normally. Same stress induction was imposed at anthesis stage of reproductive phase. The stress was induced for a period of 10 days.

Table-1 Analysis of variances for three characters in pyramided root-QTLs and single root-QTL lines under well-watered (WW) and low moisture stress (LMS) conditions during vegetative stage

Parameters	Well-watered (WW)			Low moisture stress (LMS)		
	PHT	NOT	DF	PHT	NOT	DF
GM	55.82	12.35	104.77	56.72	11.67	99.39
TEM	54.57±5.65	12.77±1.86	104.07±1.57	54.75±6.59	11.76±2.17	98.82±1.03
CM	64.81±2.82	9.31±0.93	109.75±0.79	70.93±3.29	11±1.08	103.44±6.52
Significance						
Lines	*	NS	**	NS	NS	**
Checks	**	**	**	**	**	**
Lines v/s lines	**	**	**	**	NS	**

*, ** marks significant at p=0.05 and 0.01 respectively, NS= Non significant at p=0.05

GM= General mean CM= Check mean TEM= Test entries mean

PHT= Plant height (cm) NOT= Number of tillers DF= Days to 50% flowering

Traits and measurements

Plant height (PHT), Number of tillers per plant (NOT), Days to 50 per cent flowering (days), Leaf rolling score (LRS), Leaf drying score (LDS), Number of panicles (Productive Tillers) (NP), Panicle length (PL), Number of seeds per panicle (SPP), Spikelet sterility percentage (SP), Test weight (TW), Water use efficiency (WUE) and Grain weight per plant (GWT) were recorded.

The observations were recorded by randomly selecting six plants each in all the genotypes. All the above said traits were measured under both stress and control conditions. LRS, LDS and WUE were recorded only under LMS condition. Leaf rolling score was visually scored with the scale from "0" to "9" at noon on the 10th day of stress period when the difference among lines became most obvious by following International Rice Research Institute (IRRI) standard evaluation pattern. A score of "0" indicates leaves healthy (no leaf rolling) and the score "9" indicates the leaves tightly rolled. Leaf drying score (LDS) was also visually scored by giving scores from "0" to "9" just prior to irrigation (on the 10th day of stress). A score "0" depicts healthy leaves and "9" depicts a complete drying (death) of leaves. A subset of genotypes were selected based on their yield per se and subjected them for $\Delta^{13}C$ determination. The plant material was oven dried at 80°C after which the samples were powdered in a ball mill. The powdered sample was stored in labeled Eppendorf tubes until analysis. The $\Delta^{13}C$ of the leaf samples were determined using the Isotope Ratio Mass Spectrometer (IRMS) at the National Facility for stable isotope studies in biological sciences in the Department of Crop Physiology, UAS, Bangalore, India.

The WUE was analyzed by Isotope Ratio Mass Spectrometer (IRMS) by determining $\Delta^{13}C$ Carbon isotope of the leaf samples. Carbon isotopic

discrimination is measured as the deviation of the molar ratios between ^{13}C and ^{12}C from an international standard – PDB (Pee Dee Belemnite, California, USA). Carbon isotopes are fractionated due to diffusion through stomata denoted as 'a' and at the carboxylation site by RuBisCO, denoted as 'b'. The total isotope discrimination (in parts per million) is the deviations of these fractions from the isotopic ratio in the air. Therefore, the discrimination $\Delta^{13}C$ is computed as follows:

$$\Delta^{13}C (\text{‰}) = \frac{\delta_a^{13}C - \delta_b^{13}C}{1 + \delta_b^{13}C/1000}$$

Evaluation of root traits

The evaluation of root morphology of QTL pyramids, single QTL lines (parents of Pyramids) along with parents (parents of single QTL lines/NILs) and checks was conducted in the green house. The seeds of these genotypes were sown in PVC pipes measuring 1 metre in length and 18 cm diameter, both in LMS as well as in WW conditions. The pipes were filled with a mixture of sandy clay loam and FYM in 4:1 proportion. The soil was uniformly filled and they were placed in subsurface pits so that the tops of the PVC pipes were at ground level. The soil was fertilized according to the recommended package of practices. One hundred and thirteen genotypes were sown in each PVC pipe with two replications for each treatment. All the genotypes were randomized within a block. Only one seedling per pipe was retained after 10 days of sowing. The WW set was irrigated on every alternate day, while LMS condition was given normal irrigation till 64th day and moisture stress imposed between 65 to 75 days after sowing (DAS). Rainout shelter was provided during the imposition of low-moisture stress. Roots were sampled on

76th day. Pipes in which the plants were grown were soaked in water overnight and the roots were washed with a force of water to remove the soil from the roots. The cleaned roots mass was stored in paper bags for recording observation after oven drying. The following observations were recorded on roots viz., Maximum root length (MRL) (cm), Total root number (RN), Root volume (RV), Root fresh weight (RFW) and Root dry weight (RDW) apart from Plant height (PHT) (cm) at sampling, Number of tillers per plant (NOT).

