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Identification of QTLS for root and agronomic traits in rice under water stress condition conditions

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Abstract
DNA markers have enormous potential to improve efficiency and precision of conventional plant breeding via MAS. QTLs mapping studies for diverse crop species have provided an abundance of DNA marker trait associations. Two F3 populations with parental designation of Dagaddeshi (Land race) was tolerant to drought and MTU1010, is the most popular high yielding variety in central India, however is susceptible to water stress (Drought), and used for validation of root and yield related markers in kharif-2015. These populations were evaluated under stress and non-stress conditions, SSR linked markers within DTY QTL 1.1 present on chromosome #1, chromosome # 5. A total 40 SSR linked markers within DTY QTL 1.1 present on chromosome #1, chromosome # 5 and some markers adjacent to RM 242 regions on chromosome # 9 were used for validation. Among 40 markers 2 markers were linked to chromosome 1, 3 markers for chromosome no #5, 5 chromosomes for QTL on chromosome 9 adjacent to RM 242. A total of 10 markers were used for validation studies. The marker, RM242 showed 1% significant association with seedling height during the irrigated (transplanted) condition whereas flag leaf width showed 5% significant association and TSD (transplanted) RM242 showed 5% significant association with total root length.

Keywords: QTLs, SSR markers, drought tolerance, breeding populations

Introduction
Drought is a major abiotic stress that limits rice productivity in rainfed, upland ecosystems (Bimpong *et al*., 2011) [2] and worldwide, drought affects approximately 27 million ha of rainfed. In India, area under rice cultivation remained stagnant and even declined in the recent years due to water availability. Drought reduces yield by 15–50 per cent depending on the stress intensity and crop growth period at which the stress occurs in rice Verulkar *et al*., 2010) [12]. Plant response to drought stress is one of the most complex biological processes, and it involves numerous changes at the physiological, cellular, and molecular levels. Many genes have been identified to be involved in the response of drought stress in plants. Root traits are key component in rice plant adaptation to drought stress (Courtois *et al*., 2009) [3].

Root traits related to drought response are complex and controlled by many genes, each with a small genetic effect (Sharma *et al*., 2011) [9]. In recent years more attention has started to be given to mapping of QTLs for grain yield under managed stress environments Selection and breeding for desirable root traits associated with drought tolerance have been practiced in rice and the differential response of rice genotypes to drought has been related to root system characters (Steele *et al*., 2006, Kanbar and Shashidhar, 2011) [11, 7]. It is acknowledged that a deeper, thicker and more branched root system with a high root to shoot ratio can enhance the tolerance of rice to water deficits (Gowda *et al*., 2011) [5]. Among the root morphological traits, maximum root length, root dry weight, root volume, root to shoot weight and length ratios are associated with drought tolerance in upland rice (O'Toole 1981; Babu *et al*., 2003 and Kanbar *et al*., 2009) [8, 1, 7].

Materials and Methods
The parents of mapping population Danteshwari was moderately susceptible to drought, high yielding, dwarf, early high tillering, resistant to gall midge, long slender grain whereas Dagaddeshi (Land race) was tolerant to drought. The recombinant inbred lines (RILs) were developed by using modified single seed descent method (SSD) to F14 and F15 generation was used for identification of QTLs for root, yield and yield related traits as depicted. Another cross was made between the parents MTU1010 and Dagaddeshi during summer 2013.

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In Chhattisgarh, MTU1010, covers about 4, 43, 920 ht/area and is the most popular high yielding variety in central India, however is susceptible to water stress (Drought), leading to significant yield losses. The population from these two parents was made for validation of root and yield related trait study in further experiments.

Screening for drought tolerance

The observations for physiological traits contributing for yield were recorded at plant specific stage during their maximum tillering stage, vegetative stage, maturation stage and some important post-harvest observations were also recorded according to SES, IRRI 2002 for root, yield and its contributing traits. The fixed five plants were selected from each line and observations were recorded for all physiological traits.

