IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR ROOT AND YIELD RELATED TRAITS IN RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Root system is one of the important components of drought tolerance. Improvement our understanding of interaction between root and drought in rice could have a significant impact on global food security. Marker-trait association were studied for eleven yield and root traits using 17 polymorphic SSR markers with a set of 24high yielding and 24 low yielding F4 population along with two parents (IR84984-83-862-B x MTU1010) of rice. Out of 17 SSR polymorphic markers five SSR markers show association with QTL. Three markers associations for root traits, viz. root length, root volume and root diameter followed by two markers associated with yield characters like plant height, flag leaf length and panicle length. RM3825 was found associate with total root length after root scanning with WinRhizosoftware. RM431 and RM 28076 associated with root volume and average root diameter respectively, while RM 28048for plant height and flag leaf length and RM 28130 related for panicle length and the plant height. Moreover, phenotypically these characters have more association with each other. Hence these markers may be useful for marker assisted breeding programme.

INTRODUCTION

Rice (*Oryza sativa* L.) is a "Global Grain" (Shalini and Tulsi, 2008 and Reddy *et al.,* 2013) cultivated widely across the world feeding millions of mankind. Rice is a model plant with a small completely sequenced whole genome in cereal crops. Grain yield is major important component for breeder and farmers. Grain yield is a complex character and is controlled by many factors. Roots play a key role in absorbing water and nutrients essential for the plant (Devendra *et al.,* 2015). The relationships between root growth and grain yield under drought are complex. Positive associations between root length and grain yield have been documented in rice (Mambani and Lal, 1983a; Lilley and Fukai, 1994). In contrast, Ingram *et al.* (1994) found no significant association between the two traits. It may be that a simple correlation between root growth and yield could be expected only in well-defined target environments root traits are generally controlled by many genes through quantitative trait loci (QTL). Since the first study by Champoux et al. (1995) to locate genes controlling rice root traits with molecular markers, many QTLs related to root traits have been identified in rice using 12 different mapping populations, with QTLs, identified and analyzed in rice for more than 30 root morphological parameters. The most studied root traits in all QTL mapping studies are maximum root depth, root diameter, and root to shoot ratio. Root traits that result in improved plant water status through a stress-prone growing season could confer non-stage-specific drought resistance. For example, conventional breeding for root-related drought resistance in rice was conducted using farmer-participatory plant breeding approaches (Shashidhar, 2008).

However the Courtois *et al.* (2009) analyzed the conditions of detection of 675 QTLs in 24 studies conducted on 12 different populations. Yue *et al.* (2006) reported that genetic linkage map consisting of 245 SSR markers was constructed for mapping QTL for these traits. Total of 27 QTLs were identified for 7 traits of relative performance of yield, 36 QTL for 5 root traits under control, and 38 for 7 root traits under water stress conditions. Till date, many QTLs have been reported in rice, including those responsible for yield , roots and grain quality; salt, drought and submergence tolerance; disease and insect resistance and nutrient utilization efficiency. Map-based cloning techniques have enabled scientists to successfully fine map and clone approximately 17 QTLs for various traits. (Hao and Lin, 2010).

In this study, F4 population was developed from the cross of IR84984-83-862-B one of the drought-tolerant breeding lines, was used as the donor and the recipient variety was MTU1010 (Cottondora Sannalu). Objectives of this study were to evaluate the root and yield characteristics of thispopulation at terminal stage drought (TSD) conditions based on the analysis of genotypic and phenotypic data.

MATERIALS AND METHODS

Experiments were conducted at the Indira Gandhi Agricultural University Raipur, in kharif 2014 and summer 2015 is situated at latitude 14º13'N and longitude 121º15'E. The performance of genotypes in Rainout shelter screens at IGKV has been reported to be correlated with wet-season screens.

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Population development

In this present investigation, a set of IR84984-83-862-B one of the drought-tolerant breeding lines, short duration (90-95 days) was used as the donor and MTU1010 is a semi-dwarf high-yielding medium-duration (112-118 days) variety developed at the Regional Agricultural Research Station, Maruteru, Andhra Pradesh, India. It is grown in large areas in central, southern, and eastern India. By using these two parents the population was developed. Single seeds from each F2 plant were selected and F3 seeds obtained were grown under TSD (Terminal Stage Drought) and harvested individually. Two hundred Twenty plants from the population were harvested and grown in KH- 2014 wet season.

Selection of lines on the basis of phenotypic data

The selection of 220 lines was on the basis of yield data. The 220 lines were grown under TSD condition and the data were recorded on five randomly selected plants for yield traits viz, plant height, flag leaf length, width, panicle length, number of tillers, biological yield and root pulling strength (RPS) using root puller machine. After recording data for all above mentioned traits on that basis the 48 lines were selected containing each of 24 high yielding and low yielding that were again screened in summer 2015 for all yield and root traits.