Statistical analysis

The analysis of variance as per [25] was carried out for different characters. The statistical analysis of the data on individual characters using AGRISTAT package was carried out on the mean values of each genotypes and checks. Regression 5 analysis for yield and other independent variables was carried out on selected genotypes by using SPSS software V12.0. The regression model was fitted to explain the variability and model is as follows,

$$Y = a + bx \text{ or } \hat{Y} = a + bx$$

Correlation studies

The degree of association between shoot, WUE and root traits studied, phenotypic correlation was computed by using the formula given by [26]. The significance of correlation co-efficient was tested by referring to the table value at n-2 df given by [27].

Results

Shoot and yield related parameters of root-QTL pyramids

The mean values of root QTL pyramids obtained for various plant growth and yield traits taken during vegetative phase were analysed individually. The analysis of variance for plant height, number of tillers per plant, days to 50% flowering are presented in the [Table-2]. As evident from the table, the significant difference among the means of different genotypes (root-QTLs, parents and checks) for all the traits observed under both the conditions except for the number of tillers under LMS condition. The significant difference was observed among root-QTL pyramids for plant height and days to 50% flowering under WW condition. At the same time significant difference was observed for days to 50% flowering among root-QTL pyramids under LMS condition. This could be due to differential expression and effect of QTLs controlling drought traits, which are localized on different chromosomes. The results of the analysis of variance for various traits under WW [Table-3] condition and under LMS recorded during reproductive phase revealed significant differences among the genotypes. Between the two conditions (WW and LMS) significant difference among root-QTL pyramided and root-QTL parental lines was observed for the characters viz., plant height, number of tillers per plant, panicle number, panicle length, grains per panicle, spikelet sterility percentage and grain weight. There was an increased mean performance of root QTL pyramided lines compare to IR64 for panicle number, panicle length, grains per panicle and test weight under WW condition. All the genotypes had significant difference for all traits except for plant height, panicle length in comparison with Azucena. This is because of Azucena is a Japonica variety with taller stature and higher panicle length. Under low moisture stress, increased mean performance of root QTL pyramided lines for plant height, panicle number, panicle length, grains per panicle, grain yield was 6 recorded compared to IR64.

Table-2 Performance of pyramided root-QTLs and parental root-QTL lines under well-watered (WW) and low moisture stress (LMS) condition during reproductive phase

Parameters	Well-watered (WW)							
	PHT	NOT	PNO	PL	GPP	STP	TW	GWT
GM	67.94	9.95	8.26	19.14	75.42	41.4	2.44	7.58
EM	66.56±2.43	10.22±0.67	8.41±0.85	18.97±0.57	75.43±3.09	43.65±3.3	2.41±0.12	7.47±1.24
CM	77.87±4.87	8±1.35	7.23±1.71	20.32±1.15	75.41±6.19	25.26±6.61	2.61±0.24	8.38±2.48
Significance								
Lines	**	*	NS	*	**	**	NS	*
Checks	**	**	**	**	**	*	*	**
Checks v/s lines	NS	**	*	**	NS	**	*	NS
Parameters	Low moisture stress (LMS)							
	PHT	NOT	PNO	PL	GPP	STP	TW	GWT
GM	65.51	11.5095	14.091	19.159	90.907	48.823	2.169	7.465
LM	63.54±3.91	11.50±0.97	14.50±2.38	19.17±0.56	89.39±7.4	49.03±8.64	2.149±0.1	7.03±1.71
CM	79.54±1.95	11.54±1.95	11.15±4.77	19.07±1.12	101.74±14.81	47.36±17.28	2.313±0.2	10.56±3.42
Significance								
Lines	**	NS	*	**	*	NS	NS	NS
Checks	**	**	NS	*	**	*	**	**
Checks v/s lines	**	NS	*	NS	**	NS	*	**

*, ** marks significant at p=0.05 and 0.01 respectively, NS= Non significant at p=0.05

GM= General mean, EM= Entries mean, CM= Checks mean, PHT= Plant height (cm), NOT= Number of tillers per plant, PNO= Number panicle per plant
PL= Panicle length (cm), STP= Sterility per cent, GPP= Grains per panicle, TW= Test weight (g), GWT= Grain weight (g)

Table-3 Performance of pyramided root-QTLs and single root-QTL lines for shoot and root characters under well-watered (WW) and low moisture stress (LMS) conditions during vegetative stage

Parameters	Well-watered (WW)						
	PHT	NOT	RL	RN	RV	RFW	RDW
GM	40.82	4.38	14.95	16.89	6.21	3.62	2.47
LM	39.04±2.42	4.19±1.14	14.31±2.04	15.67±3.01	5.89±2.09	3.70±0.54	2.56±0.24
CM	53.50±1.21	5.75±0.57	15.44±1.02	25.63±1.50	8.53±1.04	3.05±0.23	1.83±0.12
Significance							
Lines	**	*	**	**	**	**	**
Checks	**	**	**	**	**	**	**
Checks v/s lines	**	**	NS	**	**	**	**
Parameters	Low moisture stress (LMS)						
	PHT	NOT	RL	RN	RV	RFW	RDW
GM	45.46	5.15	17.75	24.81	6.37	6.94	4.01
LM	43.86±1.48	4.94±0.45	16.51±0.99	21.67±1.45	5.56±0.82	6.27±0.05	3.48±0.34
CM	56.75±2.96	6.59±0.91	26.5±1.98	46.96±2.91	12.08±1.64	11.71±1.10	7.72±0.67
Significance							
Lines	**	**	**	**	**	**	**
Checks	**	**	**	**	**	**	**
Checks v/s lines	**	**	**	**	**	**	**

