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Molecular marker analysis and interval mapping of QTL for root and shoot traits in the Danteshwari/ Dagad Deshi RIL population of rice (*Oryza sativa* L.)

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ABSTRACT

Nine hundred and thirty seven (788 HvSSR, 108 RM, 5 RGNMS and 36 SNP) markers were surveyed for parental polymorphism between indica cultivars Danteshwari and Dagad Deshi. One hundred and four (11.9 %) markers exhibited polymorphisms which were further analyzed for marker segregation on F7 275 RILs. Thirty eight (36.54%) markers showed expected 1:1 segregation and rest 63.46 % of the markers showed distorted segregation. Linkage map was constructed, using 104 (SSR and SNP) markers across the 12 rice chromosome covering 2665.5 cM in length. Interval mapping analysis identified 12 putative QTLs for root and shoots traits explaining phenotypic variability from 4.41% to 27.49%. For total plant length, 5 putative QTLs were identified on chromosomes 1 and 4. Three putative QTLs for shoot length were detected on chromosome 1. Out of 2 putative QTLs for shoot dry weight, 1 was located on chromosome 5 and another on chromosome 10. Whereas single putative QTL for shoot fresh weight and root volume was positioned on chromosome 5 and 6, respectively. The result also suggests that the different analysis model played a role in detection of QTLs. Further, the identified QTLs may be used in rice breeding programme for the improvement in the root and shoot traits.

Key words: *Molecular marker, QTL, rice, root, shoot*

INTRODUCTION

Decoding of the rice genome has changed radically our understanding of rice evolution and its synteny with other cereal genomes. This information has been utilized extensively for mining molecular markers to impart markerassisted breeding (http://www.gramene.org). Molecular marker technology has helped in developing highly saturated molecular maps in rice and studies on genetics of inheritance pattern of several complex traits. Among the different molecular markers, simple sequence repeats (SSR) of 2-6 bp motifs are of great value due to their relative abundance, codominant inheritance, multiple alleles, uniform genome coverage and simple reproducible assays (McCouch et al., 2002; Singh et al., 2010). Different types of SSR markers are available for rice like rice microsatellite (McCouch et al., 2002), HvSSR (Singh et al., 2010) and RGNMS (Parida et al., 2009). Simple sequence repeat (SSR) with longer repeat-motifs are more polymorphic due to high length dependent replication slippage (Temnykh et al., 2000; Xu et al., 2000). Highly variable SSR markers are derived from hypervariable region of the rice genome. HvSSR loci with repeat lengths in the range of 51-70 bp are able to discriminate between rice varieties most expeditiously reported (Singh et al., 2010). Rice genic non coding microsatellite (RGNMS) are derived from the promoter, 5' UTR, intronic and 3' UTR sequences of protein coding genes and are more robust and polymorphic as compared to genic coding and random genomic SSR markers (Parida et al., 2009). In recent years, due to the advent of next generation sequencing and faster genotyping technologies during the last decade, new marker systems such as single nucleotide polymorphisms (SNPs) have attracted real attention and have emerged as the marker of choice in rice breeding because they are more abundant, stable, dispose to automation, efficient and cost effective. Molecular markers are most useful in complex trait analysis through QTL mapping. QTL mapping rely on the phenotypic data, genotypic data and the model used for QTL analysis.

In particular, a first step in QTL mapping is to survey polymorphism in order to create a molecular linkage map. This implies screening of several hundred molecular markers to acquire sufficient number of polymorphic loci covering the whole genome. The availability of molecular markers in rice offers opportunities to increase the density of locus specific polymorphic marker for generating genotypic data. Therefore, the objective of the work here reported was to generate genotypic data using molecular markers (SSRs and SNPs) and mapping of QTLs associated with root and shoot traits in the RIL population of rice.

MATERIALS AND METHODS

Plant materials

Danteshwari, a shallow rooted high yielding, gall midge resistance, long slender grain and moderately susceptible to water stress was used as female parent. Dagad Deshi, a tall deep rooted poor yielder and tolerant to water stress was used as male parent (Fig. 1). The 275 F_7 RILs developed from the cross between Danteshwari and Dagad Deshi using singleseed descend were used for the mapping of QTLs for root and shoot traits.

Plant DNA Extraction and genotyping

DNA extraction

Genomic DNA was isolated from 30 days old plant leaf samples using miniprep method (Doyle and Doyle, 1987) and used for PCR amplifications.

SSR genotyping using agarose gels

Polymorphism survey were conducted between the parents Danteshwari and Dagad Deshi using 901 SSR markers randomly distributed on all 12 rice chromosomes. Polymorphic markers were used for genotyping of all 275 RILs along with parents. The PCR amplifications were performed according to Sinha et al. (2015a).

SNP genotyping using Sequenom MassARRAY system

Thirty six SNP markers (SNPs from conserved single copy rice genes) were used for genotyping using Sequenom MALDI-TOF MassArray system as suggested by Sinha et al. (2015a).

Fig. 1. Morphological polymorphism for root traits in parents Danteshwari and Dagad Deshi.

Data analysis

χ^2 test

The genotypic data generated from markers were tested for χ^2 goodness of fit test against 1:1 segregation ratio. The Chi-square test was computed using QTL Cartographer software v2.5 software (Wang et al., 2007)

Linkage map construction and identification of QTLs

Genotypic data generated from markers were used for linkage map construction using computer software MAPMAKER/EXP, version 3.0. Kosambi function was used to calculate the genetic distances between the markers (Kosambi, 1944). The mean phenotypic data reported by Sinha et al. (2015a) were utilized for QTL identification using QTL Cartographer v2.5 (Wang et al., 2007) following interval mapping analysis at LOD scores of 2.5.