Root studies under soil-filled glass rhizotrons

A set of selected lines based on phenotypic performance were grown for 45 days in thin rhizotron made up of transparent glass plates filled with a mixture of cocopeat (60%), sand (20%) and field soil (20%). In order to increase the number of roots growing along the rhizotron surface, rhizotrons were stored at an angle of ~15° (Table 3.2). After 45 days rhizotrons were open and plants were taken out. Root systems were separated from the shoot and cleaned from the substrate. Finer soil particles still attached to the root were removed using a small painting brush. Plant roots were stored in a 25% ethanol solution until the scanning procedure.

The scanning was performed with a custom flatbed scanner by placing a transparent acrylic made flat container over the surface of the scanner using Winrhizo software. Once the fragment was positioned, its lateral roots were carefully untangled (with a painting brush if needed). The scan was done with a 600 DPI resolution. The same procedure was repeated for every fragment of the root system. The final outputs of the scanning were therefore saved as an image and Microsoft excel data files for different root parameters. Detailed procedure is outlined below.

Materials used

- Plates of 4 mm thick clear glass cut to 450×300 mm.
- Soil sieved using a coarse sieve (approximately 5 mm mesh) to remove stones and large clumps.
- Duct tape, two straight 15 mm thick and 400 mm long wood, 15 mm thick plastic ring.

Method

- Two clean glass plates were taken. One was placed on a work surface with two of the four edges slightly overhanging. Two lengths of 15mm thick wood were placed on top of the first plate, a 15mm thick plastic ring was placed at the top and the bottom of the glass as spacers and then the second sheet of glass was placed over the top.
- Duct tapes were used to join the two sheets of glass together at the overhanging edges. The sheets were turned so that the remaining long edge was overhanging, and that was then sealed with duct tape. Three of the four sides were therefore completely sealed with duct tape.
- The empty rhizotrons was set vertical, a single strip of duct tape was wound right around the rhizotrons at the top and bottom, and the two lengths of wood were removed. The two plastic ring prevented glass from coming together and empty rhizotrons was stacked.

- One at the top ring was removed at a later date during the soil-filling process. The plastic ring at the bottom remained in the rhizotron. The empty rhizotrons was stood upon a soft support such as expanded polystyrene sheet and sieved soil was then encouraged into the rhizotrons. When the rhizotrons was nearly full, the upper ring was removed. When full, the rhizotron was lifted and then gently dropped onto the support, caused the soil level drop by 10-15 cm due to packing of the soil. The rhizotrons was refilled, gently dropped once more and refilled to within 5 mm of the top for a final time.
- A small drainage hole was made at each side at the bottom using a sharpened pencil.
- Rhizotrons were placed in stacks of eight and was leaned at an angle of 150 to encourage roots to grow on the lower face. The exposed face of the first stack was backed with an insulation sheet to reduce heat exchange and prevent light penetration.
- Three seeds were sown in each rhizotron and thinned to two when they have emerged. Proper watering was done up to 60 days.
- At the end, the rhizotrons was photographed with a high-resolution digital camera.
- At the end of the experiment, shoots were removed in a single day and dried to assess shoot dry weight.
- Roots were washed and then scanned for analysis using software WinRhizo, and dried to assess root dry weight.

Root washing

Materials required for root washing

Large plastic containers, 50 ml Tarson tube, metal forceps, small plastic container, markers for labelling and 25/50% ethanol.

Procedure for root washing

Tarson tubes for root samples were prepared first, with the exact sample name. This can be the most difficult and laborious step in the experiment. For root scanning the roots were washed with tap water two times.

Roots were preserved in ethanol solution (25%) in 50 ml Tarson tubes for root scanning. The procedure was conducted cautiously to prevent supplementary root damage and losses. Debris and dead roots were removed from vital roots.

Root scanning of rice lines

Root studies were followed according the root scanning protocol given by International Rice Research Institute (IRRI), Philippines. After 45 days, samples were collected for root scanning. Protocol used for root scanning (www.irri.org) under three steps (a) Root studies under soil filled glass rhizotrons (b) root washing and (c) root scanning 3.1.5.3.4 Materials used for root scanning Plastic forceps, Water, Plexiglas trays (Acrylic trays) for Win Rhizo Reg 2009 scanner (clean with no scratches), computer, Win Rhizo Reg 2009 USB key.