*, ** marks significant at p=0.05 and 0.01 respectively, NS= Non significant at p=0.05

GM= General mean, EM= Entries mean, CM= Checks mean, PHT= Plant height (cm), NOT= Number of tillers, RL = Root length (cm)
RN = Root number, RV = Root volume (cc), RFW = Root fresh weight (g), RDW = Root dry weight (g)

This is due to the functioning of root QTL under stress condition; whereas increased performance was observed for a number of tillers, panicle numbers, panicle length, spikelet sterility and grain yield per plant under LMS. This could be due to all the root QTL lines in the background of high yielding parent IR64 and functioning of root QTLs under drought condition. Highest leaf drying was noticed in IR64 i.e., 3 (tip drying extended up to ¼ of a leaf) followed by Azucena (1), Buddha (1) and Rasi (1), among check varieties. Almost all the root QTL pyramided lines have shown values in between 1 to 3. Highest score of leaf rolling was recorded in IR64. It was taken score of 3 indicating leaves folding to become deep V-shape. Azucena has taken score of 1 depicting leaves start to fold (shallow). All the QTL pyramided lines have taken the score in between 0–5.

Root morphology of root-QTL pyramids

In this experiment, we attempted to evaluate root-QTL introgressed parental lines, root QTL pyramided lines and checks for root morphological traits. Genetic evaluation of root-QTL pyramided and NILs along with four checks undertaken during the wet season for root morphological traits. The mean values obtained for various root morphological characters of root-QTL lines and checks were taken for analysis. Individual analysis of variance of WW and LMS for root morphological characters of root QTL and checks were carried out. The comparative analysis of variance for WW data and LMS data is presented in [Table-4]. As evident from the table, the analysis of variance indicated significant differences among entries under both the conditions for plant height (PHT), number of tillers (NOT), root length (RL), root number (RN), root volume (RV), root fresh weight (RFW) and root dry weight (RDW). But non-significant differences were observed for root length under a WW situation between checks and QTL pyramided lines.

Table-4 Carbon-13 isotope discriminated values for single and multiple root-QTL pyramided lines

Sl#	Genotype	$\Delta^{13}C$	# of QTLs	Result	Sl#	Genotype	$\Delta^{13}C$	# of QTLs	Result
1	qrt 1-1	19.82	1		37	qrt1+7-18-3	17.22 ^{*1,7}	2	High WUE
2	qrt 1-2	19.64	1		38	qrt1+7-2-1	17.07 ^{*1,7}	2	High WUE
3	qrt 1-3	19.05	1		39	qrt1+7-2-3	18.45 ^{*1,7}	2	High WUE
4	qrt 2-4	19.5	1		40	qrt1+7-25-2	19.98	2	
5	qrt 2-5	18.6	1		41	qrt1+7-25-3	20.04	2	
6	qrt 2-6	19.42	1		42	qrt1+7-25-4	19.77	2	
7	qrt 2-7	19.09	1		43	qrt2+7-10-1	17.06 ^{*2,7}	2	High WUE
8	qrt 2-8	19.19	1		44	qrt2+7-10-2	19.59	2	
9	qrt 7-9	19.33	1		45	qrt2+7-14-2	19.29	2	
10	qrt 7-10	18.98	1		46	qrt2+7-14-4	17.30 ^{*2,7}	2	High WUE
11	qrt 7-11	18.8	1		47	qrt2+7-15-2	16.70 ^{*2,7}	2	High WUE
12	qrt 7-12	18.3	1		48	qrt2+7-15-4	17.30 ^{*2,7}	2	High WUE
13	qrt 7-13	19.39	1		49	qrt2+7-15-5	17.82 ^{*2,7}	2	High WUE
14	qrt 7-14	19.42	1		50	qrt2+7-26-2	18.43 ^{*2,7}	2	High WUE
15	qrt 7-15	18.98	1		51	qrt2+7-26-5	17.51 ^{*2,7}	2	High WUE
16	qrt 1+7-16	19.59	2		52	qrt2+7-27-1	16.76 ^{*2,7}	2	High WUE
17	qrt 1+7-17	18.42 ^{*1,7}	2	High WUE	53	qrt2+7-27-2	16.60 ^{*2,7}	2	High WUE
18	qrt 1+7-18	18.66 ^{*1,7}	2	High WUE	54	qrt2+7-9-2	16.41 ^{*2,7}	2	High WUE
19	qrt 1+7-19	19.04 ^{*1}	2	High WUE	55	qrt2+7-9-4	16.30 ^{*2,7}	2	High WUE
20	qrt 1+7-20	19.9	2		56	qrt2+9-19-1	18.48 ^{*2,9}	2	High WUE
21	qrt 1+7-21	19.88	2		57	qrt2+9-19-2	18.41 ^{*2,9}	2	High WUE
22	qrt 1+7-22	19.28 ^{*1}	2	High WUE	58	qrt2+9-19-3	18.17 ^{*2,9}	2	High WUE
23	qrt 1+7-23	18.94 ^{*1,7}	2	High WUE	59	qrt2+9-19-4	18.62 ^{*2,9}	2	High WUE
24	qrt 9-24	19.66	1		60	qrt2+9-19-5	19.23	2	
25	qrt 9-25	19.37	1		61	qrt7+9-6-1	16.66 ^{*7,9}	2	High WUE
26	qrt 9-26	19.58	1		62	qrt7+9-6-5	16.47 ^{*7,9}	2	High WUE
27	qrt 9-27	19.03	1		63	qrt7+9-7-2	21.07	2	
28	qrt 9-28	19.22	1		64	qrt7+9-7-3	20.64	2	
29	qrt 9-29	19.52	1		65	qrt7+9-7-5	20.86	2	
30	qrt1+7-1-1	18.50 ^{*1,7}	2	High WUE	66	qrt7+9-8-2	19.35 ^{*9}	2	High WUE
31	qrt1+7-1-3	20.35	2		67	qrt7+9-8-4	18.23 ^{*7,9}	2	High WUE
32	qrt1+7-16-4	15.68 ^{*1,7}	2	High WUE	68	qrt7+9-8-5	18.79 ^{*7,9}	2	High WUE
33	qrt1+7-16-5	18.78 ^{*1,7}	2	High WUE	69	qrt1+2+7-3-1	19.43 ^{*1}	3	High WUE
34	qrt1+7-17-2	20.07	2		70	qrt1+2+7-3-2	19.03 ^{*1,7}	3	High WUE
35	qrt1+7-17-5	18.88 ^{*1,7}	2	High WUE	71	qrt1+2+7-3-3	17.81 ^{*1,2,7}	3	High WUE
36	qrt1+7-18-1	18.31 ^{*1,7}	2	High WUE	72	qrt1+2+7-4-1	18.27 ^{*1,2,7}	3	High WUE
					73	qrt1+2+7-4-3	18.82 ^{*1,2,7}	3	High WUE

