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Research Article

Molecular tagging of genomic regions influencing root phenomics for improving drought resistance in rice

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Abstract
Drought, with its complex genetic nature possesses great challenge for resource deficient agriculture of this century. Even the climate change with its unpredictable weather pattern causes peak water scarcity. With these current challenges, genotypes with better root plasticity prove an effective way for sustainability in agriculture. Root, the 'hidden half' has high complexity for screening but serves as critical role for water and nutrient acquisition. Root phenomics was assessed by screening the RILs developed from Norungan and IR64 in modified transparent soil filled root box. RILs exhibiting extreme root phenome were screened under natural moisture stress condition in the field. Several candidate genes governing root development have been identified in rice. The genomic location of six candidate genes involved in root development pathway was obtained from OrygeneDB and specific microsatellites falling within and flanking these candidate genes were acquired from Gramene. Out of twenty-two microsatellites recovered and used for parental polymorphism survey, 3 microsatellites in the 3 candidate gene loci *viz., OsMADS61, OsMADS23* and *OsMADS27* exhibited polymorphism between IR 64 and Norungan. Association analysis of phenotypic and genotypic data indicated that all the three polymorphic markers significantly influenced the trait, maximum root length. The gene specific marker RM3558 (*OsMADS61),* and RM23170 (*OsMADS23)* also controlled the trait root volume, while the microsatellite marker, RM5789 linked to *OsMADS27* had its influence on root: shoot ratio. This study identified significant candidate gene-linked microsatellites for utilization in drought tolerance breeding through marker assisted selection approach.

Keywords: Rice- drought- root phenomics

Introduction

Rice, the staple food for 3.5 billion people, is adapted to the wide range of ecosystems ranging from flooded condition to low moisture tract. About half of the world rice area is rainfed, where drought is the major constraint. Existing climate variabilities affects rice growth in larger extent of 32 percent fluctuation (Ray *et al.,* 2015). Drought is the major abiotic stress limiting rice production and productivity in rainfed low-land and upland ecosystems. But, Rice is well adapted to be grown in flooded fields, helping it cope with water stress and enabling it to produce economically good yields under drought is a great challenge. Achieving drought resistance in rice will be necessary for meeting the growing water shortage of the world, and it requires a deeper understanding of the mechanisms that could facilitate drought resistance (Serraj *et al.,* 2011). Productivity gains in this water-limited environment involve many traits that tend to show a complex interaction with a number of environmental factors. This complex interaction along with multiple pathways involvement possesses great challenge for developing lines for drought tolerance (Nachimuthu *et al.,* 2017). Root systems have a critical role in whole plant metabolism. Previous studies has indicated that the shallow roots might be required for improved phosphorus acquiring efficiency (Lynch, 2007) or deep roots had more access to stored soil water (Henry *et al.,* 2011). Both constitutive and adaptive root growth have been implicated in the improved performance of rice under rainfed lowland conditions (Kamoshita *et al.,* 2002). Root growth improves drought resistance by hastening the water uptake from deeper layer of the soil. Hence, it has been a target of research objectives aiming at improving crop yields (Gowda *et al.,* 2011). Utilization of this trait in drought resistance improvement is lacking due to the absence of efficient screening techniques. Many traditional root phenotyping platforms *i.e.* PVC cylinder, wax petroleum layer system, rhizotrons, hydroponics, soil box method, pot culture method *etc.* have been reported in various studies (Gowda *et al.,* 2011). Several mapping populations were developed to identify quantitative trait loci (QTLs) and candidate genes influencing root morphology and other drought-related traits that could then be used in marker-assisted selection (MAS) to improve upland varieties. Meta QTL analysis was done for QTLs controlling rice root morphological parameters and significant meta QTLs were identified (Courtois *et al.,* 2009). Plasse *et al.* (2009) sequenced the segments of eight genes co-segregating with meta-QTLs for root development in rice and identified CRL1/ARL1, 4 transcription factors belonging to the MADS box family

(*OsMADS23*, *25*, *27* and *61*), 2 Auxin Efflux Carriers, and the early auxin responsive gene of auxin/indoleacetic acid component 8 (IAA8). This study was formulated to evaluate root phenomics of F_{11} RILs developed from Norungan, drought tolerant landrace and IR64, elite variety by the modified transparent soil filled root box. Then, RILs exhibiting extreme root phenotypes were screened under natural moisture stress condition in the field to study the influence of root parameters on field performance. Association was analyzed among the traits as well as the candidate gene polymorphism with the field performance.

