GENETIC ANALYSIS AND VALIDATION OF TRAIT SPECIFIC SSR MARKERS FOR YIELD AND ITS ATTRIBUTING TRAITS IN F_3 FAMILY OF IM 90/ IR 64 CROSS IN RICE (*Oryza sativa* L.) UNDER AEROBIC CONDITION

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INTRODUCTION

Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, Gramineae. It is one of the three major food crops of the world and forms the staple diet of about half of the world's population. The global production of rice has been estimated to be at the level of 650 million tones. Asia is the leader in rice production accounting for about 90% of the world's production (FAOSTAT, 2013). Hybridization is one of the way through which enormous variability can be created (Shailaja Hittalmani and Shivashankar, 1987). It has been postulated that advancement in breeding programme depends on extent of variability created during hybridization (Shantavva *et al.*, 2014). Segregating populations are more important for improving plant types by operating selection (Farhad Kahani and Shailaja Hittalmani 2015). Therefore the acquaintance of genetic parameters present in segregating population for the improvement of character under study has paramount importance. Phenotypic coefficient of variation(PCV), genotypic co-efficient of variation (GCV), heritability, genetic advancement will be useful for efficient exploitation of variability (Shantavva *et al.*, 2014).The genotypic coefficient of variation measures the range of variability available in a crop and also enables to compare the amount of variability present in different characters. The phenotypic expression of the character is the result of interaction between genotype and environment. Hence, the total variance needs to be partitioned into heritable and non-heritable components to assess the inheritance pattern of the particular character under study. Heritability indicates the relative degree at which a character is transmitted from parent to offspring. Heritability estimates along with GA are normally more helpful in predicting the gain under selection than heritability estimates alone. As both will serve as important selection parameters (Ogunbayo *et al.*, 2014). One of the main constraints of rice cultivation and production is water shortage during periods of low rainfall, which affects the vegetative growth rate and grain yield (Manjappa *et al.*, 2014).Aerobic rice cultivation is one of the alternative wherein the water required is half of that of irrigated puddled rice, without compromising the productivity (Gandhi *et al.*, 2011a;Gandhi *et al.*, 2011b;Nareshbabu *et al.,* 2010).It has been reported that, validation of markers helps in determining the reliability and practical applicability of the markers in predicting the phenotype (Collard *et al.*, 2005).Among the markers SSR markers are considered as marker of choice,because it has the advantage of being co-dominant; they are simple PCR based and extremely polymorphic. They are also highly informative due to number and frequency of alleles detected and ability to distinguish between closely related individuals. They find application as marker for mapping, cultivar identification, protecting germplasm, determination of hybridity, analysis of gene pool variation and as diagnostic marker for traits of economic value (Powell *et al.*, 1996).In this context the present investigation was undertaken to evaluate the genetic variability present in F_3 segregating populations and also to validate trait specific SSR markers linked to yield and related traits in rice.

ABSTRACT

Thirty F_3 families derived from IM 90/ IR 64 cross in rice were evaluated under aerobic condition for 14 quantitative traits to assess the genetic parameters and validate SSR markers linked to yield related traits. The estimates of GCV were lower than the estimates of respective PCV, indicating the influence of environmental factors on the expression of the traits studied. Further the traits like spikelet fertility (94.99%, 14.62), plant height at maturity (91.22 %, 12.09) and Days to 50% flowering (81.45%,12.20) have high heritability coupled with moderate genetic advance as percent mean. Indicates greater role of additive gene action in their inheritance. The results of correlation revealed positive and significant correlation of grain yield per plant with productive tillers per plant (r = 0.490), spikelet per panicle(r = 0.422), harvest index (r = 0.369) and biomass(r = 0.294). Whereas, the highest direct effects on grain yield per plant by productive tillers per plant (1.083), biomass(2.76) and panicle weight(2.22) was observed indicated that during selection these traits has to be given prime importance. Further the six validated SSR markers associated with yield related traits could be useful for selection in segregating populations of rice.