Procedure

Root scanning of selected rice lines

The stored roots of each line in two replicated were used for root scanning. The roots of three plants from each line were used for root scanning which given the detailed information about all root parameters including root length, root volume, root diameter, root tips, number of forks etc. The root scanning was done by using root scanner machine Epson Perfection V700/ V750, 3.81 Version, Win Rhizo Reg 2009,

the data was recorded automatically in the computer for different root parameters including root length, average root diameter, root volume, number of tips, forks, surface area etc.

Following procedure used for root scanning
a) Preparing Roots for Scanning
The acrylic trays were first washed with water and dried completely, the tray was filled with clear clean water and the roots were placed in tray. Roots were then floated in water in acrylic trays on the scanner, this allows the roots to be arranged so as to reduce overlap and crossing of roots. Plastic forceps was used tool for arranging the roots in specific manner, this is delicate work; good lighting and steady hands are helpful.

b) Scanning Roots
For best results, Win Rhizo Reg 2009 with an approved scanner used for root scanning, which allows the roots to be light from above and below while the root being scanned. This is an important feature (called "Dual Scan" in Regent's documentation), which reduces shadows on the root image. The Regent Positioning System allows the trays to be consistently placed, thus obviating the need to preview each scan. Optimum scanning resolution depends on the type of samples. Generally roots scanned at 600 dpi in 10x15 cm trays. Root length analysis was carried out with gray scale images.

c) The Right Threshold Value is Important
Analysis results can be sensitive to the threshold parameters used. Win Rhizo can automatically set these, one you may manually tweak them from time to time. The color traces on the root indicate where roots have been detected.

d) Analyzing Scanned Images
The image was analyzed by selecting the region of interest, and it is analyzed. When scanned images are analyzed, the software uses thresholding to determine what is root and what not root is. A few second later, the analysis was complete and roots found by Win RHIZO were identified by colored line in image. The colors used for drawing them are coded according to root diameter. Portions of the image can be excluded from analysis if necessary, and there are basic editing tools if minor image editing is required.

e) Save the measurement data
The last step of the analysis was data saving Win RHIZO knows when data was easily recordable by many programs including spread sheet style like Excel. Image and their analysis were also saving to file for later validation, reanalysis, or for visualization in other software programs.

Observations recorded under soil filled Glass rizhotron
The observations for physiological traits contributing for root traits were recorded at plant specific stage from each rhizotron plate. Two replicated plants from each line were taken for recording the observation such as Plant Height, Total number of tillers, Total root length, Fresh/dry weight of root and shoot (Table 3.2).

Table 1: Observations recorded after Root scanning using Win Rhizo software

S.N.	Trait	Stage and Observation
1	Average diameter (mm)	45 DAS
2	Total root length	45 DAS
3	Total root tips	45 DAS
5	Root volume (WinRhizo)	45 DAS
6	Total surface area (WinRhizo)	45 DAS

Total root length
Sum of the length of the entire roots (Primary, secondary, tertiary) present on a single plant; which was analyzed by Win Rhizo software after scanning of root in root scanner.

Root Volume: It is the area occupied by roots which is analyzed by Win Rhizo software after scanning of root in root scanner.

Root Diameter: Average diameter of all type of roots (Primary, secondary, tertiary) present in a single plant; which was analyzed by Win Rhizo software after scanning of root in root scanner.

Development of genotypic data using SSR and In-del DNA markers

Genomic DNA isolation
Total rice genomic DNA was extracted from single tagged plant during wet season 2014, by MiniPrep method (Doyle and Doyle, 1987) and used for PCR amplification to test the gene for yield contribution traits using the different functional/ gene tagged DNA markers. The step wise protocol for isolation is as follows:

1. Approximately 0.2gm of leaf sample from each plant was cut into small pieces with the help of sterile scissor and transferred into 2.0 ml micro centrifuge tube.

2. Once the sample was prepared 0.5 ml of extraction buffer was added and crushed using Tissue Lyzer (Mo Bio Laboratories ltd. Pawerlyzer TM 24).

3. After crushing, 600μl of chloroform: isoamyl alcohol (24:1) was added and gently vortex for 30 sec.

4. The tubes were centrifuged at 13000 rpm for 10 min. to separate the phases and the upper phase was transferred to new tube.