Materials and Methods

Norungan, a drought tolerant landrace and IR64, an elite rice variety was used as donor and recipient respectively. One hundred and forty four F_{11} RILs was used in the study. Low cost modified root box technique was developed for the evaluation of different root traits. It was constructed to a dimension of 0.6 m x 0.5m x 0.15 m with the help of wooden planks on the sides and transparent acrylic solid sheets on the front and rear side. Base of the box was attached with khada cloth which facilitates water movement through the soil. A set of 144 RILs derived from the cross between IR64 and Norungan was screened in the root box. It was screened in three runs, each time with 48 RILs and 2 parents. In order to reduce error, each entry was screened with three replication randomized across the root box. Three set of experiments have been carried out in different environmental condition due to seasonal variation. Irrigation was stopped at 3 leaf stage. Forty days after planting, the root boxes were dismantled and roots clearly but slowly washed for each plant. The traits such as root length, root thickness, root volume, root density and root shoot dry weight ratio were measured.

Selected 30 extreme RILs were sown directly with a uniform spacing of 20 x 20 cm in a randomized block design with 6 replications (3-Control, 3-Stress) in the plot size of 2.4 m x mention the size in m. At 50 DAS, the experiment was divided into two, each with three blocks. The field was divided with the help of 2m buffer channel lined with impermeable high density polyethylene sheets. One part with three replications was irrigated and another part with three replications was imposed with stress by withholding irrigation. Soil moisture content was measured using IRROMETER. Six IRROMETER of two different depths *viz.*, 30 cm, 15 cm were installed in stressed blocks. Each replication had two IRROMETERs of 30 cm and 15 cm respectively. Threshold for irrigation was setup when the soil moisture has depleted around 30 percent available moisture (140 Centibars soil suction). Plant height (cm), DFF (Days to 50% flowering), panicle length (cm), panicle exertion, leaf rolling, relative water content (%), spikelet sterility, 100 grain weight, harvest index (HI) and plot yield were recorded.

DNA was extracted from the leaf samples of the two parents and the progenies following CTAB method developed by Saghai-Maroof *et al*. (1984) with suitable modifications suggested by Hoisington *et al*. (1994). It was quantified in gel electrophoresis (0.8 per cent agarose gel) and diluted to 30 ng/µl. PCR amplifications were performed in Bio-Rad (MyCycler thermal cycler) and AB PCR machine. The reaction was carried out in a total reaction volume of 20 µl with 30 ng DNA, 1× PCR buffer, 100 µM dNTPs, 250 µM primers, and 1 unit *Taq* polymerase enzyme. Bands were visualized in 2.5 - 4 % agarose gel and Polyacrylamide Gel Electrophoresis. Six candidate genes (*OsMADS 23, OsMADS 25, OsMADS 27, OsMADS 61, IAA8, OsARL 1*) which have significant effect on root parameters were selected. The genomic location of six candidate genes involved in root development pathway was obtained from OrygeneDB and specific microsatellites falling within and flanking these candidate genes were acquired from Gramene database. Parental polymorphism survey was performed for the two parents IR 64 and Norungan using 22 SSR primer pairs. Population was genotyped with the polymorphic primers. Fishers Z transformation analysis was employed to assess the candidate loci specific microsatellites-morphological trait correlation.

Result and discussion

Among the several traits contributing to enhance stress tolerance, root characters are considered to be a vital component of dehydration postponement mechanism since they contribute to regulation of plant growth and extraction of water and nutrients from deeper layers (Thanh *et al.*, 1999). Toorchi *et al.* (2006) and Kanbar *et al*. (2009), based on canonical correlation studies conducted under contrasting moisture regimes, suggested that maximum root depth, root shoot ratio, and root dry weight conferred an advantage to grain yield under stress. Hence the RILs were first screened for root traits using root box and the selected RILs were subjected to field stress, which indicated the presence of genetic variation for drought response among the parents and RILs in the study.