*1, 2, 7 and 9 marks increased WUE (lower c-13 value) over single QTL lines having QTL on chromosome 1, 2, 7 and 9, = equal WUE

a. Well watered condition

Plant height was highest in qRT 1+7-2-1 root-QTL pyramid and lower in qRT 2+7-10-1 pyramid. Number of tillers per plant was more in qRT1+7-25-3 root-QTL pyramid and less in qRT 7+9-20-2, qRT 1+7-16-3, qRT 1+7-1-5, qRT 1-2-4, qRT 1+2+7-4-2, qRT 1+2+7-4-3 and qRT 1+2+7-4-5. The overall mean of the genotypes for this trait was minimized when compared to checks. The maximum root length was recorded in qRT 1+7- 18-4 and minimum in qRT 2+9-19-2. The overall mean of the genotypes have shown that checks were having lesser mean values. Almost all the root-QTL pyramids had longer roots when compared to checks. There was a significant difference found in total root number. The higher root number was recorded in qRT 1+7-19 and lower in qRT 9-26, qRT 1+7-2-3. With respect to this trait, mean value of QTL pyramided genotypes were high compared to checks.

b. Low moisture stress condition

All the characters showed highly significant differences for mean performance of

the genotypes. But some of the QTL introgressed lines showed significant increase in all characters observed as compared to checks. Increase in plant height and number of tillers per plant was reported in comparison with checks. The same results were noticed by [17].

Water use efficiency/Carbon-13 (C-13) isotope discrimination

Carbon-13 isotope (^{13}C) discrimination in plant tissue has a potentially important role in the selection and breeding for drought tolerance via water use efficiency. A set of 79 lines out of which 29 single root QTL lines (NIL's), 44 QTL pyramided lines, 2 parents (IR64, Azucena) and 2 checks (Buddha, Rasi) were subjected for carbon -13 isotope discrimination [Table-5]. The best 44 QTL pyramided lines were selected based on their yield level and analysed for C-13 isotope. From the [Table-5], we can clearly see that some of the pyramids are best water users when compared to single QTL lines. The average value of QTL on chromosome 1 is 19.51 and QTL on chromosome 7 is 19.03, pyramids were compared with these

values. Those pyramids that have a lesser value than these values are considered to be high water use efficient genotypes. The most efficient genotype in this pool is qRT 1+7-2-1 having a C-13 value of 17.07. Out of 21 pyramids having QTL on chromosome 1 and 7, more than 60% of pyramids are showing increased performance over their parents or single QTL lines. This clearly depicts the additive nature of the QTLs in a single line. The comparisons were also made between single QTL lines (NILs) and root-QTL pyramids carrying QTL on chromosome 2 and 7. Out of 13 pyramids, 11 pyramids showed increased water use efficiency (WUE) over single QTL lines. The most efficient genotype is qRT 2+7-9-4 having C-13 value to the tune of 16.30. Likewise, the performance of the single QTL lines having QTL on chromosome 2 and 9 individually and in combination (2+9) was compared. Out of 5 pyramids, 4 pyramids are showing the lowered C-13 value and in turn depict high water use efficiency than single QTL

lines. Similarly, It is imperative from the table that some of the pyramids have shown increased WUE and some have not shown any increase between lines carrying QTL on chromosome 7 and 9. The best water user is qRT 7+9-6-5 (16.47) followed by qRT 7+9-6-1 (16.66), qRT 7+9-8-4 (18.23) and qRT 7+9-8-5 (18.79). Pyramid qRT 7+9-8-2 has shown increased performance over average of single QTL on chromosome 9. The same trend was observed when we compared the performance of single QTL lines with pyramids for 3 QTLs. All the 5 pyramids showed increased performance over single QTL lines. The best water user is qRT 1+2+7-3-3 (17.81) followed by qRT 1+2+7-4-1 (18.27) and qRT 1+2+7-4-3 (18.82) when compared to single QTL lines. qRT Pyramid qRT 1+2+7-3-2 (19.03) has also shown improved performance over QTL on chromosome 1 and equal performance over QTL on chromosome 7.

