

Original Research Article

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Identification of QTLs and Markers Linked to Root Traits in Rice (*Oryza sativa* L.)

Sahoo Abinita¹, R. Gudadhe Kalyani¹, Sunil K. Verma², Ritu R. Saxena²,
Satish B. Verulkar¹ and Ambika Prasad Mishra^{3*}

¹Department of Plant Molecular Biology and Biotechnology, ²Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C. G.), India

³Department of Soil Science & Agricultural Chemistry, Odisha University of Agriculture & Technoly, India

*Corresponding author

ABSTRACT

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Root is an important plant part mainly responsible for absorption of water and nutrient, and hence root traits affect the plant growth and development under normal as well as under water stress and nutrient stresses. A RIL population consisting of 116 lines developed using a land race with deep and thick root was grown in mini-rhizotron for measuring root volume and root length. The genotypic data of this population was also developed using SSR markers. The QTL analysis leads to the identification of twenty four QTLs for root length and three QTLs for root volume, the markers linked to these traits were also identified.

Introduction

Rice (*Oryza sativa* L.) is the world's most important wetland food crop. Large areas of rice are grown under lowland and upland rainfed conditions. Compare to other cereals, rice requires more water. Drought is one of the most important and highly unpredictable abiotic stresses causing drastic reductions in yield under rainfed rice environments, affecting 20 % of the total rice growing area in Asia (Pandey and Bhandari, 2008). Root systems form one of the important components of drought resistance. Therefore,

improving our understanding of the interaction between root function and drought in rice could have a significant impact on global food security (Gowda *et al.*, 2011).

Root systems influence the amount of water available to the crop depending on their distribution in the soil (Hemamalini *et al.*, 2000). Every plant part has unique functions. Root has a large range of functions, including acquisition of water and nutrients, as well as structural support (Rebouillat *et al.*, 2009). It is also the site of major hormone biosynthesis (Courtosis *et al.*, 2009). Root length and

surface area are important indicators for a potential uptake of water and nutrients. Root volume indicates that a plant can permeate a particular volume of soil or that it has a proportion of thick or thin roots. Such a plant would have greater water gathering potential for growth and survival (Zurio-Altoveros *et al.*, 1990).

QTL mapping provides a powerful tool for conducting physiological and genetical research to understand and possibly improve drought resistance. It eases screening for traits that are difficult to quantify and influence by environmental stimuli (Hanson *et al.*, 1990). DNA markers have been successfully used for screening plant genomes for quantitative trait loci (QTLs) controlling complex traits, including tolerance to abiotic stresses (Ribaut *et al.*, 1997; Zhang *et al.*, 2001). Development of various types of DNA markers by different techniques has provided a new platform for rice genome research. Among the various types of markers available for genetic mapping, SSR has number of advantages over others. They are highly polymorphic, single locus, co-dominant and multi-allelic (Temnykh *et al.*, 2000). Considering the importance of root traits, this study was undertaken with the major objectives to develop precise phenotypic data and identify the QTLs for these traits.

Materials and Methods

Plant material

Recombinant inbred line population in F₁₃ generation having 116 lines were developed from a cross between Danteshwari and Dagad Deshi. Danteshwari is high yielding variety and moderately susceptible to water stress condition while Dagad Deshia local land race is tolerant to water stress condition with deep and thick root system.

Phenotyping

The plant material was grown in mini-rhizotron (soiled filled in between two glass-plates of 1'x1.5') at University Research and Instructional farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during the month of December to January to generate the phenotypic data. In each glass rhizotron three seeds were sown, later thinned to two when they emerged. Proper watering was done up to 60 days. After 60 days the glass plates were opened, soil removed carefully in water to get the intact root. The root was then again washed with water. The root was then scanned with root scanner to record total root length and root volume. It was done with the help of software WinRhizo root scanner.

Genotyping

DNA from 116 RILs along with parents was isolated using mini prep method (Doyle and Doyle, 1987). Each DNA samples were quantified on Nano Drop Spectrophotometry and diluted to 40 ng/μl. SSR and HvSSR primers were used for genotyping of this whole population. For DNA amplification, reaction mixture as given in table 1 was used and temperature profile as elaborated in table 2 was used for PCR amplification in an Applied Biosystem PCR machine. To each amplified PCR product, 3 μl of loading dye was added and then electrophoresed in 5% PAGE. After electrophoresis gels were stained with Ethidium Bromide for 5 minutes, washed with distilled water and photographed using gel doc unit (*BIO RAD*).