RESULTS AND DISCUSSION

SSR genotyping

Nine hundred and one SSR markers were screened for parental polymorphism, 93 (10.32 %) markers (76 HvSSR, 14 RM and 3 RGNMS) generated informative polymorphism between the parents and further used to genotype RILs (Table 1, Fig. 2a & 2b). Polymorphic

Table 1. Simple sequence repeat (SSR) markers used for parental polymorphism.

Chromosome	SSR Markers			Total markers	Polymorphic markers	Polymorphism %
	HvSSR	RM	RGNMS			
1	97(11)	12(4)	5(3)	114	18	15.79
2	91(6)	8(0)	-	99	6	6.06
3	96(8)	14(1)	-	110	9	8.18
4	55(6)	11(1)	-	66	7	10.61
5	70(11)	2(0)	-	72	11	15.28
6	77(6)	4(1)	-	81	7	8.64
7	54(4)	5(0)	-	59	4	6.78
8	53(4)	1(1)	-	54	5	9.26
9	57(7)	8(1)	-	65	8	12.31
10	45(4)	5(0)	-	50	4	8.00
11	42(4)	26(3)	-	68	7	10.29
12	51(5)	12(2)	-	63	7	11.11
Total	788	108	5	901	93	10.32

( ) - figure in parenthesis indicates polymorphic markers

SSR markers exhibited di-, tri and tetra nucleotide motif of which the largest proportion corresponds to poly (AT/TA) motif (46.25 %) followed by poly (TC/CT) motif (16.13 %) and poly (GA/AG) motif (13.98 %). Similarly among the length of the repeat motif the largest tract of repeat motif corresponds to di-nucleotide AT/TA (44-70) and TC/CT (28-70). The AT motif (Fig. 3) is the most abundant di-nucleotide motifs in the present study due to which it is a major potential source of polymorphic SSR markers. The SSR with longer repeat-motifs is expected to be more polymorphic due to high length dependent replication slippage reported by Xu et al. (2000) and Temnykh et al. (2000), which therefore justifies the validation of SSR markers in our population derived from the parents involving indica genotypes (Danteshwari/ Dagad Deshi).

SNP genotyping

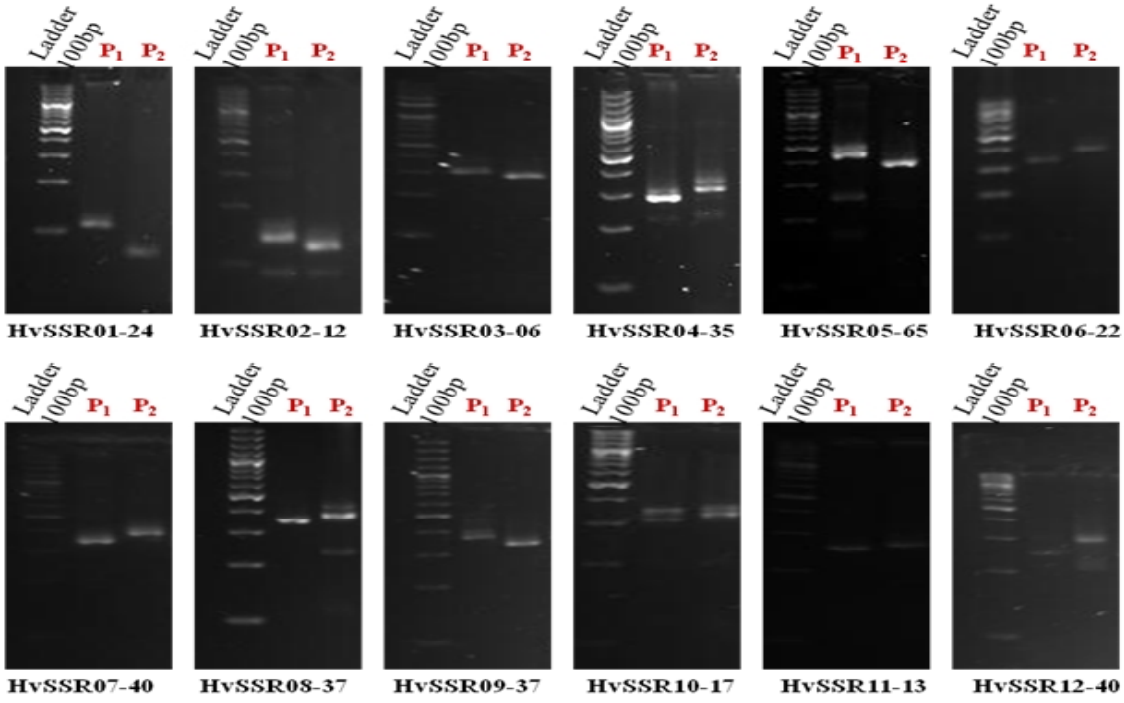

Fig. 2a. Polymorphic banding pattern of SSR markers between the parents Danteshwari (P 1) and Dagad Deshi (P2).