MATERIALS AND METHODS

Plant material

The thirty F$_3$ families along with parents and checks were evaluated during *kharif* 2013, in augmented design (Federer 1965), under aerobic conditions in K-block, Dept. of Genetic and Plant Breeding, UAS, Bangalore which represents the eastern dry zone, with all proper crop management practices. The spacing was kept at row-row 20 cm and plant to plant 25 cm.

Aerobic rice cultivation

Aerobic rice cultivation involves direct sowing of seeds in un-puddled land with a spacing of 30 cm × 25 cm. Irrigation was given at 5 days interval to maintain soil moisture at field capacity throughout the crop growth. The soil is maintained under aerobic condition unlike the anaerobic condition in puddled soils with standing water in irrigated transplanted rice. During reproductive stage the irrigation is given once in 3 days interval.(Raghavendra and Shailaja Hittalmani 2015).

Observations recorded

In order to obtain the information on the performance of families, 20 randomly selected plant data was recorded for 14 quantitative traits as per SES 1996. Among those growth traits like plant height at 30days after transplanting, plant height at maturity, days to 50 % flowering, total number of tillers per plant and spikelets per panicle. Yield traits like productive tillers per plant, spikelet fertility, panicle length, panicle weight, grain yield per plant, 1000 grain weight, biomass, harvest index were recorded.

DNA extraction and SSR marker analysis

Leaf samples of twenty five days old seedlings were used for DNA extraction. DNA was isolated as Dellaporta method (Dellaporta, *et al.*, 1983).Total of 500 rice microsatellite markers used for screening IM 90 and IR 64 for polymorphism survey. Further, twenty reported SSR markers associated with yield and related traits, with high LOD score markers were used for genotyping of F$_3$ progenies (Table 1). PCR amplification was carried out following the standard procedures. The bands generated by polymorphic SSR primers were scored by giving code 'A' for Parent-1 (IM 90) (upper/ lower band), 'B' for Parent-2 (IR64) (upper/ lower band) and – for missing samples (Fig.1).

Statistical analysis

The data recorded on 20 randomly selected plants were subjected to ANOVA (Panse and Sukhatme, 1967). Variance, heritability and genetic advance (Robinson and Comstock, 1949) were estimated. Single marker analysis was done with the help of "student's" t-distribution and one-way analysis of variance (Edwards *et al.,* 1987).

RESULTS AND DISCUSSION

ANOVA

The mean sum of squares due to families showed significant difference for 14 quantitative traits (Table 2) indicates the presence of adequate genetic variability (Paikhomba *et al.,* 2014). This would prove to be beneficial as available variability could be further utilized in crop improvement programme.

Table 1: List of SSR trait specific markers used for validation for yield and its contributing traits in Rice