5. The chloroform extraction (step 4) was repeated one more time.

6. Two third volume of pre-chilled isopropanol was added and incubated at room temperature (or -200C) for 30 min. or longer until DNA precipitated.

7. DNA pellet was collected by centrifuging for 10 min. at 13000 rpm.

8. DNA pellet was resuspended in 50-100 μl of TE buffer and the pellet was allowed to dissolve. Stored at -200C until use.

Quantification of DNA
The DNA samples were quantified using Nanodrop Spectrophotometer (ND1000). Nucleic acid has maximum absorbance of ultra violet light at 260 nm. The ratio between the readings at 260nm and 280nm (OD 260 / OD 280) provides an estimate for the purity of nucleic acid. Pure preparation of DNA and RNA has a ratio of approximately

1.8 and 2.0, respectively. If there is contamination with protein or phenol the ratio will be significantly less than this value (< 1.8). A ratio greater than 2.0 indicates a high proportion of RNA in the DNA sample.

Procedure for quantification of DNA
Two µl of TE buffer was loaded on to the lower measurement pedestal and the sampling arm was lowered into the 'down' position and the blank was set. The buffer was wiped from both the pedestals using tissue paper or blotting paper. Then two µl of DNA sample was loaded on to the lower pedestal and reading showing the quantity of DNA was recorded. Both the measurement pedestal surfaces was cleaned with four µl of autoclaved distilled water and gently wiped with tissue

paper. Similar procedure was repeated for other DNA samples.

Dilution of DNA
The crude DNA after quantification was diluted to 50ng/ul with TE buffer. The diluted DNA was subsequently used for PCR amplification.

Polymerase Chain Reaction (PCR) Amplification
A set of primers located on different chromosomes of rice (Table 3.4) were used for PCR based DNA genotyping of RI lines and. The reaction mixture and the temperature profiles used were summarized in Table 3.5 and Table 3.6 respectively. 5% PAGE was performed to separate the PCR amplified products.

Table 2: List of DNA primers and their sequences used in the present investigation