Table-5 Correlation coefficients among eight yield related traits studied in root-QTL pyramided and single root -QTL lines under well-watered (WW) and low moisture stress (LMS) in rice

[illegible]

* = Significant at 5% ** = Significant at 1%

RN = Root number

RV = Root volume (cc)

PHT = Plant height (cm)

RFW = Root fresh weight (g)

NOT= Number of tillers RL = Root length (cm)

RDW = Root dry weight (g)

Regression and correlation studies

A simple and multiple linear regression analysis was carried out to know how well independent variables influences dependent variables. The data points were plotted on a graph and a standard regression was drawn. The data points closer to the standard regression line have a stronger relationship between the independent and dependent variables. We can predict the dependent variable by studying deviation between regression line and independent variable value. The data obtained from well watered condition was subjected to regression analysis. There are seven independent variables viz., Plant height (PHT=X1), Number of tillers per plant (NOT=X2), panicle numbers per plant (PNO=X3), panicle length (PL=X4), grains per panicle (GPP=X5) sterility (STP=X6) and test weight (TW=X7) were regressed on a 8 dependent variable viz., yield per plant. The 50.6% of the variability in yield was explained by the above mentioned parameters. The R2 value of the analysis was $R^2 = 0.506$ and the multiple regression equation as follows.

$$\hat{Y} = 4.122 + 0.055X_1 + 0.309X_2 + 0.596X_3 - 0.182X_4 + 0.046X_5 - 0.118X_6 - 1.176X_7$$

From the above equation, it is imperative that, three of the independent variables viz., PNO (X3), GPP (X5) and STP (X7) have greater influence on the yield. Among these, STP is highly significant and PNO, GPP are significant. Stepwise regression analysis was performed to know more about these parameters. The STP, alone explains 27.3% variability out of 50.6% of variability ($R^2 = 0.273$), whereas STP and PNO explains 41.6% ($R^2 = 0.416$) of variability. The 47.8% of the variation in the dependent variable was explained by variations in these three dependent variables. The degree of influence and sign of the independent variable on the variation of dependent variable indicates the nature of the relationship. The coefficient values (in stepwise analysis) for STP, PNO and GPP are $\beta = -0.427$, $\beta = 0.359$ and $\beta = 0.259$, respectively. The higher value and negative sign indicates greater influence of independent variable. independent and dependent variable

move in an opposite direction, respectively [Fig-1].

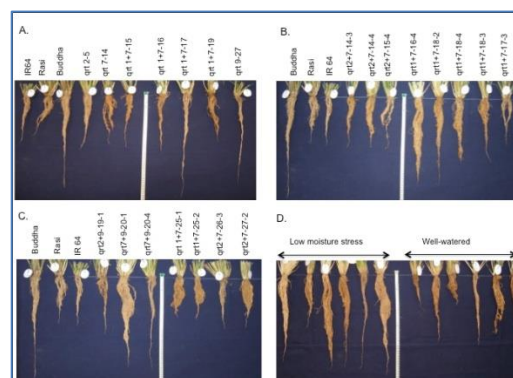


Fig-1 a) Root morphology of single QTL, b) and c) root morphology of root-QTL pyramids under LMS, and d) root growth in WW and LMS conditions

The data recorded under low moisture stress condition was subjected for regression analysis. The pooled analysis shows that 59.7% ($R^2 = 0.597$) of total variability in yield was explained by variability in 8 independent variables. The regression model for this pooled analysis is as follows.

$$\hat{Y}=11.54-0.021X_1+0.613^{**}X_2+0.036X_3-0.107X_4+0.033X_5-0.194^{**}X_6-6.05^{*}X_7-0.793X_8$$

From the above model, we can clearly see the significance effect of independent variables such as NOT=X2, STP=X6 and TW=X7. Two independent variables such as NOT and STP have shown a higher level of significance. These two parameters have greater influence on yield. The stepwise analysis revealed that

STP(X6) can explain 44.3% ($R^2=0.443$) out of 59.7% of variability in yield. From this we can infer that STP is the contributing variable for yield. The STP and TW can explain 48.8 % ($R^2=0.488$) out of 59.7% variability. The STP, TW and NOT altogether can elucidate 54.3% ($R^2=0.543$) out of 59.7% of variability. We can conclude that these three parameters have greater impact on yield when compared to other variables. The degree and direction of these three parameters influencing on yield is same as observed under WW condition. The coefficient values are $\beta=-0.744$, $\beta=0.243$ and $\beta=0.240$, respectively [Fig-2].

