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A QTL approach to determine the association between yield and lodging traits and molecular markers

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Abstract
A set of 32 rice genotypes were analyzed with 23 molecular markers. The analysis was performed by using single factor ANOVA in MS Excel software and the association between genotypic and phenotypic data was established. The significance of marker trait associations were identified by a *P-value* (<0.05) and degree of association was exhibited by phenotypic variance in per cent value. Result exhibited that a total of 11 molecular markers were significantly associated with different lodging, yield and related traits traits. RM1 on chromosome 1 exhibited the pleiotropic effect for plant height, 1000 grain weight, length of 4th internode from the top and RM 168 exhibited the pleiotropic effect for plant height, and basal internode length.

Keywords: Molecular marker, single marker analysis, quantitative traits, pleiotropic effect.

1. Introduction
After selection of a germplasm which has been identified as possessing a valuable trait for further breeding programme, the incorporation of this valuable trait in economically superior genotype is a big question. The answer largely depends on the genetic nature of the trait. Study of genetic nature of a trait could be done by establish the marker-trait association by using phenotypic data and genotypic data from molecular markers. It helps in determination of number and nature of a gene or QTL controlling a trait. To establishment of marker-trait association, data analysis approaches include single marker analysis, simple interval mapping (SIM), multiple interval mapping (MIM), and composite interval mapping (CIM). These all approaches are designated for QTL analysis (Collard *et al.*, 2005) [2]. This article focuses on single marker analysis.

Detecting marker-trait association by single marker analysis is a simple method that can be accomplished with any standard statistical analysis software package, and has the potential to identify numerous significant markers associated with the traits. Two important factors should be considered when performing the statistical analysis. The first consideration is sample size, a large sample size provides the opportunity to observe more recombinant events and to estimate parameters with greater accuracy and, therefore, a greater ability to detect QTL through a single-marker analysis. The second factor is the problem of multiple testing and arises when a large number of markers are investigated through independent statistical tests. This problem is coupled with the level of statistical significance that is used by the investigator and can lead to detection of incorrect QTL. This problem could be solved by multiple test adjustment (Doerge, 2002) [1].

Single-marker analysis is widely used for identify the markers that are segregating with a trait. Most of these applications deal primarily with detecting a single marker, rather than genomic regions, and are a quick and efficient method for to screening of large populations for specific traits.

2. Material and Methods
The experiment was conducted at Research cum Instructional Farm and MAS laboratory, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidalaya, Raipur, Chhattisgarh, India. The experimental materials adjoin of 32 genotypes of rice inclusive of four checks *viz*., IR 64, Indira aerobic 1, Pusa 1121 and Dubraj selection 1. The list of genotypes of rice is presented in Table 2.1. Different traits related to lodging and yield included in the study.

Leaves from young seedling were collected for DNA isolation and the DNA isolation was performed by using CTAB method suggested by Doyle and Doyle (1990) [3]. To accurately

assess sample quality, 260/280 ratios were analyzed in combination with overall spectral quality. Prior information was used to select the traits linked markers. A set of 23 molecular markers (Table 2.2) linked to stem diameter, yield and yield related traits were selected based on the mapping studies conducted and the results that have been documented in rice (Cho *et al.* 2003, Ookawa *et al.* 2010 and Yano *et al.* 2015) [4, 5, 6]. Polyacrylamide gel electrophoresis technique was used fir visualization of amplified products. After obtaining the gel picture, genotypic data was produced by scoring of bands.

Statistical analysis of single marker data: The fact that molecular marker genotypes can be classified into groups means that marker genotypes can be used as classifying variables for a t-test or ANOVA, or as variables for regression analysis. In this study analysis was done by using single factor ANOVA with the help of MS Excel software programme. The significance of marker trait associations were identified by a *P-value* (<0.05) and degree of association was exhibited by phenotypic variance in per cent (R^2%) value. Single marker analysis calculates whether phenotype values differ among genotypes for a given molecular marker.