S.N	Markers	Ch	Forward primer	Reverse Primer
1	RM3183	6	GTGGTGCTAGTATGGACGAGAGG	CGGTTGGTAGACTGTAAACAAAGTGC
2	RM547	8	TTGTCAAGATCATCCTCGTAGC	GTCATTCTGCAACCTGAGATCC
3	RM1369	6	CATCGATTAGCTTACATGGCAACG	ACTAGTGCGACCGTCTTCAATGG
4	RM3702	8	CCAGCACCATCTGAAATAGAAGC	CCATTGATTAGGACGGTCAAAGG
5	RM3845	8	TCGGATGAGTCCTTCGGTTTCC	TCTCAGCTCGATCTCCTCTCTAGACC
6	RM6990	8	ACTGGGTGTGATCCTTTCTGATGC	GTGATCCCAGATACACGATGTAGGG
7	RM3452	8	TGGACTTGGTCTCTCCAAACTCC	CAGTATGTGTTGGTGGGTCAAGC
8	RM214	7	GAACATGCTTTCAACCATCAGG	GATCCTCTCAGTTCAGTGCAAGC
9	RM3484	7	TCCGGTCGTCCTCATCGTATCC	GCCCTCTTGCTCCCACATCG
10	RM22418	8	GCAGTATCACGCAGTAGCACACC	CCATCCTCTTCCTCATCACACG
11	RM3394	7	GAGAGGGAAGGAGTTTCTTAGC	TAGTTTACACGTACCCATGTGC
12	RM3456	7	TTCTCCTTGCGACACAGATGAAAGG	GCCTCCTATATAAACCGGCGAACC
13	RM332	11	GAAGGCGAAGGTGAAGAAGAAGC	CCTCCCTTGCATGATACCTTGG
14	RM5979	4	GTTTGAACTCTGGTCTCAATGC	ACAGTATACGTGGCTCAATCAGG
15	RM7588	5	GCAATTTCCGAAGCCCATGACG	GCCCATGGGTACGTGCTATGATCC
16	RM3381	5	ACGAACGCGAGCTGACAGAGG	AATAGCTGCCAGCAACTGCAACG
17	RM3392	3	AGCAACCAACCCAGTAGTTAGCC	GCTCATTTGCATGCTGTGTTAGC
18	RM26643	11	CTCCCTTCTTCTGCTGAGACACC	CAAGCTACTCATTGGGCTCATCC
19	RM6327	11	GGAGCTGATACAACAATCAGACAGC	CGCTACGCTGCTCCAGATTAGG
20	RM26063	11	GATCCATATGCCTCTTCGATTGG	AACTCCAGCAGTGAGAGCGTAGC
21	RM17962	5	CAGCAAATCTCTATCACTGCAACC	GCTAGATGACCACCTGCTGTACG
22	RM24718	9	TGACGTGGCAAGTTGACTGTGG	TAGCCGATGGAGCCACTAGAAGG
23	RM26249	11	AGAAACAGCTGGAACTCACAATGG	CATGCTCGGCTCCTCTACCC
24	RM26796	11	TTCAAGACATGGTGTGGATCTGG	GCCACTGTGCCAACATTATAACC
25	RM6646	10	GGCAGTTTGATGTTGGAGAACTGC	ATTCAGCGACGACATGCACACC
26	RM1095	1	CCCATTCAGTTGATCCTGTCTGC	AGCTGGGATGCAGAAGAGTATGG
27	RM3746	1	CCTTGATCGGAAGTAGCTCAACG	GCCCTCAGAGCAGTAAGGAGAGG
28	RM10218	1	AGCTAGCCCTCTTGTCTCCATGC	CACACTAGCACAGTGGCATACTGG
29	RM8071	1	GGACAAGAGGTTCCTCAATCTCG	AAACGGGATTATCGCCACTACC
30	RM6362	7	TTATCTCCACCTTCATTGCCTTCG	AGTATTTGTCAAGGAGCGGGTTGC
31	RM12176	1	AAGACTAGTGGTCAAACAGTGC	AGGATGGAGGAAGTATGTATGC
32	RM3285	1	AAGGAACGCGAGAGAAGAGAACC	ATTCTGAGCAGGAGAAGGGAAGG
33	RM5310	1	GGGACCAAGACCTTTCCAATGC	GCGGAAGCAGGAGAATCGTAGC
34	RM7642	3	TTAGATCACGATACTCAGGGATGC	CGAAGAAAGAGAGCACGAAACG
35	RM5626	3	GGACGCCACCTTCCTCTTCTGC	CGGTCATAAACGCCATTAGACCAAGC
36	RM555	2	TTGACATGCGAAATGGAGATGG	TTGGATCAGCCAAAGGAGACC
37	RM12368	2	GAGATAAGTGCCACGATTGATTGC	GGAGCCGTACGAGTAATCTCTGC
38	RM6367	2	GCAACCACGACATCAAAGAAACC	GGAGGTTAGTGCTTCGGAGTGG
39	RM13541	2	CTCCTCGCTTCGTCCACTTCC	CCATGTGTCACCGACTCAACG
40	RM16147	3	GAGATCATCCTGAACAACCACTGC	TGTCCACCCAAACCCTCTTTACC
41	RM2488	5	NA	NA
42	RM4404	3	GAGATGGCAGTGTCAAGCTAAACAGG	TGAGGACGCCAATATGGCAAGG