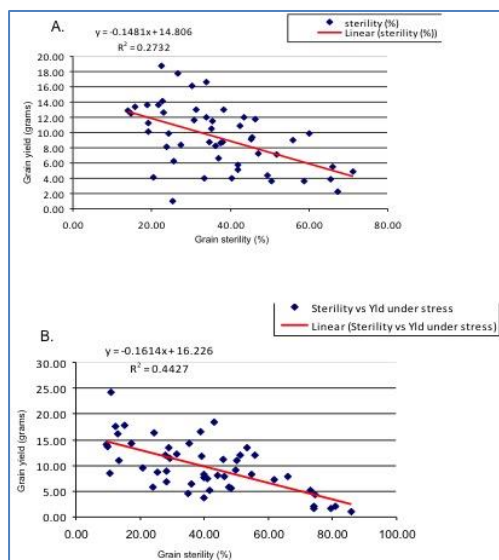


Fig-2 Relationship between yield and grain sterility measured under well-watered (WW) and low moisture stress (LMS) conditions. Grain yield was shown on 'Y' axis and sterility percent on 'X' axis

Correlation between yield and yield related parameters

Under well watered condition, grain yield per plant (GWT) was positively associated with plant height (PHT), number of tillers per plant (NOT), number of panicles per plant (PNO) and grains per panicle (GPP) [Table-6]. This understanding of association of characters helps in simultaneous selection of linked traits. Hence, selection for these traits would lead to increase in yield in an indirect way. There was a negative correlation between yield and panicle length. Panicle number was positively associated with panicle length, grains per panicle. So selection for number of panicles per plant would increase grains per panicle which in turn increases grain yield. Under low moisture stress (LMS), yield was found to be positively correlated with number of tillers per plant, 9 numbers of panicles per plant, grains per panicle and test weight (TW) but negatively correlated with plant height, panicle length and spikelet sterility percentage.

Correlation shoot and root related parameters

Among root-QTL introgressed lines, the association among root related and shoot related traits were worked out under contrasting water regimes [Table-7]. Under WW condition, plant height was positively correlated with number of tillers per plant (NOT), root length (RL), root number (RN), root volume (RV), root fresh weight (RFW), root dry weight (RDW). Number of tillers per plant was found to be positively correlated with root length, root number, root volume, root fresh weight and root dry weight. Maximum root length recorded positive association with root number, root volume, root fresh weight and root dry weight. Root volume was found to be associated with the root number, root, fresh weight, root dry weight; these results are in accordance with [17]. Under LMS condition, plant height was negatively associated with root volume. The number of tillers per plant was negatively associated with root number. From this study, we observed that a positive correlation exists among the root traits; the selection is based on any of the easily measured traits, which might provide breeders an opportunity to develop drought resistant upland rice variety.

Table-6 Correlation coefficients among seven shoot and root traits studied in root -QTL pyramided lines and single root -QTL lines under WW and LMS during vegetative stage in rice

Trait	WW	LMS	WW	LMS	WW	LMS	WW	LMS	WW	LMS	WW	LMS	WW	LMS
	PHT	NOT	RL	RN	RV	RFW	RDW							
PHT	1	1	0.31**	0.14	0.39**	0.085	0.32**	0.097	0.09	-0.183	0.36**	0.052	0.26**	0.078
NOT		1	1	0.23*	0.191*	0.37**	-0.015	0.23*	0.011	0.45**	0.076	0.38**	0.028	
RL			1	1	0.4**	0.286**	0.2*	0.182	0.49**	0.424**	0.24*	0.278**		
RN				1	1	0.23*	0.462**	0.6**	0.642**	0.4**	0.435**			
RV					1	1	0.42**	0.452**	0.39**	0.284**				
RFW						1	1	0.75**	0.782**					
RDW							1	1						

PHT = Plant height (cm)
 NOT = Number of tillers
 RL = Root length (cm)
 RN = Root number
 RV = Root volume (cc)
 RFW = Root fresh weight (g)
 RDW = Root dry weight (g)
 * = Significant at 5%
 ** = Significant at 1%

Table-7 Correlation coefficients of $\Delta^{13}C$ value among other traits studied in root QTL pyramided lines and single root -QTL lines under low moisture stress condition during reproductive stage of rice

Trait	PHT	NOT	PNO	PL	GPP	STP	TW	GWT	$\Delta^{13}C$
PHT	1.000	-0.068	0.142	0.614**	0.344*	-0.068	0.237	0.153	-0.343
NOT		1.000	0.172	0.072	-0.110	0.158	0.038	0.100	0.253
PNO			1.000	-0.001	-0.063	0.042	-0.220	-0.020	0.195
PL				1.000	0.230	0.102	0.148	-0.042	-0.179
GPP					1.000	-0.218	0.073	0.282	0.800
STP						1.000	0.210	-0.670	-0.271
TW							1.000	0.100	0.113
GWT								1.000	0.188
$\Delta^{13}C$									1.000

PHT= Plant height (cm)
 NOT= Number of tillers per plant
 PNO= Number panicle per plant
 PL= Panicle length (cm)
 TW= Test weight (g)
 GWT= Grain weight (g)
 $\Delta^{13}C$ = Carbon 13 isotope value.
 GPP= Grains per panicle
 STP= Spikelet Sterility per cent
 * = Significant at 5%
 ** = Significant at 1%

Correlation between Carbon-13 isotope (^{13}C) discrimination (WUE) and yield related traits

The ^{13}C was found to be negatively correlated with plant height (-0.343), panicle length (-0.179), spikelet sterility percentage (-0.271), therefore it is positively correlated with WUE. A positive correlation was recorded with a number of tillers

(0.195), grains per panicle (0.080), test weight (0.113) and grain weight (0.188), therefore it is negatively correlated with WUE [Table-8]. This association analysis clearly depicts the strong inverse relationship between ^{13}C and WUE. So those traits which have negative correlation with ^{13}C have a positive relationship with WUE and vice versa.