There was a notable variation between the two parents, Norungan which is a drought resistant parent and IR 64, a drought susceptible parent. Norungan possess higher root length, root volume, root thickness and root dry weight than IR 64 in all three sets of experiment. The measured traits of RILs were compared with the parents in replicated experiments. A significant variation among the RILs was observed for most of the investigated root traits *viz*., maximum root length, shoot length, root volume, basal root thickness, root density

based on dry weight, root dry weight and root shoot dry weight ratio. Mean and value ranges are presented in Table1. Broad sense Heritability was estimated for all the measured root traits. Maximum root length possessed moderate heritability (52%, 30%, 37%) in all the three root box experiments. In contrast, root shoot dry weight ratio (2%, 23%, 12%), root dry weight (21%,29%, 29%), root density based on dry weight at 10-20 cm (16%, 22%, 22%), root density based on dry weight at 20-30 cm (7%, 5%, 11%), root density based on dry weight at >30 cm (10%,18%,11%) had low heritability. Shoot length had moderate heritability (49%, 52%) in first and third set of experiments whereas in second set, it had high heritability (0.88). Low heritability was observed in first and second set of experiments for basal root thickness (4% & 18%) and it showed moderate heritability in third set of experiments (33%). Root volume exhibited high heritability (84%) in first root box experiment while in second (36%) and third (54%), it showed moderate heritability. Norungan and few RILs had recorded higher values for all root architectural traits, yield and yield related traits than the susceptible parent, IR 64 under water limited condition. Similar result was obtained by Puckridge and O'Toole, (1981) using a deep rooted cultivar Kinandang Patong and shallow-rooted cultivars (IR20 and IR36) for root length. Similar results were also obtained by Mambani and Lal (1983b), Lilley and Fukai (1994a), and Kato *et al.* (2007b). Deep rooting, an importance drought tolerance trait was mapped to chromosome 2 by genome wide association mapping and family based linkage mapping (Lou *et al.,* 2015) Most of the measure root traits has moderate to low broad sense heritability.

Selected subset of 30 extreme RILs along with parents was characterized under field control and stress condition. Significant variations were observed in the measured traits between the parents and among the RILs in both conditions of control and stress. The mean trait values, range and critical difference at 95% of the confidence interval for the RILs along with the parents are presented trait wise (Table 2). Phenology is the most important factor in determining the grain yield under stress condition (Jongdee *et al*., 2002). Delayed days to flowering under stress as compared with that under non-stress has been reported to be one of the parameter associated with drought resistance in rice (Bernier *et al*., 2007; Venuprasad *et al*., 2002). . Similar results obtained here with the resistant genotypes showing early flowering. Susceptible RILs has exhibited late flowering where as the resistant genotypes showed early flowering. Norungan exhibited early flowering than IR 64. Lafitte *et al*. (2006) reported that relationship between plant height and drought resistance was due to the presence of *sd-1* gene which often associated with the traits such as high tillering and shallow rooting due to their pleiotropic effects. Current study has exhibited marginal reduction of plant height in resistant genotypes with well-developed root system. Norungan a landrace is taller than IR 64 and the RILs has short stature than its donor parent. Norungan had lengthier panicles than IR 64 in both conditions of stress and control. In control condition, the panicle length of RILs ranged from 19.3 cm to 25.9 cm with a mean value of 21.7 cm whereas in stress condition the panicle length of RILs ranged from 18.2 cm to 22.4 cm with a mean panicle length of 20.2 cm. Susceptible parent, IR 64 had high spikelet sterility percentage when compared to drought resistance genotypes like Norungan and RILs. Boonjung and Fukai (1996) reported that poor panicle exsertion was the main cause for spikelet sterility which in turn reduces the yield. In this study, Norungan and several RILs had high panicle exsertion with low spikelet sterility coupled with higher yield under drought. The present investigation indicated that Norungan had high RWC than IR 64 and few RILs had higher RWC than Norungan. Broad sense heritability was estimated for different traits and computed across two environments. The present investigation has recorded high heritability for the traits such as days to 50% flowering, spikelet sterility, plant height, panicle length, panicle exsertion, harvest index and plot yield under control and for the characters *viz.,* days to 50% flowering, plant height, panicle length, panicle exsertion, spikelet sterility, harvest index, leaf rolling and plot yield under stress condition Norungan and few RILs recorded higher yield under stress than IR 64. Most of the traits exhibited moderate to high broad sense heritability.