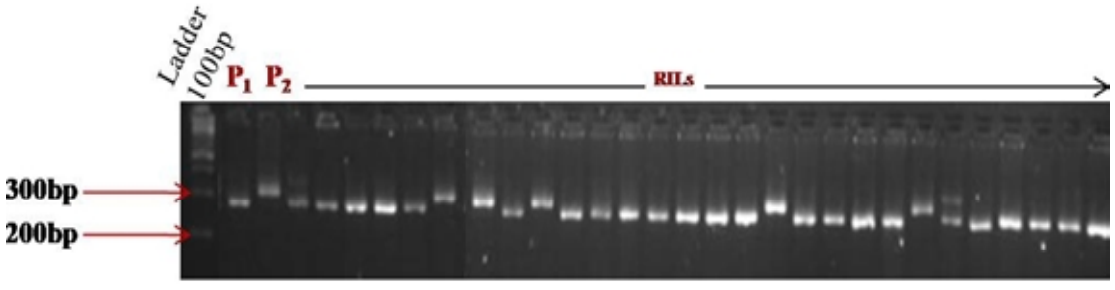

Fig. 2b. PCR amplification profile of HvSSR05-66 SSR marker on genomic DNA of RILs derived from Danteshwari (P1)/ Dagad Deshi (P2).

Single nucleotide polymorphisms (SNPs) are the most abundant form of molecular markers and are markers of choice in plant breeding programs for construction of high-resolution genetic maps and genomic selection (Varshney et al., 2009b). The development of SNP markers facilitates to automatize and enhances ten folds the effectiveness of genotyping. In the present study, out of 36 SNP markers, 11 (30.56 %) markers exhibiting polymorphism between the two parents (Danteshwari/ Dagad Deshi) were used for genotyping of 275 RILs using Sequenom MALDI-TOF MassArray system (Fig. 4). SNP marker analysis indicated low level of polymorphism due to narrow genetic variation between the parents as both were indica ecotypes.

χ^2 test

The χ^2 was performed to test the segregation pattern of two alleles using 104 polymorphic markers. The

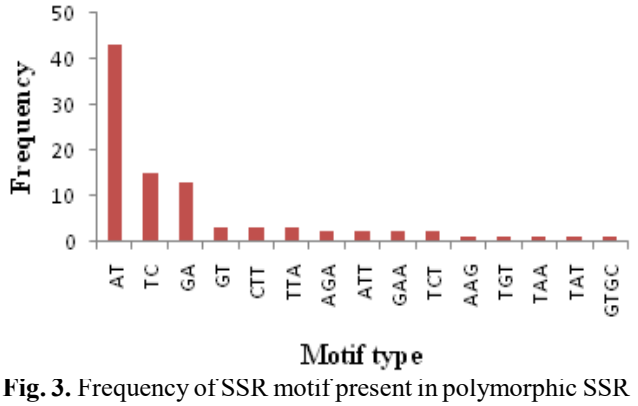

Fig. 3. Frequency of SSR motif present in polymorphic SSR markers.

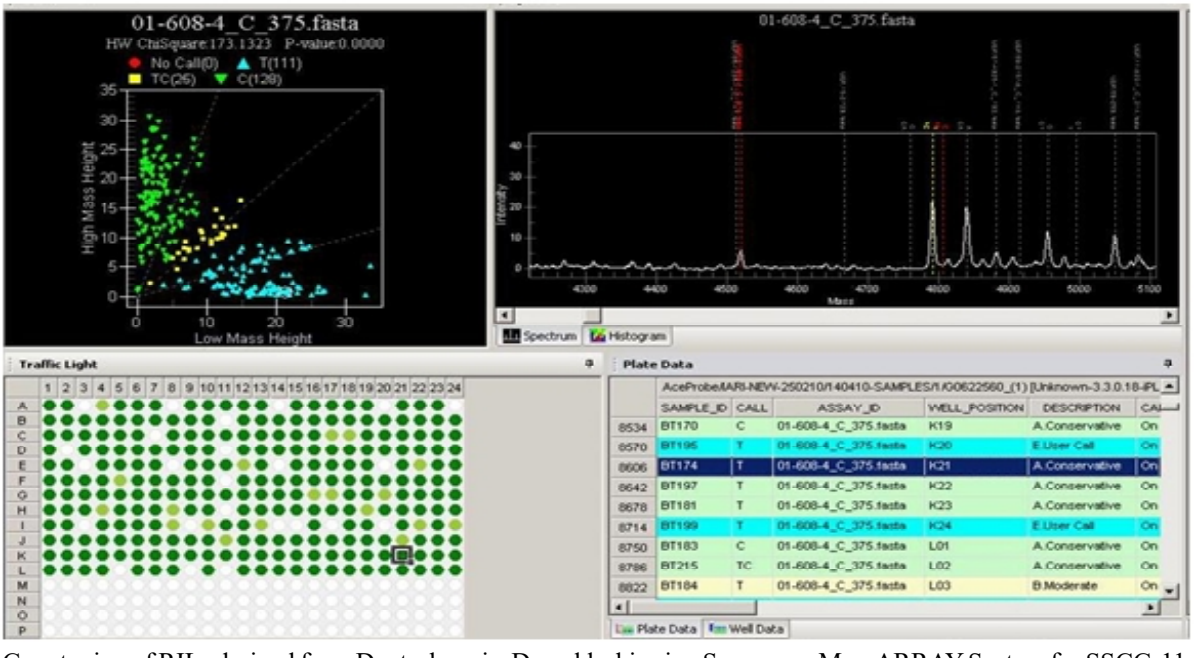

Fig. 4. Genotyping of RILs derived from Danteshwari x Dagaddeshi using Sequenom MassARRAY System for SSCG-11-522-