Results and Discussion

The different RIL lines along with parents exhibited significant variation for both the parameters i.e. root length and root volume. The lines with higher and lower root length and root volume are presented in table 3 & 4

respectively. The variation in the RIL population was expected as both the parents involved in the cross are different for these two traits. High root volume indicate that a plant can permeate a large volume of soil or that it has a high proportion of thick roots, such plants would have greater water gathering potential for growth and survival. The drought tolerant varieties having greater

root volume than drought susceptible varieties (Zurio-Altoveros *et al.*, 1990). Verma 2014, reported that the root studies using mini-rhizotron has significant correlation with performance of individual line under water stress condition. Similarly Price *et al.*, 2012 has also reported the utility of this type of morphological studies in understanding the rooting pattern (Table 5 and 6).

Table.1 PCR mixture for one reaction

Reagent	Stock concentration	Volume (µl)
Sterile and nanopure H ₂ O	-	13.5
PCR buffer	10 X	2.0
dNTPs (Mix)	1mM	1.0
Primer (reverse and forward)	5 pM	1.0
Taq polymerase	1 unit/ µl	0.5
DNA template	40 ηg/µl	2.0
Total		20.0

Table.2 Temperature profile used for PCR amplification using microsatellite markers

Steps	Temperature (°C)	Duration (min.)	Cycles	Activity
1	95	5	1	Denaturation
2	94	1	34	Denaturation
3	55	1		Annealing
4	72	2		Extension
5	72	10	1	Final Extension
6	4	24 hrs	1	Storage

Table.3 Lines with high and low root volume

S. No.	High root volume		Low root volume	
	Line No.	Root volume (cm ³)	Line No.	Root volume (cm ³)
1	31	0.65	1	0.37
2	50	0.61	30	0.34
3	53	0.87	33	0.34
4	58	0.70	46	0.19
5	60	0.73	71	0.17
6	61	0.73	73	0.20
7	77	0.60	81	0.13
8	99	0.59	85	0.30
9	106	0.61	101	0.16
10	108	0.66	107	0.03

Table.4 Lines with high and low root length

S. No.	High root length		Low root length	
	Line No.	Root length (cm)	Line No.	Root length (cm)
1	98	462.99	88	336.78
2	56	455.84	66	376.74
3	99	454.89	71	386.90
4	41	452.63	107	389.36
5	58	451.34	85	389.69
6	26	447.49	31	390.45
7	106	446.54	62	392.79
8	50	446.07	81	396.98
9	30	443.19	46	399.16
10	52	437.09	44	403.16

Table.5 List of QTLs identified for root length through scanning under mini-rhizotron condition

Chromosome no.	Left Primers	Right primers	LOD Value	Phenotypic variance %
C2	RM- 492	RM-475	4.4	5.106
C2	HvSSR2-78	RM-6375	4.0	1.6512
C3	RM-411	RM-85	3.9	0.5994
C4	HvSSR4-42	RM-564	4.3	2.082
C4	RM-348	RM-559	3.1	1.0838
C7	RM-481	HvSSR7-40	4.0	0.0158
C11	HvSSR11-1	HvSSR11-13	4.6	0.9716

Table.6 List of QTLs identified for root length through scanning under mini-rhizotron condition

Chromosome	Left primer	Right primer	LOD value	% phenotypic variance
1	RM 8071	RM 259	2.5	0.424
2	RM 492	RM 475	3.0	2.296
2	RM 109	HvSSR2-12	2.6	1.053

Fig.1 Genetic map locating three QTLs for root length under mini-rhizotron on C #2, 3, 4, 7, 11 by QTL cartographer 2.5