Path analysis gives an idea about how a trait influences grain yield directly and indirectly via other traits (Deway and Lu, 1959). This is very important in giving due weightage to major yield contributing traits while selection. In the present investigation, under control condition, path analysis revealed that plot yield registered a very high positive direct effect on single plant yield (Table 3a). Plant height and thousand grain weight showed high positive direct effect. Number of tillers showed moderate positive effect whereas panicle exsertion and spikelet sterility recorded low positive effect on single plant yield. Number of productive tillers and panicle length had high negative effects. Days to 50% flowering showed moderate negative effect whereas harvest index recorded low negative effect. In stress environment, the trait such as days to 50% flowering, leaf rolling and harvest index have high direct effect on single plant yield. Panicle exsertion, plant height, number of productive tillers, panicle length showed high negative direct effect on yield (Table 3b).

Twenty two SSR primers synthesized with the sequences retrieved from Gramene database for

candidate genes were used for conducting parental survey. Parents were surveyed with these markers for polymorphism in 3% agarose gel and PAGE (Figure 1). Among the surveyed markers, 3 markers exhibited polymorphism between the parents IR 64 and Norungan registering 13.64% polymorphism (Figure 2). These 3 SSR markers were distributed in chromosome 8, 2 and 4 in the genomic regions linked to candidate genes reported for drought resistance. Mean segregation percentage of markers for IR 64 and Norungan was 46.67 and 42.2 per cent respectively. A mean percentage of 11.11 per cent heterozygotes were recorded with regard to the three marker loci analyzed. Among the markers, RM3558 recorded the highest marker allele corresponding to the parent IR 64 and RM23170 had the highest marker allele for Norungan. The marker RM5789 reported highest percentage of heterozygotes (Table 4). It was analyzed for trait association using Z statistic - test of equality of two means. The study based on test of equality of two means by Z statistics revealed that there was a significant difference between the various root traits of two bulks at the level of 0.05 and 0.01 probability (Table 5) and all the three genes (*OsMADS 61*, *OsMADS 23*, *OsMADS 27*) influence the trait maximum root length. The gene *OsMADS 61* and *OsMADS 23* control the trait root volume while *OsMADS 27* had its influence on root shoot ratio.

Genetic control of root development has been studied mainly through quantitative trait loci (QTL) analysis, and a wide range of QTLs associated with small–medium effects on root biomass, root length, root number (either under control or abiotic stress conditions) has been identified in rice. Six candidate genes influencing root trait were selected for the present investigation. *IAA8* involved in auxin signalling pathway. *ARL1* is an auxin-responsive factor involved in auxin-mediated cell dedifferentiation, and that it promotes the initial cell division in the pericycle cells adjacent to the peripheral vascular cylinder in the stem. (Liu *et al.,* 2005). *MADS-box* genes play an important role in controlling various aspects of plant development. The study by Lee *et al.,* (2003) indicated that Rice *ANR1* homologs (*OsMADS23*, *OsMADS25*, and *OsMADS27*) were expressed in roots. Based on location specific microsatellites analysis, *OsMADS 61* and *OsMADS 23* found to control the trait root volume while *OsMADS 27* had its influence on root shoot ratio. The above result was found to be correlated with the report made by Liu *et al.* (2005) using doubled haploid population from IRAT 109 and Yuefu as they identified QTL for maximum root length in chromosome 2 (*mrl 2*) and chromosome 8 (*mrl 8*). Another correlated report was given by Yue *et al.* (2006), using F_9/F_{10} generation RILs to identify QTLs. They identified QTL for maximum root length in chromosome 2 and chromosome 4. In chromosome 4, they identified QTL for maximum root length and root volume in the same region for control/stress condition which is similar to the result that the candidate gene *OsMADS 61* at chromosome 4 is associated with maximum root length and root volume. Also, they identified QTL for root volume in chromosome 8 under control and stress, which may be correlated with the candidate gene *OsMADS 23* at chromosome 8 that is found to be associated with the trait root volume. Hence it shows that the candidate gene specific markers (*OsMADS 61*, RM 3558 and RM 23170 for *OsMADS 23*, RM 5789 linked to *OsMADS 27)* identified in this study had some likeness with previous reports involving the identification of QTLs and it would be useful for selecting elite genotypes for drought tolerance using marker assisted selection.