Table 2a. Polymorphic markers showing expected Mendenlian segregation ratio (1:1) on RILs derived from Danteshwari x Dagad Deshi

S.No.	Marker	Ch#	Allele segregation %			χ^2	Probability
			P1	P2	H		
	HvSSR markers						
1	HvSSR01-10	1	45.65	51.74	2.61	0.88	0.34
2	HvSSR01-55	1	44.23	50	5.77	0.92	0.34
3	HvSSR01-80	1	41.37	51.81	6.83	2.91	0.08
4	HvSSR02-44	2	50.6	42.57	6.83	1.72	0.19
5	HvSSR03-09	3	47.73	48.86	3.41	0.04	0.85
6	HvSSR03-40	3	42.31	48.72	8.97	1.06	0.3
7	HvSSR03-56	3	47.32	52.2	0.49	0.49	0.48
8	HvSSR03-71	3	53.05	42.37	4.58	3.14	0.07
9	HvSSR03-85	3	45.21	44.44	10.34	0.02	0.89
10	HvSSR04-26	4	54.02	45.21	0.77	2.04	0.15
11	HvSSR04-35	4	44.4	42.54	13.06	0.11	0.74
12	HvSSR05-13	5	42.91	52.11	4.98	2.32	0.12
13	HvSSR05-23	5	46.81	47.23	5.96	0	0.95
14	HvSSR05-39	5	52.67	41.98	5.35	2.94	0.09
15	HvSSR05-65	5	46.28	44.63	9.09	0.07	0.78
16	HvSSR05-66	5	40	46.92	13.08	1.43	0.23
17	HvSSR06-22	6	50.64	41.28	8.09	2.24	0.13
18	HvSSR06-30	6	50.61	48.18	1.21	0.15	0.7
19	HvSSR06-56	6	52.57	41.18	6.25	3.77	0.05
20	HvSSR06-65	6	40.75	50.94	8.3	3	0.08
21	HvSSR07-46	7	47.76	43.28	8.96	0.41	0.52
22	HvSSR09-07	9	46.94	41.63	11.43	0.78	0.38
23	HvSSR09-19	9	48.3	50.57	1.13	0.14	0.71
24	HvSSR09-37	9	45.27	48.97	5.76	0.35	0.55
25	HvSSR10-05	10	45.91	50.19	3.89	0.49	0.48
26	HvSSR10-17	10	50.2	45.75	4.05	0.51	0.47
27	HvSSR12-40	12	44.49	51.33	4.18	1.29	0.25
	RGNMS markers						
28	RGNMS341	1	49.63	47.39	2.99	0.14	0.71
	RM markers						
29	RM17	12	51.31	45.32	3.37	0.99	0.32
30	RM21	11	48.94	45.11	5.96	0.37	0.54
31	RM24	1	50.19	45.95	3.86	0.49	0.49
32	RM242	9	47.24	49.61	3.15	0.15	0.7
33	RM28305	12	41.11	42.96	15.93	0.11	0.74
34	RM572	1	50.38	46.24	3.38	0.47	0.49
35	RM7	3	54.75	44.49	0.76	2.79	0.09
	SNP markers						
36	SSCG-01-608-4	1	48.48	42.05	9.47	1.42	0.23
37	SSCG-05-4192-1	5	40.51	47.26	12.24	1.23	0.26
38	SSCG-11-522-1	11	53.03	41.67	5.3	3.55	0.06

P1= Danteshwari, P2= Dagad Deshi, H= Heterozygote, ($\chi^2 > 3.84$, $P < 0.05$)

36.54% markers segregated in the expected 1:1 ratio while 63.46% deviated from the expected 1:1 ratio ($\chi^2 > 3.84$, $P < 0.05$) (Table 2a & 2b). On chromosome 1, highest number of polymorphic markers (20) was identified out of which only 7 markers showed expected 1:1 segregation (Fig. 5). Similarly on chromosome 8 and 10, minimum number of polymorphic markers (5) was identified. Only two showed expected 1:1 segregation on chromosome 8. Marker showed allele segregation deviating from normal and therefore indicated skewed distribution of alleles towards female parent which ranged from 24.24-81.05 % (Table 3).

Table 2b. Polymorphic markers showing deviation from Mendenlian segregation ratio (1:1) on RILs derived from Danteshwari x Dagad Deshi.