43	RM3403	1	CTGCCTCCTCCATTTCCCACTCC	CGAACGACTGCTCCCTCTTCAGC
44	RM226	1	GAAGCTAAGGTCTGGGAGAAACC	AATGGCCTTAACCAAGTAGGATGG
45	RM3585	3	NA	NA
46	RM3103	12	CTGGAGTGGAGAAGAGAGAACAGG	TCTCCGCTCGGTTTCATCTAGG
47	RM1141	1	CAGAACCAGGGCTTTGTAAGAGG	AACCTGTGGTTGCATTGTGTAGG
48	RM17960	5	CTAGGTTTGTGTCTCTTTGTGG	TCCATATGCATACTCCTACAGC
49	RM3392	3	AGCAACCAACCCAGTAGTTAGCC	GCTCATTTGCATGCTGTGTTAGC
50	RM6842	2	CCGTGCATCTCGCTACCTAACC	TGCACACACAACTTAGAGGAAGAAGG
51	RM8068	1	GTGTCATATGCAAGCAACAACTCC	AGTATGTACGTCTCCTCCGTTGC
52	RM7448	12	GACTTTGGCGGATTGATTGC	GCTGGCTAATAGTGTGCTATTGTACC
53	RM7119	12	CTGAGACCATGACGGGATAAACACC	GGCCTCAGATCATCACAACTTGG
54	RM6712	3	CCAGCATCATCATTGTCATCATCG	ATCCATCCAGCAGGAGAAACAGG
55	RM6703	1	GCTTTCCTCTCCTCTCCTCTCC	CAAATCAGTGTCGTATGCAGTGG
56	RM6676	3	AGAGGAGAGATACAGATTGAGACG	GACCCTTGATGTGAGTAGTTGG
57	RM8084	1	GCGCCCAATGCATGTAAATTCC	TGCCGATACCTGTGATCAAGTCC
58	RM3329	3	AGAAATGGTGAAAGATGGTGCTACCG	CTGAATGTTCTTCAACTCCCAGTGC
59	RM566	9	AATATGGTGGCGCGTACATCC	TGATCGAGCCAACAACAACTGG
60	RM7576	3	GTGGGAAGAAGAACATCAACTGG	GCACACAAGATAAACCCAATCAGC
61	RM6959	3	GATTCCTATGGAGGATTGTTGC	AACTCCACCGGTGTTAAGAAGG
62	RM511	12	AACGAAAGCGAAGCTGTCTCC	ATTTGTTCCCTTCCTTCGATCC
63	RM4404	3	GAGATGGCAGTGTCAAGCTAAACAGG	TGAGGACGCCAATATGGCAAGG
64	RM5501	1	GTTGGCGTACGTAGAGAGGAGTACG	CTTCATTGTCGCTGCCAGAGC
65	RM6616	2	TCAAGATCAACGCACTCCTCTCC	TCGTACGAGCAACAGGTGGTAGC
66	RM3825	1	CCACTAGCAGATGATCACAGACG	GAGCACCTCATAAGGGTTTCAGC
67	RM243	1	CAGACTGCAGTTGCACGATACTACG	GAAAGCTGCAACGATGTTGTCC
68	RM1331	1	CAAACGGAGTGAGTACATTAGC	TGATGTGTTCTGTACAGGTTCC

Table 3: Reaction mixture used for polymerase chain reaction (PCR) with SSR, Hv SSR and In-dels.

S. No	Component	Stock concentration	Volume/reaction
1	DNA	50μg/ml	1.5 μl
2	ADW	-	5.25μl
3	10XBuffer	10X	1μl
4	dNTP	1mM	1μl
5	Forward Primer	10μM	0.5μl
6	Reverse Primer	10 μM	0.5μl
7	*Taq* polymerase	1U/μl	0.25μl

Table 4: Temperature profiles used for PCR

Steps	Activity	Temperature(ºC)	Cycles	Time (min)
1	Initial denaturation	95 ºC	1	5
2	Denaturation	95 ºC		1
3	Annealing	55 ºC	30	1
4	Extension	72 ºC		1
	Final extension	72 ºC	1	7
6	Store	4 ºC	1	24 hr

PCR amplification using SSR and In-del primers

1.5 μl of diluted template DNA of each genotype was dispensed at the bottom of PCR plates. As given in Table 3.5 cocktail was prepared separately in an eppend off tube. 8.5 μl of cocktail was added in each tube. PCR was carried out for 30 cycles in 96 wells PCR plate (Axygen make) in Applied Biosystems thermal cycler.

After the completion of PCR reaction, 3 μl of 6 X loading dye was added to 10 μl PCR products and 3.5 μl of it was loaded on 5 % PAGE in a mini-vertical electrophoresis system (CBS scientific, model MGV-202-33) along with 100bp or 50bp ladder.

Results

Marker-trait associations

A F3 population derived from cross between common donor as Dagaddeshi (*Oryza sativa* L. Land race) and high yielding variety MTU1010 (*Oryza sativa* L. ssp. *Indica*) was evaluated under stress and non-stress conditions showed in Fig. 4.19 and used for validation of root and yield related markers in kharif-2015. MTU1010 is highly sensitive to drought and suffer high yield losses in years with drought (Verulkar *et al*., 2010; Vikram *et al*., 2011) [12, 13] while Dagaddeshi is low yielding, drought tolerant landrace. The yield related 17 traits were recorded using this population as follows.