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Table 1. Traits mean values of parents and RILs along with their range and critical differences (CD) at 95% confidence interval

				RILs		
Traits		**IR 64**	**Norungan**	**Mean**	**Range**	**CD**
Maximum root length (cm)	Run I	18.2	27.5	24.7	5.27-47.73	9.8
	Run II	38.5	50.83	46.4	22.67-65.00	19.64
	Run III	27.83	43	38	10.00-55.533	16.98
Shoot length (cm)	Run I	44.6	67.3	56.6	16.33-73.23	18.7
	Run II	40.33	60.33	62.76	37.33-84.33	7.9
	Run III	32.7	60	48.1	5.67-68.33	19.24
Root volume (cc)	Run I	1.1	3.8	1.6	0.17-4.33	0.6
	Run II	3.67	7.67	9.69	2.33-21.33	6.76
	Run III	4.5	8.83	6.66	1.83-11.50	2.69
Basal root thickness (mm)	Run I	0.5	0.6	0.6	0.17-1.00	0.4
	Run II	0.6	1	1.13	0.70-1.80	0.53
	Run III	0.57	1.23	0.93	0.30-1.83	0.6
Root density based on dry weight (0-10) (g)	Run I	0.12	0.19	0.15	0.02-0.29	0.12
	Run II	0.35	0.42	0.52	0.20-1.04	0.39
	Run III	0.23	0.52	0.25	0.05-0.53	0.18
Root density (10-20) (g)	Run I	0.02	0.07	0.06	0.00-0.15	0.08
	Run II	0.07	0.08	0.1	0.03-0.26	0.12
	Run III	0.05	0.21	0.14	0.02-0.38	0.14
Root density (20-30) (g)	Run I	0	0.01	0.01	0.00-0.08	0.04
	Run II	0.02	0.03	0.05	0.00-0.31	0.16
	Run III	0	0.05	0.07	0.00-0.17	0.09
Root density (>30) (g)	Run I	0	0.001	0.001	0.00-0.01	0.01
	Run II	0.01	0.01	0.07	0.00-0.47	0.21
	Run III	0	0	0.02	0.00-0.09	0.05
Root dry weight (g)	Run I	0.15	0.27	0.22	0.03-0.45	0.18
	Run II	0.45	0.54	0.74	0.24-1.56	0.61
	Run III	0.28	0.78	0.47	0.10-0.99	0.34
Root shoot dry weight ratio	Run I	0.34	0.35	0.42	0.10-1.24	0.5