S.No	Marker	Ch#	Allele segregation %				
			P1	P2	H	χ^2	Probability
			HvSSR markers				
1	HvSSR01-24	1	50.42	32.35	17.23	9.39	0*
2	HvSSR01-30	1	52.53	34.24	13.23	9.91	0*
3	HvSSR01-33	1	58.49	33.58	7.92	17.85	0*
4	HvSSR01-34	1	26.17	64.06	9.77	40.73	0*
5	HvSSR01-49	1	64.86	26.64	8.49	41.35	0*
6	HvSSR01-86	1	53.91	39.06	7.03	6.07	0.01*
7	HvSSR01-87	1	37.3	52.78	9.92	6.7	0.01*
8	HvSSR01-89	1	57.74	36.6	5.66	12.54	0*
9	HvSSR02-01	2	67.8	26.14	6.06	48.79	0*
10	HvSSR02-12	2	67.23	30.67	2.1	32.49	0*
11	HvSSR02-23	2	57.48	33.46	9.06	16.11	0*
12	HvSSR02-27	2	74.38	24.38	1.24	61.26	0*
13	HvSSR02-78	2	75.31	20.5	4.18	74.94	0*
14	HvSSR03-06	3	57.68	39	3.32	8.69	0*
15	HvSSR03-35	3	55.07	37	7.93	8.04	0*
16	HvSSR03-41	3	57.53	38.81	3.65	7.97	0.04*
17	HvSSR04-32	4	24.24	57.2	18.56	35.2	0*
18	HvSSR04-38	4	52.42	29.44	18.15	16	0*
19	HvSSR04-39	4	56.45	23.39	20.16	33.96	0*
20	HvSSR04-42	4	67.87	22.89	9.24	55.5	0*
21	HvSSR05-12	5	33.2	61.78	5.02	22.26	0*
22	HvSSR05-31	5	51.79	33.47	14.74	9.89	0*
23	HvSSR05-48	5	66.67	26.22	7.12	47.03	0*
24	HvSSR05-51	5	68.68	26.04	5.28	50.87	0*
25	HvSSR05-52	5	64.4	30	5.6	31.34	0*
26	HvSSR05-56	5	55.65	41.84	2.51	4.67	0.03*
27	HvSSR06-35	6	53.67	30.89	15.44	15.9	0*
28	HvSSR06-44	6	64.81	30.74	4.44	32.81	0*
29	HvSSR07-40	7	33.86	61.42	4.72	20.25	0*
30	HvSSR07-43	7	38.77	59.03	2.2	9.53	0*
31	HvSSR07-53	7	81.05	17.34	1.61	102.31	0*
32	HvSSR08-24	8	59.43	37.3	3.28	12.36	0*
33	HvSSR08-29	8	53.52	40.23	6.25	4.82	0.03*
34	HvSSR08-31	8	74.79	20.94	4.27	70.88	0*
35	HvSSR08-37	8	59.16	36.26	4.58	14.4	0*
36	HvSSR09-05	9	28.1	65.7	6.2	36.48	0*
37	HvSSR09-25	9	55.51	39.37	5.12	6.98	0.01*
38	HvSSR09-27	9	58.33	36.9	4.76	12.15	0*
39	HvSSR09-57	9	55.06	41.3	3.64	4.86	0.03*
40	HvSSR10-01	10	34.27	58.06	7.66	15.2	0*
41	HvSSR10-34	10	39.16	56.65	4.18	8.4	0*
42	HvSSR11-01	11	61.96	35.69	2.35	18.03	0*
43	HvSSR11-02	11	59.18	39.7	1.12	10.24	0*
44	HvSSR11-03	11	35.96	59.18	4.87	15.13	0*
45	HvSSR11-13	11	55.02	35.32	9.67	11.56	0*
46	HvSSR12-35	12	42.69	56.52	0.79	4.88	0.02*
47	HvSSR12-36	12	31.4	62.81	5.79	25.33	0*
48	HvSSR12-48	12	70.31	24.61	5.08	56.33	0*
49	HvSSR12-51	12	59.02	31.58	9.4	22.11	0*

Continued.....

Fifty one (37 HvSSR, 2 RGNMS, 6 RM and 6 SNP) out of 66 markers showed 50 % or more female type allele and rest of the markers (15) showed 50 % or more male type allele against the 275 RILs genotyped.

Table 2b. Continued.....

			RGNMS markers				
50	RGNMS115b	1	71.02	28.98	0	43.3	0*
51	RGNMS313	1	59.57	40.43	0	8.42	0*
			RM markers				
52	RM206	11	56.18	36.7	7.12	10.9	0*
53	RM217	6	61	34.36	4.63	19.28	0*
54	RM243	1	54.48	34.33	11.19	12.25	0*
55	RM26334	11	54.62	39.5	5.88	5.79	0.02*
56	RM3471	4	34.12	65.88	0	21.27	0*
57	RM499	1	67.05	30.3	2.65	36.61	0*
58	RM5514	8	55.85	39.25	4.91	7.68	0.01*
			SNP markers				
59	SSCG-01-6351-1	1	59.36	27.49	13.15	29.36	0*
60	SSCG-02-267	2	54.55	35.97	9.49	9.65	0*
61	SSCG-02-3029-1	2	78.88	9.56	11.55	136.38	0*
62	SSCG-03-1691-1	3	73.71	16.33	9.96	91.75	0*
63	SSCG-03-3478-1	3	35.6	47.6	16.8	4.33	0.03*
64	SSCG-04-3787-3	4	70.68	22.09	7.23	63.38	0*
65	SSCG-05-2692-1	5	70.52	14.74	14.74	91.59	0*
66	SSCG-10-1192-7	10	37.97	53.59	8.44	6.31	0.01*

P1= Danteshwari, P2= Dagad Deshi, H= Heterozygote (?2 > 3.84, P < 0.05)

The 66 markers showed distorted segregation on all the 12 chromosomes and the distribution of the marker was 13, 7, 5, 6, 7, 3, 3, 5, 4, 3, 6, and 4, respectively. Subashri et al. (2009) and Gomez et al. (2010) also observed earlier distorted segregation of molecular markers in their mapping populations. The genetic basis of segregation distortion may be the uneven abortion of male or female gametes or the selective fertilization of particular gametic genotypes was described earlier by Xu et al. (1997) and Ali et al. (2000).