Sl. No	Marker	Chromo some No.	SSR marker linked traits	Forward primer 5' – 3'	Reverse primer 5' – 3'	Expected base pair	References
1	RM24	1	Plant height (cm)	GAAGTGTGATCACTGTAACC	TACAGTGGACGGCGAAGTCG	193	Price *et al.* (2002)
2	RM 243	1	Root dry weight (g)	GATCTGCGAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC	116	Marriet *al.* (2005)
3	RM443	1	Test weight (g)	GATGTTTTCATCGGCTACG	AGTCCCAGAATGTCGTTTCG	124	Price *et al.* (2002)
4	RM493	1	Spikelet fertility (%)	TAGCTCCAACAGGATCGACC	GTAGTAAACGCGGAAGGTG	211	Lauxman *et al.* (2010)
5	RM495	1	Plant height (cm), spikelet fertility (%)	AATCCAAGGTGCAGAGATGG	CAACGATGACGAACACAACC	159	Yueet *al.* (2005)
6	RM594	1	Root to shoot ratio	GCCACCAGTAAAAGCAATAC	TTGATCTGCTAGTGAGACCC	300	Marriet *al.* (2005)
7	RM208	2	Panicle length (cm), number of spikelets per panicle	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC	173	Venuprasadet *al.* (2009)
8	RM263	2	Grain yield (stress) (g)	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	207	Zhang *et al.* (2001)
9	RM131	4	Grain yield (g)	TCCTCCCTCCCTTCGCCCCACTG	CGATGTTCGCCATGGCTGCTCC	215	Yueet *al.* (2006)
10	RM471	4	Days to 50% flowering	ACGCACAAGCAGATGATGAG	GGGAGAAGACGAATGTTTGC	106	Marriet *al.* (2005)
11	RM248	7	Root dry weight (g), Total root weight (g),days to 50% flowering	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG	102	Price *et al.* (2002)
12	RM152	8	Spikelet fertility (%)	GAAACCACCACCTCACCG	CCGTAGACCTTCTTGAAGTAG	NA	Xing *et al.* (2002)
13	RM337	8	Plant height (cm)	GTAGGAAAGGAAGGGCAGAG	CGATAGATAGCTAGATGTGGCC	192	Lauxman *al.* (2010)
14	RM447	8	Test weight (g)	CCCTTGTCGTCTTCCTCTC	ACGGGCTTCTTCTCCTTCTC	111	Lauxman *al.* (2010)
15	RM3262	8	Grain yield (g)	CTGGAGATGCAGATCCTCAACC	TAGTACAACATGGGAGCCTGTCG	290	Lafitte *et al.*2002
16	RM205	9	Total number of tillers	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG	122	Lauxman *et al.* (2010)
17	RM242	9	Grain yield (g), root length (cm)	GGCCAACGTGTGTATGTCTC	TATATGCCAAGACGGATGGG	225	Marriet *al.* (2005)
18	RM596	10	Grain yield (stress) (g)	ATCTACACGGACGAATTGCC	AGAAGCTTCAGCCTCTGCAG	188	Lauxman *al.* (2010)
19	RM4	12	Productive tillers	TTGACGAGGTCAGCACTGAC	AGGGTGTATCCGACTCATCG	NA	Venuprasadet *al.* (2009)
20	RM519	12	Plant height (cm), productive tillers	AGAGAGCCCCTAAATTTCCG	AGGTACGCTCACCTGTGGAC	122	Venuprasadet *al.* (2009)

Table 2: Analysis of variance (mean sum of squares) for 14 quantitative characters studied in F₃ families of IM 90 / IR 64 Rice cross

| Source of variation	Progenies	Error
Degrees of Freedom (df)	29	10
Days to 50% flowering	28.39***	3.63
Plant height at 30 days after transplanting (cm)	10.05***	5.18
Plant height at maturity (cm)	16.39***	0.98
Productive tillers plant $^{-1}$	3.97*	1.58
Total number of tillers plant $^{-1}$	1.59*	0.43
Spikelets per panicle	6.15***	5.15
Spikelet fertility (%)	3.17***	1.12
Panicle length (cm)	3.44*	1.21
Panicle weight (g)	0.53*	0.38
Days to maturity	50.96*	25.26
Grain yield plant $^{-1}$ (g)	12.54*	5.16
1000-grain weight (g)	4.1*	0.64
Biomass (g)	53.54**	7.11
Harvest index	0.00*	0.00
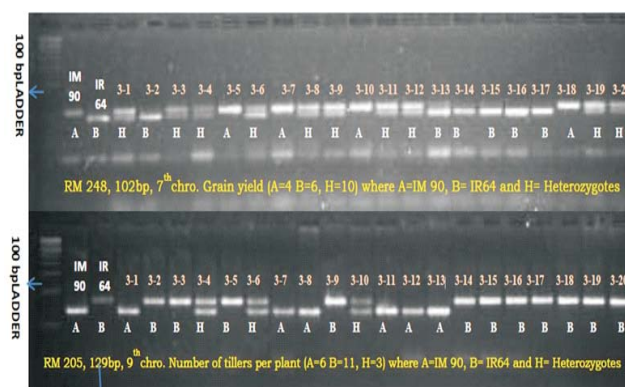
Figure1: Characterization of F₃ progenies of IM 90 X IR 64 cross in rice using SSR markers RM 248 linked to QTL's governing grain yield and RM 205 linked to QTL's governing number of tillers per plant.