Root Rhizotron study

Root characters were also studied in rhizotron and thinned to one when they have emerged. At the end of the experiment, roots were washed and then scanned for analysis of root traits using software WinRhizo. (fig1 and Table 1)., viz root length, root volume, surface area, root diameter were used for QTLs analysis.

Molecular Studies

For developing genotypic data based on SSR markers, DNA was extracted from fresh leaf with the help of MiniPrep method (Doyle and Doyle, 1987). Polymerase chain reaction (PCR) amplification was performed in a total volume of 20 μl and the reaction mixture contained 10 X Assay buffer, 1 mMdNTP mix, 5 pM forward and reverse primers, 40 ηg of template DNA and 1 unit *Taq* polymerase in Applied Biosystems thermal cycler. After an initial denaturation step of 95°C for 5 min, the amplification was carried out for 34 cycles comprising 1 min each of 94°C, 55°C and 72°C. The final elongation step was extended to 7 min at 72°C followed by 4°C.

After the PCR reaction was completed, the PCR products were resolved on 5% PAGE in a mini-vertical electrophoresis system (CBS scientific, model MGV-202-33). DNA fragments were then stained with ethidium bromide and visualized with a UV trans-illuminator Bio-rad XLR +

Genotyping of 48 lines was done using 17 polymorphic (out of 41 tested) listed in table no 3 SSR markers in Molecular Markers laboratory. The banding pattern was scored as A, B and H for female, male and heterozygous banding pattern, respectively. These markers were quite evenly distributed across the entire twelve chromosomes.

Identification of significant markers

The phenotypic and genotypic data was analyzed using Single marker analysis (t-test) for identification of markers which is associated with root length, volume, average diameter and yield traits.

RESULTS AND DISCUSSION

Selection for root traits under drought is an efficient criterion for improving the drought tolerance of rice. Recently, some drought-tolerant rice varieties have been developed using this selection criterion and successfully released for cultivation in drought-prone target environments. The process can be made more efficient and rapid through marker-assisted breeding, a well-known fast-track approach in crop improvement (Krishna Kumar Mishra *et al.*, 2013). In present investigation rice cultivar IR84984-83-862-B used as a drought-tolerant donor, out-yielded susceptible parents, MTU1010, under TSD in kharif 2014. The two hundred twenty plants were screened for Yield and root traits under drought condition. Out of 220 plants select 24 high yielding and 24 low yielding lines depends on

Table 1: Observations under Root Rhizotron

S.N.	Trait	Stage and Observation
1	Plant Height	60 DAS
2	Number of Leafs	60 DAS
3	Total tillers	60 DAS
4	Root length (WinRhizo)	60 DAS
5	Root volume (WinRhizo)	60 DAS
6	Root surface area (WinRhizo)	60 DAS
7	Root dry weight	60 DAS
8	Shoot dry weight	60 DAS

Figure 1: Root studies under soil filled glass Rhizotron

Table 2: Discriptive statistics for various yield and root Traits

PARAMETRE	HY PHT	LY PHT	HY FLL	LY FLL	HY FLW	LY FLW	HY PL	LY PL	HY NT	LY NT	HY BY	LY BY	HY RL	LY RL	HY SRL	LY SRL	HY RV	LY RV	HY SA	LY SA	HY AD	LY AD
Average	118.4	89.2	25.5	20.6	1.3	1.12	23.3	20.2	12.8	11.5	546.9	337.3	55.3	48.0	462.6	436.7	0.5	0.40	105.6	78.3	0.25	0.19
Standard Error of Mean	3.7	3.4	0.9	0.8	0.0	0.03	0.4	0.6	0.6	0.6	28.3	30.6	1.0	1.4	4.2	2.5	0.0	0.04	7.6	8.2	0.01	0.00
Standard Deviation	17.2	16.8	4.2	4.1	0.1	0.16	1.9	2.9	3.0	3.0	133.0	149.9	4.9	6.4	20.7	11.5	0.2	0.20	37.4	38.5	0.06	0.01
Variance	296.5	283.8	18.0	17.2	0.0	0.03	3.6	8.2	8.8	8.7	17680.4	22476.3	24.0	40.8	430.3	133.0	0.1	0.04	1402.3	1480.9	0.00	0.00
Coefficient of Variation	14.5	18.9	16.6	20.1	11.2	14.62	8.1	14.2	23.1	25.7	24.3	44.4	8.9	13.3	4.5	2.6	46.9	49.65	35.5	49.1	22.31	6.68
Range	73.0	66.6	16.8	20.3	0.6	0.78	7.5	14.6	10.0	11.6	469.0	587.0	24.0	23.0	73.0	53.4	0.8	0.80	118.3	147.8	0.20	0.05
Minimum Value of sample	69.0	47.2	15.9	9.5	1.0	0.54	18.4	11.1	8.8	7.4	326.0	142.0	43.0	33.0	421.1	421.1	0.2	0.10	56.9	27.3	0.18	0.17
Maximum Value of sample	142.0	113.8	32.7	29.8	1.6	1.32	25.9	25.7	18.8	19.0	795.0	729.0	67.0	56.0	494.1	474.5	1.0	0.90	175.1	175.1	0.38	0.22
Total	2605.0	2141.2	561.1	495.4	28.2	26.76	512.7	484.8	281.6	276.0	12031.0	8096.0	1327.0	1057.0	11101.4	9607.2	11.9	8.70	2533.4	1723.3	6.04	4.27