Table-8 List of single root-QTL lines, pyramided root-QTLs and checks used in the experiment

Sl.No	QTL Lines Nomenclature	QTL on chr #	Sl.No	QTLs Lines Nomenclature	QTL on chr #	Sl.No	QTL Lines Nomenclature	QTL on chr #
1	qrt 1-1	1	41	qrt1+2+7-4-4	1+2+7	81	qrt1+7-16-4	1+7
2	qrt 1-2	1	42	qrt1+2+7-4-5	1+2+7	82	qrt1+7-16-5	1+7
3	qrt 1-3	1	43	qrt7+9-6-1	7+9	83	qrt1+7-17-1	1+7
4	qrt 2-5	2	44	qrt7+9-6-2	7+9	84	qrt1+7-17-2	1+7
5	qrt 2-6	2	45	qrt7+9-6-3	7+9	85	qrt1+7-17-3	1+7
6	qrt 2-8	2	46	qrt7+9-6-4	7+9	86	qrt1+7-17-4	1+7
7	qrt 7-10	7	47	qrt7+9-6-5	7+9	87	qrt1+7-17-5	1+7
8	qrt 7-11	7	48	qrt7+9-7-1	7+9	88	qrt1+7-18-1	1+7
9	qrt 7-12	7	49	qrt7+9-7-2	7+9	89	qrt1+7-18-2	1+7
10	qrt 7-14	7	50	qrt7+9-7-3	7+9	90	qrt1+7-18-3	1+7
11	qrt 7-15	7	51	qrt7+9-7-4	7+9	91	qrt1+7-18-4	1+7
12	qrt 1+7-16	1+7	52	qrt7+9-7-5	7+9	92	qrt1+7-18-5	1+7
13	qrt 1+7-17	1+7	53	qrt7+9-8-1	7+9	93	qrt2+9-19-1	2+9
14	qrt 1+7-18	1+7	54	qrt7+9-8-2	7+9	94	qrt2+9-19-2	2+9
15	qrt 1+7-19	1+7	55	qrt7+9-8-3	7+9	95	qrt2+9-19-3	2+9
16	qrt 1+7-20	1+7	56	qrt7+9-8-4	7+9	96	qrt2+9-19-4	2+9
17	qrt 1+7-21	1+7	57	qrt7+9-8-5	7+9	97	qrt2+9-19-5	2+9
18	qrt 1+7-22	1+7	58	qrt2+7-9-1	2+7	98	qrt7+9-20-1	7+9
19	qrt 9-24	9	59	qrt2+7-9-2	2+7	99	qrt7+9-20-2	7+9
20	qrt 9-25	9	60	qrt2+7-9-3	2+7	100	qrt7+9-20-3	7+9
21	qrt 9-26	9	61	qrt2+7-9-4	2+7	101	qrt7+9-20-4	7+9
22	qrt 9-27	9	62	qrt2+7-9-5	2+7	102	qrt7+9-20-5	7+9
23	qrt1+7-1-1	1+7	63	qrt2+7-10-1	2+7	103	qrt1+7-25-1	1+7
24	qrt1+7-1-2	1+7	64	qrt2+7-10-2	2+7	104	qrt1+7-25-2	1+7
25	qrt1+7-1-3	1+7	65	qrt2+7-10-3	2+7	105	qrt1+7-25-3	1+7
26	qrt1+7-1-4	1+7	66	qrt2+7-10-4	2+7	106	qrt1+7-25-4	1+7
27	qrt1+7-1-5	1+7	67	qrt2+7-10-5	2+7	107	qrt1+7-25-5	1+7
28	qrt1+7-2-1	1+7	68	qrt2+7-14-1	2+7	108	qrt2+7-26-1	2+7
29	qrt1+7-2-2	1+7	69	qrt2+7-14-2	2+7	109	qrt2+7-26-2	2+7
30	qrt1+7-2-3	1+7	70	qrt2+7-14-3	2+7	110	qrt2+7-26-3	2+7
31	qrt1+7-2-4	1+7	71	qrt2+7-14-4	2+7	111	qrt2+7-26-4	2+7
32	qrt1+7-2-5	1+7	72	qrt2+7-14-5	2+7	112	qrt2+7-26-5	2+7
33	qrt1+2+7-3-1	1+2+7	73	qrt2+7-15-1	2+7	113	qrt2+7-27-1	2+7
34	qrt1+2+7-3-2	1+2+7	74	qrt2+7-15-2	2+7	114	qrt2+7-27-2	2+7
35	qrt1+2+7-3-3	1+2+7	75	qrt2+7-15-3	2+7	115	qrt2+7-27-3	2+7
36	qrt1+2+7-3-4	1+2+7	76	qrt2+7-15-4	2+7	116	qrt2+7-27-4	2+7
37	qrt1+2+7-3-5	1+2+7	77	qrt2+7-15-5	2+7	117	IR 64	
38	qrt1+2+7-4-1	1+2+7	78	qrt1+7-16-1	1+7	118	Azucena	1+2+7+9
39	qrt1+2+7-4-2	1+2+7	79	qrt1+7-16-2	1+7	119	Rasi	
40	qrt1+2+7-4-3	1+2+7	80	qrt1+7-16-3	1+7	120	Buddha	