A total 40 SSR linked markers within DTY QTL 1.1 present on chromosome #1, chromosome # 5 DTY QTL 12.1 present on chromosome #12 and some markers adjacent to RM 242 regions on chromosome # 9 were used for validation. Among 40 markers 2 markers were linked to chromosome 1, 3 markers for chromosome no #5, 5 chromosomes for QTL on chromosome 9 adjacent to RM 242 and 9 markers was localized on chromosome 12. A total of 19 markers were used for validation studies. The marker, RM242 showed 1% significant association with seedling height during the irrigated (transplanted) condition whereas flag leaf width showed 5% significant association and TSD (transplanted) RM242 showed 5% significant association with total root length (Table 4.8 and Fig. 4.20).

RM18641 detected to be significantly associated with panicle length during irrigated condition. Significant associations were observed to the marker RM27493 with panicle length, number of tillers during different conditions. RM24587 was found 1% and 5% level of significant associated with plant height, Flag leaf length, spikelet fertility and spikelet sterility.

RM1261 was significantly associated with flag leaf length, tiller number and second flag leaf length during both the conditions irrigated and TSD. RM27576 was significantly associated with spikelet fertility and sterility under TSD and irrigated conditions. RM 28048 was detected to be significantly associated with second flag leaf length.
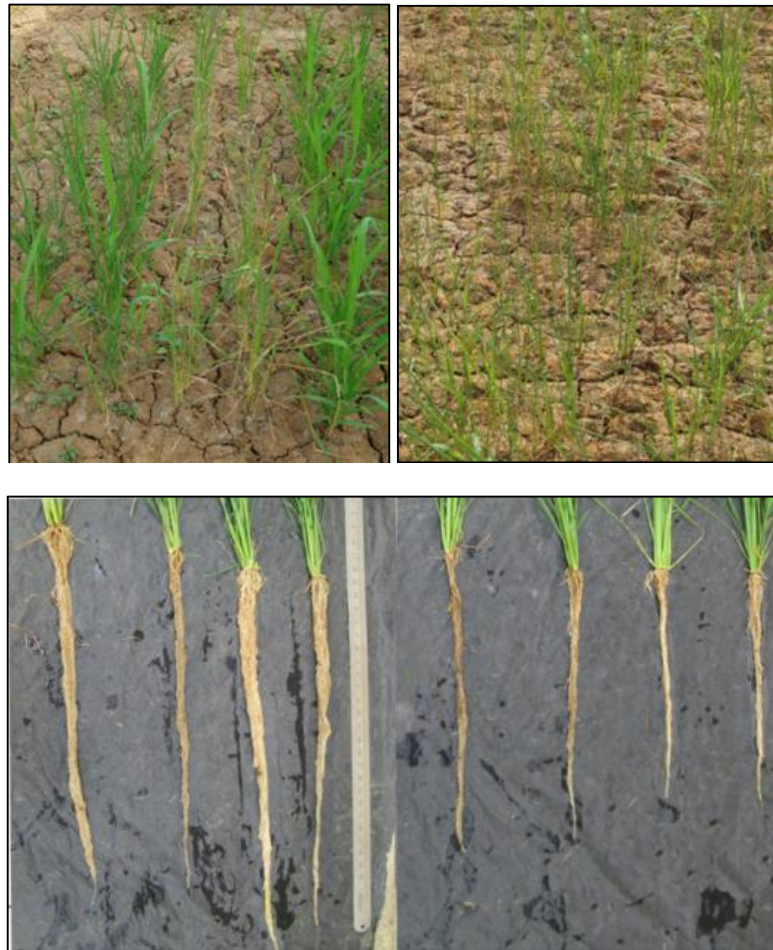