Table 2.1: List of genotypes used in study

S.N.	Accessions	S.N.	Accessions
1	IC 459207	17	Morobreaken
2	IC 459643	18	Badshah Bhog (B:2504)
3	IC 125622 (Dashehra matiya)	19	Chini Kapoor (C:30 ll)
4	IC 125666 (Deshi safri)	20	Dubraj (D:1284)
5	IC 378466 (Luchai)	21	Dudh Nag (D:668 l)
6	Jasmine Scented	22	Dudi Kanth (D:839)
7	Dudhkhasa	23	Ganga Baroo (G:230)
8	Malagkit sung song	24	Ganga Prasad (G:230)
9	Amajhopa (A:200)	25	Ganjo (G:1035)
10	Katina (K:1591)	26	Garra Kat (G:113 ll)
11	Pratiksha	27	Rajim-14 (R:170 lV)
12	Ganga Godavari	28	Shri Kamal (S:663 l)
13	Basha Bhog	29	IR-64
14	Shree Ram	30	Indira Aerobic 1
15	Parewa Dhan (P:469 lV)	31	Pusa-1121
16	Pihi kirwa (P:368)	32	Dubraj Selection 1

Table 2.2: List of molecular markers used for varietal polymorphism and marker –trait association.

S.N.	Marker name	Forward Sequence	Reverse Sequence	Chromosome	Traits	Expected product size	Annealing temperature (^{0}C)
1	RM15764	aacatcctgtaacctgaacgg	attgcaaccttacttcaacgg	3	SC	150	58.50
2	RM15767	ttgctgcagattctctctgatcc	aagggaggaaggaaacatcacg	3	SC	284	61.90
3	RM20546	tgagcaggagacggggacagc	tatccgtttctgcaacgctacgc	6	SC	169	57.50
4	RM20562	gggttaattaggtttgctcgctacc	cgacaagtattatctgcccagaagg	6	SC	450	55.00
5	RM20557	tcatgagggttatgggagtcaagg	agtggtcgttgaaatccttgtgc	6	SC	295	55.80
6	InDel-2	catcttaccaagctataccaagt	ggtgtctctagcttcatgttaat	3	SC	320	48.60
7	InDel-8	aacgtaacttttcgtttttcata	gctaactacttactccctccttc	3	SC	140	45.10
8	RM472	ccatggcctgagagagagag	agctaaatggccatacggtg	1	TGN	296	52.40
9	RM208	tctgcaagccttgtctgatg	taagtcgatcattgtgtggacc	2	GW	173	51.70
10	RM168	tgctgctgcctgcttccttt	gaaacgaatcaatccacggc	3	TLN, PLH	116	52.30
11	RM30	ggttaggcatcgtcacgg	tcacctcaccacacgacacg	6	SSP	105	53.80
12	RM214	ctgatgatagaaacctcttctc	aagaacagctgatctcacaa	7	PLH	112	46.90
13	RM228	ctggccattagtccttgg	gcttgcggctctgctt	10	GW	154	50.30
14	RM21	acagtattccgtaggcacgg	gctccatgagggtggtagag	11	SPY	157	54.30
15	RM18	ttccctctcatggactccat	gagtgcctggcgctgtac	5	TGW	157	52.50
16	RM1	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac	1	GW	157	47.70
17	RM5	tgcaacttctagctgctcga	gcatccgatcttgatggg	1	GPP	113	51.00
18	RM213	atctgtttgcaggggacaag	aggtctagacgatgtcgtga	2	GPP	139	52.00
19	RM273	gaagccgtcgtgaagttacc	gtttcctacctgatcgcgac	4	NPP	207	53.50
20	RM302	tcatgtcatctaccatcacac	atggagaagatggaatacttgc	1	GW	156	48.60
21	RM303	gcatggccaaatattaaagg	ggttggaaatagaagttcggt	4	SSP	200	48.60
22	RM411	acaccaactcttgcctgcat	tgaagcaaaaacatggctagg	3	GS	110	50.60
23	RM520	aggagcaagaaaagttcccc	gccaatgtgtgacgcaatag	3	GW	247	51.80

3. Results and discussion

From the result of scoring a total of 45 alleles with the average of 1.95 alleles per locus were detected in 32 genotypes of rice. The highest number of alleles (2) was recorded for 12 markers *viz.* RM 214, RM 18, RM 208, RM 228, RM 472, InDel-8, RM 213, RM 273, RM 302, RM 411, RM 520 and RM 303 whereas, the lowest number of alleles (3) was recorded for five markers *viz.* RM21, RM168, RM1, RM5 and InDel-2.