Run II	0.3	0.5	0.31	0.14-0.59	0.21
Run III	0.45	0.64	0.38	0.09-0.79	0.3

Table 2. Mean and Range of yield and yield components of parents and RILs

				RILs		
Traits	**Experimental condition**	**IR 64**	**Norungan**	**Mean**	**Range**	**CD**
Days to 50% flowering	(C)	94	82	82	70.00-99.00	2.01
Days to 50% flowering	(S)	96	84	85	73.00-104.00	2.59
Plant height (cm)	(C)	90.8	102.3	97.8	73.40-117.90	16.05
Plant height (cm)	(S)	59.8	89.5	83.6	61.80-102.30	19.41
Panicle length (cm)	(C)	21.1	23.4	21.7	19.30-25.90	2.56
Panicle length (cm)	(S)	16	20.9	20.2	18.20-22.40	2.47
No. of tillers	(C)	10.3	11.6	10.6	7.70-14.00	3.61
No. of tillers	(S)	10	13	10.2	7.50-13.40	3.01
No. of Productive tillers	(C)	9.7	11.3	10.1	7.00-13.00	3.58
No. of Productive tillers	(S)	11	10	8	6.00-11.00	2.66
Panicle exsertion	(C)	2	1	3	2.00-5.00	1.38
Panicle exsertion	(S)	5	3	4	2.00-6.00	1.49
Spikelet sterility (%)	(C)	24.5	21.9	18.2	7.60-18.20	9.76
Spikelet sterility (%)	(S)	35.8	22.9	31.74	16.78-52.53	18.19
1000 grain weight	(C)	24.6	25.1	29.1	19.40-50.40	10.08
1000 grain weight	(S)	18.4	28.1	19.9	12.60-31.00	9.34
Plot yield	(C)	121.3	172.4	117.86	60.02-172.35	16.09
Plot yield	(S)	41.9	70.2	47.8	13.10-82.20	28.07
Harvest index	(C)	30.7	35.5	34.73	24.10-49.60	3.05
Harvest index	(S)	11.5	29.2	24.47	18.45-33.24	3.57
Leaf rolling	(S)	6	2	3	2.00-6.00	1.43
Relative water content	(S)	66.3	85	70.87	60.00-91.86	10.01
Leaf senescence	(S)	9	4	7	4.00-9.00	3.17
Single plant yield	(C)	3.7	4.8	3.51	1.48-4.89	1.01
Single plant yield	(S)	1.1	2.1	1.3	0.40-2.60	0.73

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Table 3a,b. Path coefficient of all traits on single plant yield under control and stress

Control	DTF	PE	PH	NOT	NOPT	PL	SS	1000GW	HI	PY
DTF	**-0.25066**	0.01426	0.03831	-0.09051	0.15927	0.00357	0.0173	-0.02341	0.01994	0.25763
PE	-0.02168	**0.1649**	-0.08818	-0.10715	0.16071	0.0739	-0.01702	-0.01058	0.00416	-0.02546
PH	-0.02822	-0.04274	**0.34024**	0.09591	-0.14783	-0.21602	0.00469	-0.06084	0.00227	-0.28521
NOT	0.08071	-0.06286	0.11609	**0.2811**	-0.44347	-0.08118	0.09365	0.05747	0.01751	-0.36507
NOPT	0.09214	-0.06116	0.11608	0.28768	**-0.43332**	-0.12367	0.07797	0.07731	0.01658	-0.29218
PL	0.00262	-0.03562	0.21482	0.0667	-0.15664	**-0.34213**	-0.01346	0.09346	0.00454	0.23912
SS	-0.02343	-0.01517	0.00863	0.1423	-0.18263	0.02489	**0.18499**	-0.06356	0.02025	-0.25511
1000GW	0.0182	-0.00541	-0.0642	0.05011	-0.10391	-0.09918	-0.03647	**0.32239**	-0.03446	0.0237
HI	0.03589	-0.00493	-0.00556	-0.03534	0.05158	0.01116	-0.02689	0.07977	**-0.13927**	0.49483
PY	-0.04923	-0.0032	-0.07397	-0.07823	0.09651	-0.06236	-0.03597	0.00583	-0.05253	**1.31186**