Fig 1: Rainout screening and root length of selected lines of drought susceptible and Resistance lines
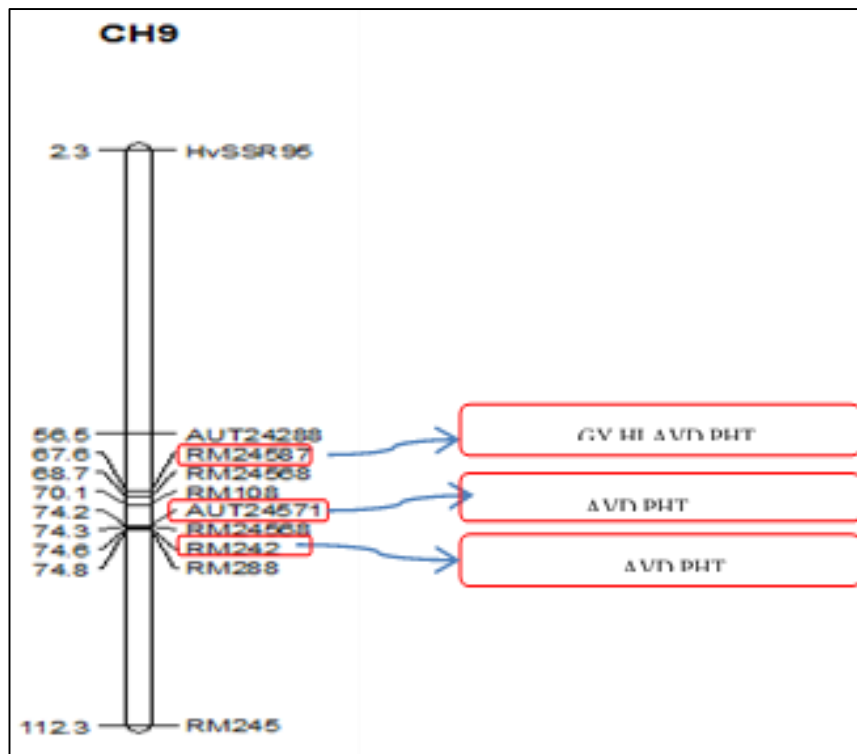

Fig 2: Marker-trait associations at different significance level on chromosome # 9

Table 5: Marker-trait associations at different significance level

12.1	I			RF			TSD		
Traits	5% (*)	1% (**)	0.1% (***)	5% (*)	1% (**)	0.1% (***)	5% (*)	1% (**)	0.1% (***)
SH	-	RM28048	-	RM28048	-	-	RM7195	-	-
SB	-	-	--	RM1261	-	--	RM28099	-	--
50%FLW	RM28160	RM28076		RM28076	1		M28160	RM28076	-
PHT	RM8215 RM28199	-	-	RM8215 RM28199	-	-	RM28199	-	-
PNL		RM1261	RM28160	RM28160	RM1261	---	-	RM1261	RM28160
FLL	RM28160 RM28199	RM1261	-	RM28160 RM28199	RM1261	-	RM8215 RM28076 RM28199	-	-
2FLW	RM8215 RM28076	-	-	-	-	-	-	-	-
NTT	-	-	-	-	-	-	RM28076 RM28099 RM28199	-	-
BY	-	-	-	RM28160	RM1261	-		-	-
HI	-	-	-	RM431	-	-	RM7195	-	-
GY	-	-	-	RM28160	-	RM1261		-	-
TWT	-	-	-	-	-	-	RM28076	-	-
SPF%	-	RM8215	-	-	RM8215	-	RM8215 RM28199	-	-
SPS%	-	RM28099	-	-	RM28099	-	-	-	-
RV	-	RM28130	-	-	-	-	-	-	-
RTL	-	RM1261	-	-	-	-	-	-	-

Where SH=Seedling Height, SB= Shoot Biomasss, RPR= Root Pooling Resistance, FLW= Days To 50% Flowering, PHT= Plant Height, PNL= Panicle Length, FLL And FLW= Flag Leaf Length and Width, 2FLL And 2FLW= Second Flag Leaf Length and Width, NTT= Number of Tillers, BY= Biological Yield, HI= Harvest Index, TWT= 100 Seed Weight, SPF% and SPS%= Spikelet Fertility and Sterility, TRL=Total Root Length, AVD= Average Root Diameter, RV= Root Volume and RTL= Maximum Root Length.

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