Table 3:Estimates of genetic parameters for yield and its attributing traits in F₃families of IM 90 / IR 64 of rice cross

Sl. No.	Plant traits	Range Max.	Min.	Mean	PCV%	GCV%	h²(bs)%	GAM
1	Days to 50 % flowering	116.00	94.65	103.37	4.28	3.86	81.45	12.20
2	Plant height at 30 DAT (cm)	40.80	29.70	35.42	17.50	16.55	76.25	15.11
3	Plant height at maturity (cm)	74.00	59.75	66.83	15.02	14.79	91.22	12.09
4	Productive tillers per plant	13.00	8.75	10.50	10.40	8.05	73.14	11.10
5	Total tillers per plant	15.00	8.00	12.15	8.89	7.07	63.24	14.84
6	Spikelets per panicle	94.30	85.15	90.23	2.24	2.14	81.32	15.40
7	Spikelet fertility (%)	88.58	80.88	84.66	1.84	1.79	94.99	14.62
8	Panicle length (cm)	21.00	15.95	18.56	15.83	13.77	41.90	6.45
9	Panicle weight (g)	5.00	1.06	3.26	21.33	19.65	20.48	11.53
10	Days to maturity	146.60	116.25	132.66	14.87	13.06	39.60	17.09
11	Grain yield per plant (g)	46.45	21.70	29.10	20.79	17.45	47.66	19.58
12	1000 grain weight (g)	29.92	20.84	22.25	17.63	16.72	51.08	17.56
13	Biomass (g)	82.75	37.60	63.63	21.50	19.33	32.75	21.45
14	Harvest index	0.52	0.31	0.47	16.22	11.59	44.32	18.87

Min.	Minimum	h²(bs)%	Broad sense heritability
Max.	Maximum	GAM	Genetic advance as percent mean
S.E	Standard error	PCV (%)	Phenotypic coefficient of variation (%)
		GCV (%)	Genotypic coefficient of variation (%)

The estimates of genetic parameters like mean, coefficient of variation, heritability and genetic advance as percent mean deserve attention in deciding selection criteria for improvement in the concerned characters. (Debnath *et al.,* 2015)

Mean and Range

The mean performance of families for different traits are noticed as follows days to fifty per cent flowering ranged between 94.65 to 116 days with a mean of 103.37 days, Plant height at 30 days after transplanting varied between 29.70 to 40.80 cm with a mean value of 35.42 cm, plant height varied from 59.75 to 74.00 cm with a mean value of 66.83 cm, productive tillers per plant varied from 8.75 to 13.00 with a mean value of 10.50, total number of tillers per plant varied between 8.00 to 15.00 with a mean value of 12.15.Spikelets per panicle varied from 85.15 to 94.30 with a mean value of 90.23 cm, spikelet fertility ranged from 80.00 to 88.58% with a mean value of 84.66%, panicle length ranged from 15.95 to 21.00 cm with a mean value of 18.56cm, panicle weight varied from 1.06 to 5.00 g with a mean value of 3.26 g.

Days to maturity varied between 116.25 to 146.30 days after transplanting with a mean value of 132.66 days, grain yield per plant varied between 21.70 to 46.45 g with a mean value of 29.10 g, 1000 grain weight varied between 20.84 to 29.92 g with a mean value of 22.25 g, biomass ranged from 37.60 to 82.75 g with a mean value of 63.63 g, harvest index varied from 0.31 to 0.72 with a mean value of 0.47%.This finding corroborated with Kumar *et al.,* 2015

PCV and GCV

The estimates of GCV were lower than the PCV estimates (Khalid *et al.,* 2012 and Gangashetty *et al.,* 2013, Paikhombha *et al.,* 2014) indicating there is influence of environmental factors on the expression of traits (Table 3). The PCV estimates ranged from 1.84 to 21.50% and GCV estimates ranged from 1.79 to 19.65%.