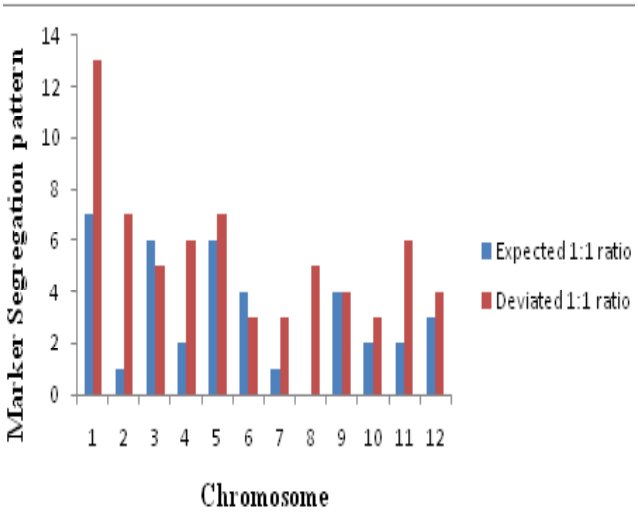

Fig. 5. Segregation pattern of polymorphic markers on 12 rice chromosome.

Our finding using 104 markers against 275 RILs indicated some residual heterozygosity which may probably be attributed to insufficient number of self pollination cycles at the F_7 generation. Segregation of two parental alleles at marker locus showing normal allele segregation showed 0.49 to 15.93 % heterozygosity, where as the heterozygosity ranged from 0 to 20.16 % in marker showing allele segregation deviating from normal (Table 3). Out of 36 markers exhibited10 % or higher heterozygosity. Six out of 36

Table 3. Polymorphic marker segregation for parental types and heterozygosity.

	% Markers with normal allele segregation			
Range	Danteshwari	Dagad Deshi	Heterozygotes	Total no. of Markers
Min	40.00	41.18	0.49	38
Max	54.75	52.20	15.93	
	% Markers with allele segregation deviating from normal at 0.05%			
Min	24.24	9.56	0.00	66
Max	81.05	65.88	20.16	

Table 4. Marker interval with gaps larger than 50 cM on different chromosome.

S.No.	Chrom-osome	Marker interval	Gaps
1	1	HvSSR01-33-HvSSR01-34	53.2 cM
2	1	RGNMS341-HvSSR01-80	58.9 cM
3	4	RM3471-HvSSR04-26	57.6 cM
4	4	HvSSR04-26-HvSSR04-32	83.5 cM
5	6	HvSSR06-56-HvSSR06-65	56.1 cM
6	7	HvSSR07-46-HvSSR07-53	56.0 cM
7	10	HvSSR10-05- HvSSR10-17	57.4 cM
8	10	SSCG-10-1192-7-HvSSR10-34	55.5 cM
9	11	HvSSR11-01-HvSSR11-02	55.9 cM
10	11	SSCG-11-522-1-HvSSR11-13	54.5 cM
12	11	HvSSR11-13-RM26334	54.0 cM
13	11	RM26334-RM21	54.0 cM

markers markers (showing normal allele segregation) (HvSSR03-85, SSCG-05-4192-1, HvSSR04-35. HvSSR05-66, HvSSR09-07 and RM28305), where as 12 markers (RM243, SSCG-02-3029-1, SSCG-01-6351-1, HvSSR01-30, HVSSR05-31, SSCG-05-2692-1, HvSSR06-35, SSCG-03-3478-1, HvSSR01-24, HvSSR04-38, HvSSR04-32 and HvSSR04-39) out of 66 (showing deviation from normal) exhibited 10 % or more heterozygosity in RILs.

The high frequency of heterozygous genotypes would have also imparted to this segregation distortion. Xu (2008) reported the segregation distortion loci (SDL) which express similar to quantitative trait loci (QTL) and have important function in evolution because they control the viability of individuals bearing different genotypes of the locus. Furthfermore, the segregation of marker loci appears to be distorted due to linkage between the neutral markers and the segregation distortion loci. Segregation distortion loci also affect the estimated recombination fractions between marker loci, but it is not clearly understood how segregation distortion loci affect the order of marker loci. Wang et al. (2005) reported that the regions of genome with segregation distortion are more likely to contain QTL. Considering these facts during the course of present investigation, 66 markers which showed segregation distortion were also included for the construction of molecular linkage map and QTL analysis.

Linkage map construction

A linkage map was constructed and 104 markers were assigned to linkage group. Randomly distributed 104 (SSR and SNP) markers across the 12 rice chromosome covered 2665.5 cM in length, representing 622.7 cM, 196 cM, 273.7 cM, 198.7 cM, 254.9 cM, 211.3 cM, 112.7 cM, 133.9, 132.5 cM, 157.7 cM, 293.6 cM, 114.2 cM, respectively for Chromosome 1 to12. There were gaps of more than 50 cM on chromosome 1, 4, 6, 7, 10 and 11 (Table 4, Fig. 6) due to low level of polymorphism and poor genome coverage. This finding therefore indicates that the monomorphic regions are present in the genome of a RIL population derived from Danteshwari/ Dagad Deshi (closely related parents). The large gaps in the linkage map was also found earlier by Price and Tomos (1997), Ali et al. (2000), Amaravathi et al. (2008) and Gomez et al. (2010).

Identification of QTLs associated with root and shoot traits

The present study has generated genotypic data was used in QTL analysis and determination of relative magnitude of their effect on the root and shoot traits in

Table 5. Putative QTLs identified by interval mapping for root and shoot traits in Danteshwari x Dagaddeshi RIL population.