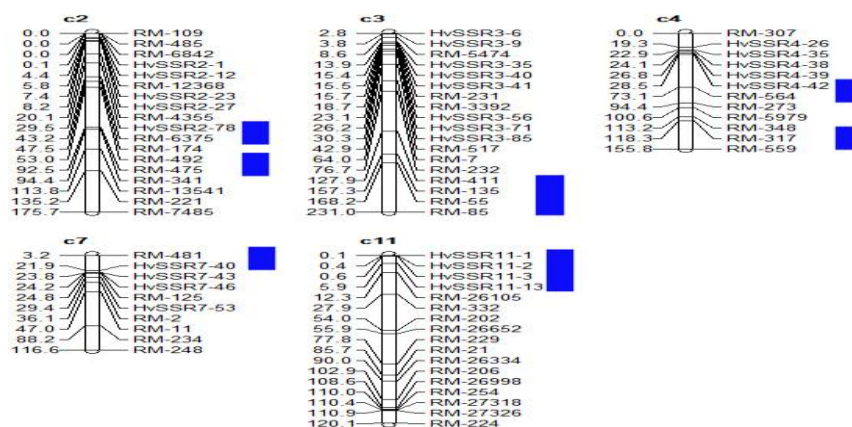
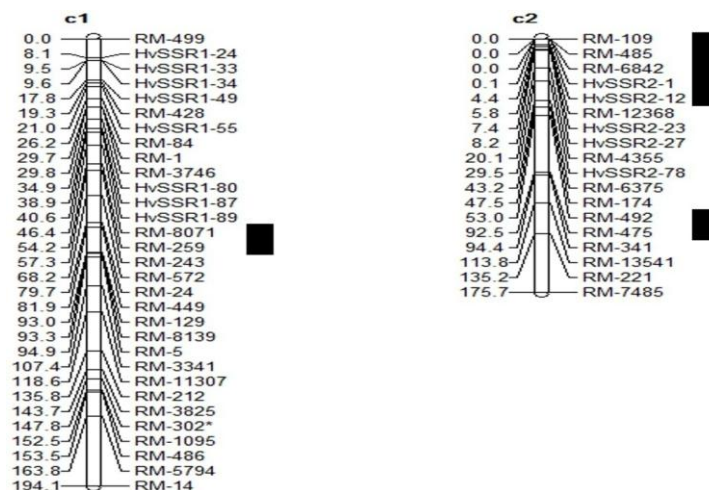


Fig.2 Genetic map locating three QTLs for root volume under mini-rhizotron on C #1 and C #2 by QTL cartographer 2.5



The DNA of 116 recombinant inbred lines along with the parents was subjected to PCR based simple sequence repeat (SSR) technique to generate genotypic data using rice SSR primers. The markers were taken from previously published rice genetic and sequence map (Singh *et al.*, 2009; McCouch *et al.*, 2002 and Temnykh *et al.*, 2001).

The phenotypic data along with genotypic data of 192 SSR and HvSSR marker was then used for QTL analysis using QTL Cartographer 2.5. Three QTLs were identified for root volume and 24 QTLs for root length. The result of QTL analysis is presented in figure 1 and 2. The perusal of figure 1 indicate that the identification of three QTLs for root volume which were located between RM 8071 to RM 259 on chromosome #1, with LOD score of 2.5, another QTL was present between RM 492 to RM 475 on chromosome #2 with LOD score of 3.0 and the next QTL was present between RM 109 to HvSSR2-12 on same chromosome #2 with LOD score of 2.6. Hemamalini *et al.*, (1990) identified two QTLs associated with root volume on chromosome 2 and 3 namely qRTV2-1 and qRTV3-1 respectively which shows the response at low moisture stress. Similarly for

root length QTLs were detected on chromosome 5 between RM 163 and RM 440; chromosome 6 between HvSSR 6-35 and HvSSR 6-44 with good LOD score of 5 or above was associated with root traits *i.e.*, total root length, and average root diameter (Verma 2014). QTL for root length on chromosome 6 (*qRL 6.1*) was also reported by Obara *et al.*, 2010. QTL for average diameter on chromosome 12 between HvSSR 12-51 to RM 277 was also associated with QTL for grain yield under rainfed condition. Root traits showed positive significant correlation under rainfed condition, so there QTL play role in water uptake. Bernier *et al.*, (2009a) reported *qtl12.1* on chromosome 12 which increase water uptake under upland condition. These QTLs affecting root length and root volume will be very useful in breeding program for developing high yielding drought tolerant rice varieties.

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