A total of 11 markers showed significant marker-trait association (*P*<0.05) with different traits (Table 3.1). Highest marker traits association was obtained between number of productive tiller per plant and RM5 (R^2 = 35.6%) whereas lowest association was observed between panicle dry weight per plant and RM 303 (R^2 = 12.29%). Grain yield per plant was the most important trait of present study and InDel-2 was found associated with this trait. RM208 located on chromosome 2 was associated with spikelet fertility. 1000

grain weight is one of the most important trait related to yield and was found associated with RM1 and RM 303. RM 303 and RM 273 were found associated with basal internode diameter and RM 273 was also linked with diameter of 4th internode from the top. Significant marker-trait association was found between basal internode length and RM 168 and length of 4th internode from the top and RM 1.

RM1 on chromosome 1 exhibited the pleiotropic effect for plant height, 1000 grain weight, length of 4th internode from the top and RM 168 exhibited the pleiotropic effect for plant height, and basal internode length. RM 303 showed pleotropic effect for plant height, culm length, 1000 grain weight and basal internode diameter. It reveals that more than 1 marker were segregating with some traits in the population. To observe more recombinant events and to estimate parameters with greater accuracy, a larger population could be used

Table 3.1: Association between molecular markers and different agronomic traits

Traits	Markers	P value	R^2 % value
Plant height	RM1	0.01	30.09
	RM168	0.015	25.86
	RM303	.04	12.75
Culm length	RM1	.01	26.96
	RM168	0.033	21.57
	RM303	0.04	12.90
Productive tillers/plant	InDel-2	0.01	29.62
	InDel-8	0.002	35.30
	RM5	0.004	35.6
Panicle dry weight/ plant	InDel-2	0.03	23.35
	RM303	0.04	12.20
Paddy length	InDel-2	0.01	18.05
	RM520	0.001	32.10
1000 grain weight	RM1	0.02	26.70
	RM303	0.02	15.73
Flag leaf length	RM21	0.002	35.26
	RM303	0.005	23.30
Basal internode diameter	RM303	0.003	21.60
	RM273	0.001	30.40
$4^{th}_{(from\ top)}$internode diameter	RM273	0.01	17.18
Basal internode length	RM168	0.006	18.20
$4^{th}_{(from\ top)}$ internode length	RM1	0.02	26.96
Upper biological yield	InDel-2	0.04	20.80
Grain yield/ plant	InDel-2	0.02	25.26
Total spikelets/ panicle	InDel-8	0.01	19.47
Filled spikelets/ panicle	InDel-8	0.008	22.10
Spikelet fertility %	RM208	0.008	22.33
Number of internodes/ plant	RM273	0.01	18.29