Highest GCV was recorded for panicle weight (19.65) followed by Biomass (19.33), grain yield per plant (17.45) and 1000 grain weight (16.72), highest PCV is recorded for traits are Biomass (21.50), panicle weight (21.33), grain yield per plant (20.79), and1000 grain weight (17.63) indicated presence of

Table 4: Correlation and direct effects of 14 quantitative traits on grain yield per plant of families derived from IM 90/IR 64 cross of rice

Traits	Correlation	Direct effects
Days to 50% flowering	-0.056	-0.1884
Plant height at 30 days after transplanting (cm)	0.450*	-0.0601
Plant height at maturity (cm)	0.358*	0.0530
Productive tillers plant $^{-1}$	0.490*	1.0830
Total number of tillers plant $^{-1}$	-0.129	0.0759
Spikelets per panicle	0.422*	0.0086
Spikelet fertility (%)	0.458*	0.0646
Panicle length (cm)	-0.026	-0.0431
Panicle weight (g)	0.012	2.2292
Days to maturity	0.200	0.1761
1000-grain weight (g)	0.553**	-0.0302
Biomass (g)	0.294*	2.7658
Harvest index	0.369*	-0.1422

predicting the resultant effect of selection on phenotypic expression, without genetic advance the estimates heritability will not be of practical use.

Heritability and genetic advance

The data on heritability (bs), depicted that heritability estimates % for 14 quantitative traits, under study ranged from 20.48% to 94.99%. Highest value of heritability was noticed for spikelet fertility (94.99%), followed by plant height at maturity (91.22%)and days to 50 % flowering (81.45%) indicted least influence of environment on these traits. Whereas, Least was observed for panicle weight (20.48), followed by biomass (32.75%) and harvest index (44.32%) indicated higher influence of environment on expression of these traits(Prajapati *et al.,*2011).

Genetic advance on percent mean was high for biomass

Table 5: R² values for SSR markers showing significant association with different phenotypic characters in rice

Sl. No.	Marker	Chromosome No.	Trait linked to SSR marker	t value (2 tailed)	R²
1.	RM495	1	Spikelet fertility (%)	2.576*	19.73%
			Plant height (cm)	2.172*	18.15%
2.	RM242	9	Grain yield (g)	1.493*	15.31%
3.	RM152	8	Spikelet fertility (%)	2.029*	12.06%
4.	RM263	2	Grain yield (g)	1.993*	12.04%
5	RM 248	7	Days to 50% flowering	1.162*	10.23%
6	RM596	10	Grain yield (g)	2.216*	4.45%

*significance at 5%, **significance at 1% and *** significance at 0.1% level

Table 6: Number of genotypes carrying positive alleles for the traits attributing to yield

Sl. No.	SSR Markers	Traits linked	Positive alleles carrying genotypes like IM 90 parent
1	RM4	Productive tillers	5
2	RM248	Root dry weight (g), Total root weight (g), days to 50% flowering	5
3	RM131	Grain yield (g)	7
4	RM596	Grain yield (stress) (g)	7
5	RM242	Grain yield (g), root length (cm)	8 and 4
6	RM263	Grain yield (stress) (g)	8
7	RM24	Plant height (cm)	10
8	RM152	Spikelet fertility (%)	10
9	RM443	Test weight (g)	10
10	RM495	Plant height (cm) and spikelet fertility (%)	10 and 7
11	RM519	Plant height (cm), productive tillers	10
12	RM205	Total number of tillers	11
13	RM493	Spikelet fertility (%)	11
14	RM447	Test weight (g)	14

high variability for these traits (Bidhan *et al.,* 2011;Ghoshandsharma, 2012).

Least GCV was recorded for spikelet fertility (1.79), spikelets per panicle (2.14)and days to 50 % flowering (3.86). However, least PCV values are observed for spikelet fertility (1.84), spikelets per panicle (2.24) and days to 50 % flowering (4.28)indicated presence of low variability for these traits (Table.3).GCV along with heritability gave an idea of expected genetic gain from selection (Kumar *et al.,* 2009). Although GCV and PCV is the indicative of the presence of degree of genetic variation, which the amount of heritability portion of variation can only be determined (Bidhan *et al.,* 2011;Idris *et al.,* 2012).In

(21.45) followed by Grain yield per plant (19.58) and harvest index (18.87)indicated preponderance of additive gene action (Bidhan *et al.,* 2011). However, Least was observed for panicle length (6.45) followed by productive tillers per plant (11.10)and Panicle weight (11.53) indicated majority of non-additive gene action in these traits inheritance.