Trait	QTL	Ch. No.	Marker Interval	Additive effect	LOD	R^2
Shoot length	*qshl1-1*	1	RGNMS341-HvSSR1-80	-8.08	2.88	22.35
	qshl1-2	1	HvSSR1-80- HvSSR1-86	-8.09	4.68	22.38
	qshl1-3	1	HvSSR1-86- HvSSR1-87	-5.79	3.97	11.49
Root volume	*qrv6-1*	6	HvSSR6-35- HvSSR6-44	-1.99	2.63	7.43
Shoot dry weight	*qsdw5-1*	5	HvSSR5-12- HvSSR5-13	-1.58	3.15	9.59
	qsdw10-1	10	SSCG-10-1192-7-Hv1034	2.64	2.66	27.49
Shoot fresh weight	*qsfw5-1*	5	HvSSR5-12- HvSSR5-13	-4.12	3.34	10.25
Total Plant length	*qtpl1-1*	1	RM572-HvSSR1-49	-7.71	2.72	15.38
	qtpl1-2	1	HvSSR1-80-HvSSR1-86	-9.06	4.74	21.87
	qtpl1-3	1	HvSSR1-86- HvSSR1-87	-6.43	3.77	11.03
	qtpl1-4	1	HvSSR1-86- SSCG-01-6351-1	-7.33	2.77	13.74
	qtpl4-1	4	RM3471- HvSSR4-32	-4.08	2.62	4.41

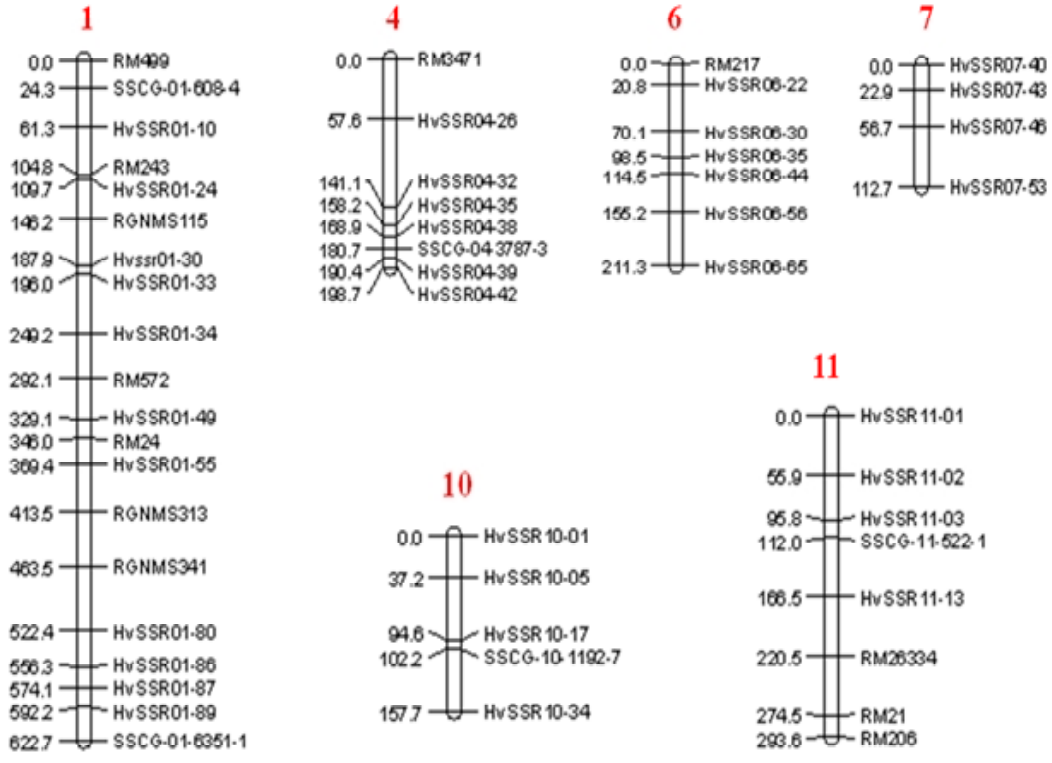

Fig. 6. Linkage map of rice chromosome with gaps larger than 50 cM on the recombinant inbred lines derived from a cross between Danteshwari and Dagad Deshi.

rice. Our previous result suggests that single marker analysis is a simplest analysis of genomic regions associated with traits of interest but it can not explain recombination fraction and QTL effect, gives false positive result and has low QTL detection power (Sinha et al., 2015b). Therefore, interval mapping was performed using the same data to overcome the disadvantages of single marker analysis. Interval mapping is a commonly used method because each marker interval is analyzed independently. Single marker analysis identified 5 QTLs putatively associated to three traits on chromosome 1, 4 and 5 whereas a total of 12 putative QTLs were detected for various traits on chromosome 1, 4, 5, 6 and 10 explaining 4.41% to 27.49% phenotypic variance in case of interval mapping (Table 5, Fig. 7). Our previous study detected fourteen putative QTLs for 7 root and shoot traits on chromosomes 1, 2, 4, 5, 6 and 8 by following composite interval mapping (Sinha et al., 2015a). Our data indicated that a shift in the analysis methods from single marker analysis to interval mapping and composite interval mapping on the same raw data led to different conclusions on the number of QTLs and result is in support of the previous studies conducted by Shen et al. (2001). In all methods, higher number of QTLs identified for shoot traits as compared to root traits. This is because it is much easier to phenotype shoot traits than root. The result from present investigation depicting that for total plant length, 5 putative QTLs identified (*qtpl1-1, qtpl1-2, qtpl1-3, qtpl1-4, qtpl4-1*) whereas, 3 for shoot length (*qshl1-1, qshl1-2, qshl1-3*), 2 for shoot dry weight (*qsdw5-1, qsdw10-1*), 1 for root volume (*qrv6-1*) and shoot fresh weight (*qsfw5-1*). But at a threshold limit of 2.5, we fail to detect QTL for root length, root fresh weight, root dry weight, tiller number, root: shoot fresh weight ratio, root: shoot dry weight ratio and root: shoot length ratio. Additionally, we found favorable alleles in the QTL for all the traits except shoot dry weight were contributed by the water stress susceptible parent Danteshwari. A work of Lanceras et al. (2004) suggests positive alleles for the trait were contributed by the stress-susceptible