HY-High Yield, LY-Low Yield, PHT-Plant Height, FLL-Flag leaf length, FLW-Flag leaf width, PL-Panicle Length, NT-No. of Tillers, BY-Biological Yield, RL-Root Length, SRL-Root Length after scanning, RV-Root Volume, SA-Root Surface Area, AD-Root Average Diameter.

Root pulling strength (RPS) and the Harvest index (HI). In summer 2014 those selected lines 24 each sown in field and recorded yield traits,and grown in rizotron for root studies simultaneously and also analyzed using the descriptive statistics (Table 2).

As the literature says that root length and diameter distribution are important characteristics to be considered when describing and comparing root systems.Ekanayake*et al* (1985)reported a significant positive correlation across diverse rice genetic materials between root pulling force and dehydration avoidance as expressed in leaf water status maintenance and visual scored of drought resistance under severe drought stress in the field. Long fibrous roots have long been recognized as an important dehydration avoidance mechanism in rice and such roots evidently also ascribe stronger anchorage and greater resistance to pulling force.

Analysis of marker-trait association

The association between trait and markers were identified using single marker analysis (SMA). DNA markers that show specific molecular weight bands in the selected genotypes across the total population were genetically linked to the loci determining the quantitative trait.Marker trait association was identified in both high yielding and low yielding lines. The result revealed that the two markers marker RM3825 and RM431 was link to root length and root volume in high yielding lines present on chromosome #1 while the marker RM28076 was associated with average root diameter in low yielding lines on chromosome #12. No one marker was identified with root length in low yielding lines. This result suggests that, denser the root system of plant ultimately give the higher yield means the roots of plant play important and beneficial role in increase in rice yield. Because the roots of plants absorb the soil nutrients so as to increase the rate of photo assimilation towards the sink. The marker RM 28048 was associated with plant height and flag leaf length and RM28130 was show the association with plant height in high yielding and low yielding lines respectively that enhances yield under drought.Krishna Kumar Mishra et al, 2013 reported a large effect of qDTY12.1 under lowland RS vis-à-vis upland RS reported earlier by Bernier *et al.* (2007) in aVandana × Way Rarem population. He reported that qDTY12.1 is found to be located onchromosome 12 flanked by markers RM28089 andRM28199, whereas the QTL interval in anearlier study in a Vandana × Way Rarem population wasRM28048 to RM511. The consensus region between thetwo studies was RM28099-RM511. This intervalcould be very important for further genomic studies related to qDTY12.1.

In this study, IR84984-83-862-B accessions have contributed as a drought-tolerant donor enhancing alleles for several root related traits in the background of popular rice variety MTU1010, indicating their potential use in yield improvement programmes.

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Table 3: Markers used for the analysis

Sr no	SSR markers	ChroNo.	Forward	Reverse
1	RM 212	1	CCACTTTCAGCTACTACCAG	CACCCATTTGTCTCTCATTATG
2	RM 3825	1	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
3	RM 431	1	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC
4	RM 242	1	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG
5	RM 11943	1	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC
6	RM 18616	5	TCTAGGCAGTTGGTGTAACTCAGTGG	AACTCAAGTCTCAAGCCATCTACAGG
7	RM 18621	5	GGCATGCATGTGTCTAACATTCG	AAGCAGAATTTGGCCGTGTTAGC
8	RM 18639	5	CATCATGTGGTAAGTGTGCAACG	GGTTGCGATGAGATTACGAGACC
9	RM 18640	5	AGGGAGATAGGCACACGAAAGC	ACCAACAACTCCTTGGCTTCTCC
10	RM 28048	12	TTCAGCCGATCCATTCAATTCC	GCTATTGGCCGGAAAGTAGTTAGC
11	RM 28076	12	GGGACTTGGGACCAGTTTATGG	TCAGGTCTGTTGGATTCCATGC
12	RM28086	12	CCCGCTGCAGCAGTTTATTGAGG	GATCTGGTACCTGCATGGGTTGC
13	RM 28099	12	TGTGCGGATGCGGGTAAGTCC	CCACCTGTCAACCACCGAAACC
14	RM 28130	12	CAGCAGACGTTCCGGTTCTACTCG	AGGACGGTGGTGGTGATCTGG
15	RM 7195	12	CGCGTGAGAGCTCCTAAAAG	TCCTTGTGTAACTACCGCCC
16	RM 511	12	CTTCGATCCGGTGACGAC	AACGAAAGCGAAGCTGTCTC

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