Residual effect = 0.427

Stress	DTF	PE	LR	PH	NOT	NOPT	PL	RWC	SS	1000GW	SEN	HI	PY
DTF	**1.76**	-2.944	0.028	0.102	0.582	-0.049	0.281	0.04	0.013	0.039	0.031	-0.477	0.381
PE	0.984	**-5.265**	0.857	1.083	1.575	0.592	0.42	0.055	0.046	0.315	-0.216	-0.474	0.025
LR	0.013	-1.17	**3.858**	0.678	-0.657	-0.492	-0.054	0.026	0.034	0.146	-0.226	-0.314	-1.103
PH	-0.068	2.172	-0.997	**-2.625**	0.856	0.767	-1.069	-0.053	0	0.161	0.177	0.461	0.164
NOT	-0.367	2.969	0.908	0.804	**-2.793**	-1.908	0.365	-0.004	0.026	-0.153	-0.373	0.796	-0.183
NOPT	0.04	1.43	0.87	0.923	-2.444	**-2.18**	0.733	0.008	0.065	-0.279	-0.159	0.491	0.307
PL	-0.324	1.448	0.137	-1.839	0.667	1.048	**-1.526**	-0.02	-0.021	0.061	0.662	0.195	-0.294
RWC	-0.543	2.234	-0.761	-1.063	-0.084	0.142	-0.23	**-0.13**	0.011	-0.104	-0.141	0.552	0.049
SS	0.174	-1.798	0.983	-0.009	-0.543	-1.057	0.234	-0.011	**0.134**	0.175	0.317	0.499	0.628
1000GW	-0.097	2.339	-0.796	0.595	-0.603	-0.858	0.132	-0.019	-0.033	**-0.709**	0.352	0.08	-0.135
SEN	-0.066	-1.376	1.053	0.563	-1.262	-0.421	1.223	-0.022	-0.051	0.302	**-0.826**	-0.318	0.755
HI	-0.383	1.139	-0.553	-0.553	-1.016	-0.489	-0.136	-0.033	0.031	-0.026	0.12	**2.188**	-0.151
PY	-0.393	0.076	2.492	0.252	-0.299	0.392	-0.263	0.004	-0.049	-0.056	0.365	0.193	**-1.708**

Residual effect = 0.517

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Table 4. Segregation percentage of polymorphic markers

S.No	Markers	Homozygotes - IR64 allele	Homozygotes- Norungan allele	Heterozygotes
1	RM3558	50	40	10
2	RM5789	46.67	40	13.33
3	RM23170	43.33	46.67	10
	Mean	**46.67**	**42.22**	**11.11**

Table 5. 'Z' Statistic associating the marker segregation with the root phenotypic data

	MRL	RV	RT	RDW	RSR
RM3558	3.152**	2.796**	1.236	1.43	0.234
RM5789	1.697*	0.779	0.145	0.049	2.371**
RM23170	3.140**	2.283**	1.512	0.627	0.938

Figure 1. Survey of parental polymorphism in Agarose and Polyacrylamide gel electrophoresis

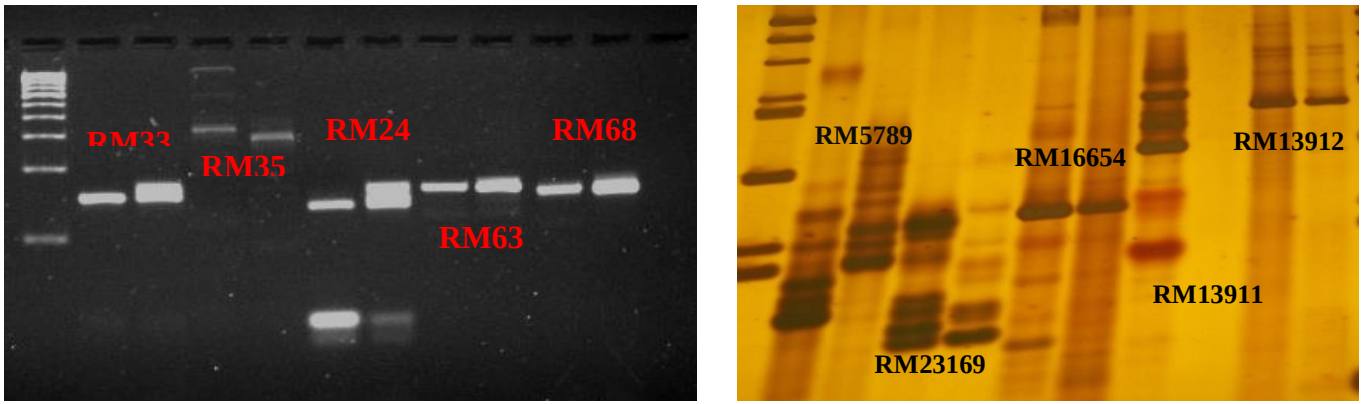

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Figure 2. RILs segregating for marker allele amplified with SSR primer pair RM5789 and RM3558 (P1-IR64, P2- NORUNGAN)