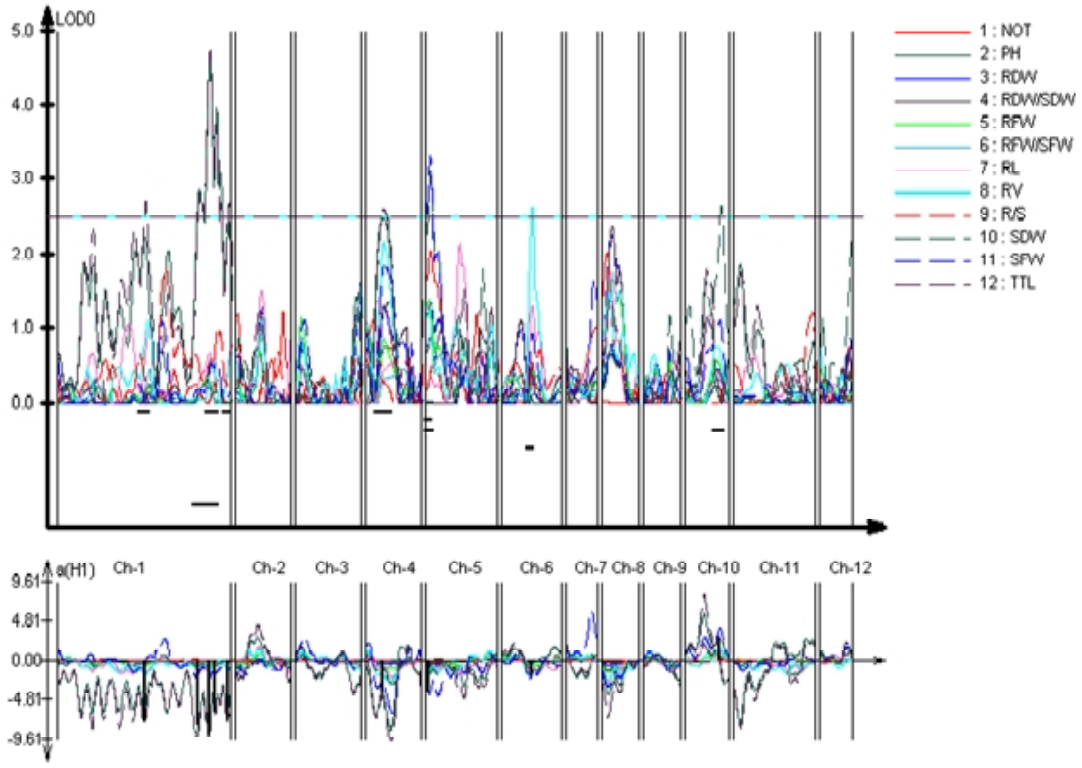

Fig. 7. QTL likelihood curves indicating putative QTLs associated with one root and five shoot traits following interval mapping analysis with a default cutoff LOD score of 2.5.

parents.

In our study, chromosome 1 was associated with shoot length and total plant length. Kumar et al., (2011) has reported that chromosome 1 (RGNMS341-SSCG-01-6351-1) was frequently been associated with grain yield and various drought response traits. Marker interval RM3471- HvSSR04-32 on chromosome 4 was associated with QTL for total plant length. QTLs for plant-type traits such as tiller number or plant height were observed in some populations in this region (Gomez et al., 2010). Marker interval HvSSR05-12 - HvSSR05-13 on chromosome 5 was associated with shoot fresh weight and shoot dry weight. Yadav et al. (1997), Kamoshita et al. (2002b) and Price et al. (2002b) reported the QTL for root thickness and root weight (RW) on chromosome 5. The marker interval HvSSR06-35 - HvSSR06-44 on chromosome 6 was associated with root volume. Ray et al. (1996) reported QTLs for root penetration on chromosome 6. Chromosome 10 had associated with shoot dry weight. Courtois et al. (2000) also reported QTL for tiller number and shoot dry weight on chromosome 10.

CONCLUSION

Our result suggests that the QTLs detected on different chromosome, therefore root and shoot traits are governed by multiple loci. Further, the detection of QTL is based on statistical model used in the analysis. The validation of more number of SSR and SNP markers can provide better genome coverage and greater arsenal of tools for QTL mapping and marker assisted selection. QTLs very often exhibits high QTL x E interaction, the detected QTLs need to be cross validated across the different environment and crosses along with the cross validation of linked molecular markers.

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