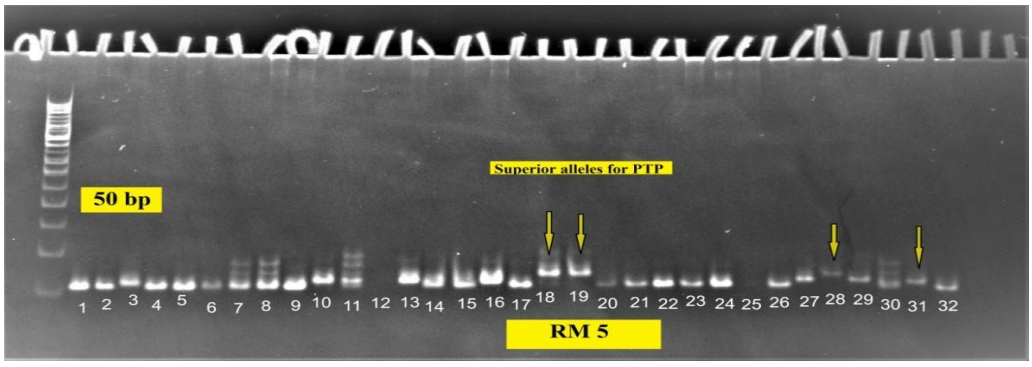

Fig 1: Banding pattern of RM 5 marker

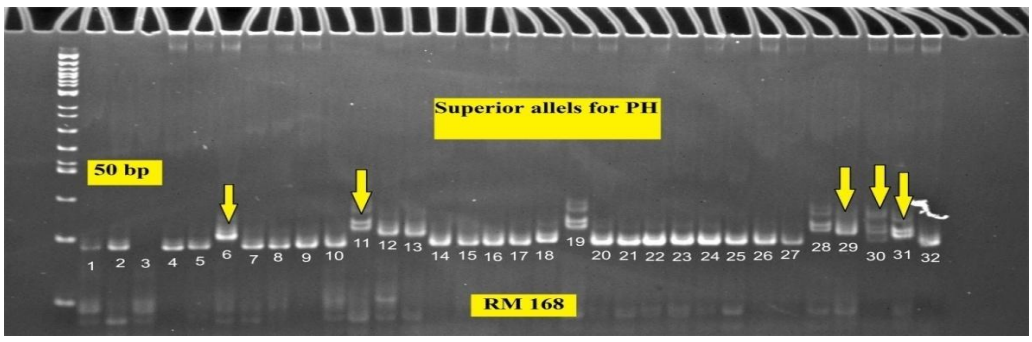

Fig 2: Banding pattern of RM 168 marker

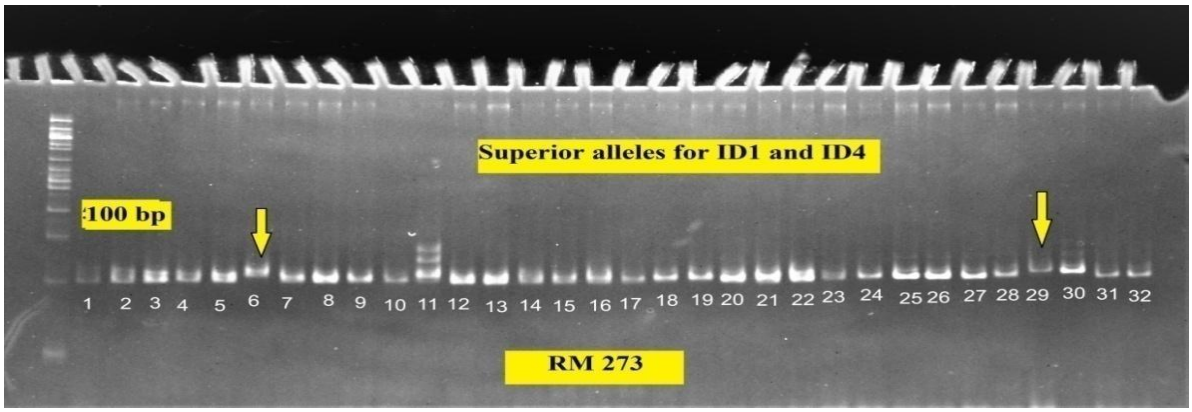

Fig 3: Banding pattern of RM 273 marker

4. Conclusion

Single marker analysis is a very easy and useful method for establishment of marker-trait association. On the basis of association of markers and quantitative traits found in this study, the result provides a very good frame for future breeding programme. Out of 23 molecular markers 11 were linked to the traits in study and some of them exhibited pleotropic effect. It makes easy to select markers for future programme and selection of genotypes became easy in short duration. 45 alleles with the average of 1.95 alleles per locus were detected in 32 genotypes of rice. A total of 11 markers showed significant marker-trait association. RM 1, RM 168 and RM 303 exhibited pleotropic effect.

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