Most of the characters under study were having moderate-high heritability coupled with moderate-high genetic advance as percent of mean indicated the preponderance of additive gene effects in its inheritance and such characters could be improved by mass selection and other breeding methods based on progeny testing (Kumar *et al.,* 2015). However,

panicle length showed moderate values of heritability coupled with low genetic advance also indicated presence of additive gene action suggest that The high heritability is being exhibited due to favorable influence of environment rather than genotype and selection for such traits may not be rewarding. (Kumar *et al.*,2015). Whereas for panicle weight low values of heritability coupled with moderate genetic advance was observed reveals that the trait is governed by additive gene effects. The low heritability is exhibited due to high environmental effects so selection may be effective in this case (Debnath *et al.*, 2015)

Correlation and path analysis

The results indicated positive and significant correlation of grain yield per plant with plant height at 30 days after transplanting (r = 0.450*), plant height at maturity (r = 0.358*), productive tillers per plant(r = 0.490*), spikelets per panicle (r = 0.422*), spikelet fertility (r = 0.456*), harvest index (r = 0.369*) and 1000 grain weight (r = 0.553**) indicated that while practicing selection these traits has to be give prime importance in early generations (Table 4). Similar results reported by Hari Rama Krishnan *et al.,* 2006 and Lokhendra singh *et al.,* 2008)

The path analysis revealed that the highest direct effect on grain yield per plant is exhibited by biomass (2.7658) followed by panicle weight (2.2292) and days to maturity (0.1761). The traits with significant and positive correlation can be used for selection to improve the grain yield and the traits with highest direct effects also helpful in improving grain yield through the selection for those traits (Karim *et al.,* 2007; Debnath *et al.,*2015).

Validation of trait specific markers

Out of 500 markers, 78 were polymorphic on 3.5% percent agarose gel. On an average, 14.18 per cent polymorphism was obtained. Similar type of results were obtained by Shishido *et al.*, 2006 indicating use of these polymorphic markers for identification and selection of good progeny plant types for associated traits and also for fingerprinting of parents. Among the polymorphic markers twenty trait specific SSR markers were used for validation, among the twenty marker chosen for the study, only six were found to be significantly associated with yield traits. Details of SSR markers association found for different characters are furnished in table.5 . It has been found that RM495 is associated with yield contributing traits spikelet fertility (%) and plant height at maturity (cm) scores with phenotypic data under field conditions showed significance at 5% level. RM 242 was found to be significantly associated with grain yield at 5% level of significance. RM 152 was found to be significantly associated with spikelet fertility (%) at 5% level of significance. RM 263 found to be associated with grain yield at 5% level of significance. Only one marker RM 248 showed significant association with days to fifty percent flowering at 5% level of significance. RM 596 was found to be associated with grain yield at 5 % level of significance similar results reported by Kanagaraj *et al.* (2010).

The percentage contribution of the significantly associated markers to total phenotypic variance (R^2) varied from 4.45 in case of RM 596 for grain yield at harvest to 19.73 in case of RM 495 spikelet fertility. In case of RM 242 phenotypic variance

explained is (R^2) is 15.31 for grain yield, RM 152 explained phenotypic variance of 12.06 (R^2) for the trait spikelet fertility, RM 248 explained days to 50% flowering phenotypic variance (R^2) is 10.23. Low R^2 values indicates large environmental component of variation. This low R^2 is due to large QTLs are controlling the traits. The identified polymorphic markers will be further utilized for development of linkage map of cross IM90 X IR 64.The genotypes carrying number of positive alleles were given in table 6. These genotypes could be selected and advanced to next generation as they were carrying alleles similar to parent IM90 which is high yielding genotype.

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