Discussion

Development of NILs and QTL pyramiding

The development and use of near isogenic lines (NILs) are more effective for gene/QTL pyramiding. The transfer of several QTLs to common genetic background by conventional breeding methods is a tedious process and involve lot of time [28] and phenotypic selection of segregating progenies [29]. The marker assisted backcrossing approach plays a major role in gene pyramiding since it increases the selection efficiency and reduces the breeding cycles. The four QTLs for deeper roots from drought tolerant genotype (Azucena) were transferred to the high yielding lowland variety IR64 [24] to develop NILs. These NILs were used to develop 94 root-QTL pyramided lines and during development, QTL region was selected by linked SSR markers. Important QTLs of rice pertaining to biotic stress, abiotic stress and grain quality have been identified and effectively deployed in marker assisted selection (MAS) [30]. QTLs conferring resistance to diseases like blast [31]; bacterial blight [32,33] and gall midge resistance QTLs 10 [34] have been efficiently pyramided. This is one such effort to develop rice genotypes that survive and yield well under water scarce situation. Performance of root-QTL pyramided lines for the shoot and yield related parameters indicated that though it is possible to develop the pyramided

genotypes the genetic interactions cannot be avoided in our study [35,36]. We evaluated the performance of NILs, root-QTL pyramids, parents (IR64, Azucena) and checks (Rasi, Buddha) under contrast moisture conditions. The results of the analysis of variance of various shoot and yield related traits under WW and LMS condition during vegetative and reproductive phase revealed significant differences among these genotypes indicating the variation due to the stacking of the genes and QTLs. Significant differences among NILs and root QTL pyramids for non-target traits like PHT, NOT, PL, GPP, STP, GWT under WW condition was observed indicating the residual effect of the donor linked traits. This difference in phenotypic responses could be attributed to the effect of QTLs combinations on the four chromosomes in new genetic background (IR64) [37].

Evaluation for root morphology and WUE

The root system is a fundamental organ for plants and helps in anchoring the plant, water uptake, nutrient uptake, storage organs, and secretes lot of plant growth promoting substances. We have evaluated NILs and root-QTL pyramids along with parents and checks during the vegetative phase of crop growth for various root morphological parameters in green house condition. The analysis of variance showed significant differences among NILs and root-QTL pyramids for

PHT, NOT, RL, RN, RV, RFW and RDW under contrasting water regimes. The uptake of water and nutrients by roots and transporting the same to the aerial parts of the plant is the most important phenomenon as it affects yield, especially under low moisture stress condition [16]. We have observed non-significant differences for RL between checks and NILs and QTL pyramids under WW condition. This could be due to absence of abscisic acid production in the presence of sufficient water around the root zone. Whenever the plant experiences low moisture stress, ABA pathway triggers and root development becomes more prolific. The same results were reported by [38] in rice variety TN-1. Individual root parameters like root thickness, root depth and root penetrate has been associated with drought avoidance [39]. It was suggested that increased root depth and root density in rice increases the water extraction capacity from deeper layers of soil and may contribute to increased drought avoidance in some rice 11 genotypes. We performed correlation among root related traits like RL, RN, RV, RFW and RDW. We observed that a positive correlation exists among the root traits, the selection based on any of the easily measurable trait might provide breeders an ample opportunity to develop drought resistant upland rice variety. We selected and subjected 29 NILs and 44 root-QTL pyramids for carbon 13 isotope discrimination based on their yield performance. In general, a wide range of variability was noticed for leaf $\delta^{13}C$ composition from 14.765 to 21.067 and showed a strong inverse relationship with water use efficiency (WUE). Analogous results were also reported by [23,40]. Among all genotypes, Azucena recorded lowest $\delta^{13}C$ value indicating high WUE, when compared to IR64 exhibiting its water use efficient capacity. Whereas, IR64 recorded high value of $\delta^{13}C$, depicting lower WUE. This clearly implies that Azucena is drought tolerant and IR64 is drought susceptible. Due to this reason, Azucena was used as one of the parent in detecting QTLs for drought. Among pyramided lines, lowest $\delta^{13}C$ was recorded in qRT1+7-16-4 and highest in qRT7+9-7-2. This indicated qRT1+7-16-4 genotype had high WUE when compared to qRT7+9-7-2 genotype. Overall the results of the QTL pyramids for root morphological characters indicated that the two QTL combinations with 1+7 combination was performing superior under stress condition as compared to other parents or other combination and was superior than three QTL pyramids. It is possible that there exists both additive gene interaction and epistatic interaction when QTL are brought together in a single genotype. Pyramids also exhibited stress response as compared to well water situation. This indicates that genes underlying root QTL are operative and function during moisture stress situation rather than in well watered condition. Hence pyramiding genes underlying QTL are useful for exploitation in developing superior genotypes for combating the drought situation in rice.

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Conflict of Interest: None declared

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