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Inheritance Study of Red Kernel Colour in Rice (*Oryza sativa* L.) Using Microsatellite Markers

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ABSTRACT

The investigation comprised of one red kernel and one white kernel fine grained rice cultivars. Crosses between White rice x Red rice cultivars and their reciprocal were made during Kharif 2013. The F_1s were evaluated during Rabi 2013-14 and F_2 seeds of each cross were obtained. The BC_1 and BC_2 i.e. back crosses of F_1 with both the parents were effected during Rabi 2013-14. The parents, F_1s, F_2s, BC_1s and BC_2s were evaluated during Kharif 2014. The F_1 progenies of both crosses under study were examined for the kernel colour and it was observed that all the F_1 progenies were having red coloured kernels. This indicates the dominance of red kernel colour over white kernel colour in rice in F_1 generation. For these studies molecular markers linked to the red kernel were used for parental polymorphism and for foreground selection.

The parental validation using SSR markers were also studied. Marker RM 206 was found as the best marker in screening F_2 population for red kernel colour followed by RM 251 as the distance between two fragments are more. The F_2 population of both crosses was screened using selected two SSR markers i.e. RM 206 and RM 251. A representative 100 individuals derived from the crosses Karjat-2 x Munga and their reciprocal was estimated for white and red kernel colour. Out of 100 individual plants 81 and 19 were found to exhibit red and white kernel colour respectively and these individuals were segregated in 3:1 chi square ratio for kernel colour trait.

Key words: *Rice, Inheritance, Quality parameters, SSR markers*

INTRODUCTION

Rice is one of the most important cereal crops in the world, providing 21% of the food for the world population and up to 76% calorie intake for Southeast Asian. It has become a useful model crop, largely because it has a small genome size (400 mb) compared with other major crops. Not only for smaller genome size but also for the availability of high precision genome sequencing and saturated molecular markers, rice is the target crop for several map-based gene discoveries. It has been estimated that a 40% increase in rice production by 2030 will meet the demand of the predicted world population.

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Increase only in rice productivity will not be sufficient as consumers' preference shifted towards specific quality parameters of rice. That's why instead of availability of several high yielding rice varieties only a few of those are accepted by the farmers. Farmers accept only those which can fetch higher market price and meet consumers preference.

So, now an ideal superior rice cultivar should have high grain-yield potential with improved grain quality, nutritional value, disease resistance and stress tolerance. Quality of rice depends on the consumers' preference and it changes according to the region. Therefore, landraces which were adopted for a specific region by hundreds of years may be a source of alleles for quality parameters and can be utilized by the breeders for improvement of quality parameters specific for that niche. Along with colour of rice, kernel aroma and kernel elongation, are also an important characteristic in commercial market. Rice kernels having white colour are preferred in the national and international markets. The red colour of the kernels is preferred by consumers in some regions of India particularly in the Southern state. However, now a day white collered people have good preference for red rice for making rice soup and hence red rice is only available in malls of the cities.

Therefore, development of rice varieties with specific kernel colour become an important plant breeding objective. Even though a number of red and white kernel varieties have been bred, no systematic attempt has been made to understand the extent of diversity within and between these two distinct phenotypic classes and inheritance patterns of it which help to formulate breeding strategies systematically. These two parameters primarily control the market value of rice grain. Over the past ten years, the development of DNA markers and genome sequencing technology have led to rapid development in the mapping and cloning of genes underlying grain quality parameters of rice. Four hundred QTLs have been assigned for explaining the variation in quality parameters of rice. Thirteen of them already cloned and their role in controlling quality parameters have been ascertained by reverse genetics approach. Not all of these genes or QTLs are responsible for controlling quality parameters of a particular rice variety. Rather, it depends on the genetic background of the specific variety and its growing environment. Thus, inheritance pattern of quality parameters is more complex than it was expected.

In this study, a population (F_2) comprising of one hundred heterogeneous lines and two rice genotypes were used for assessing the quality parameters when grown in Experimental farm of Agricultural Research Station, Karjat, Raigad and Plant Biotechnology Centre, College of Agriculture, Dapoli. Two parents used for developing population were famous for their quality parameters and they were Karjat 2 (White Kernel colour) and Munga (Red kernel colour) obtained from breeding section of Regional Agricultural Research Station, Karjat, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. To understand the inheritance of quality parameters the present experiment was undertaken.

MATERIAL AND METHODS

The parents were collected from the breeding section of Regional Agricultural Research Station, Karjat. Two parents Karjat-2 and Munga were selected for phenotypic and molecular analysis. The genotypes were grown in Randomized Complete Block Design with three replications during *Kharif* season for two consecutive years with spacing of 20 cm between lines and 15 cm between plants were provided. The standard recommended agronomic practices were followed for raising the crop. Observation on different quantitative characters and other phenotypic characters were recorded.

Isolation of rice genomic DNA

Leaf samples were collected and subsequently stored at –20°C for isolation of genomic DNA. The genomic DNA was extracted from young leaves using N-Cetyl-N, N, N-trimethyl ammonium bromide (CTAB) method described by Doyle and Doyle[7], with slight modifications. 2g of fresh leaf material were washed in distilled water and subsequently rinsed with 80% (v/v) ethanol and then ground in liquid nitrogen. 10 ml of preheated extraction buffer [4 % (w/v) CTAB, 0.2% β-mercaptoethanol (v/v), 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl] were then added per 2 g of leaf powder material and incubated for 2h at 65°C. The lysate was purified with chloroform: isoamylalcohol (24:1). The DNA pellet was resuspended in 200 to 300 µl of Tris-EDTA buffer (10 mM Tris – HCl, 1 mM EDTA, pH = 8.0). DNA was reprecipitated by adding 80% ethanol in the presence of 0.3 M sodium acetate, and pelleted by centrifugation. The pellets were lyophilized and resuspended in TE buffer. The RNA was removed by RNAse treatment at 37°C for 1 h. For further purification, DNA solution was extracted once with equal volume of phenol and chloroform: isoamyl alcohol (24:1:1) followed by two extractions with chloroform: isoamylalcohol (24:1). The upper aqueous phase was separated after centrifugation and mixed with 1/10th volume of 3 M sodium acetate. DNA was precipitated by adding two volumes of chilled absolute alcohol, pelleted, dried in vacuum and dissolved in TE buffer. Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% (w/v) agarose gel electrophoresis along side diluted uncut lambda DNA as standard. DNA was further diluted with TE to a concentration of 20 mg/µl for use in PCR analysis.

PCR analysis

For polymerase chain reaction (PCR) analysis 13 simple sequence repeat (SSR) markers linked to the red kernel trait of rice were selected. These primers were selected based on the available genome information of rice. The sequence information and other information pertaining to primers is given in Table 1.

Preparation of master mixture

The master mix was distributed to 20 tubes (19 µl tube-1) and 1 µl of template DNA of the respective genotype was added to make the total reaction volume to 20 µl and the mixture was given a short spin to mix the content. The reaction mixture for PCR analysis is prepared as desired and given in Table 2.

Standardization of annealing temperature:

Based on Tm value of forward and reverse primer the annealing temperature was adjusted in gradient PCR machine. Thermal cycling profile are as follows: denaturation was at 94°C for 5 min, followed by 35 cycles consisting of denaturation at 94°C for 20 Sec., followed by annealing range with no greater difference in Tm between primers of each pair of respective SSR primer for 30 Sec., and extension at 72°C for 45 Sec., with final extension at 72°C for 7 min.

DNA Amplification:

The DNA amplification was carried out with the help of SSR markers linked to the kernel colour of rice. The amplification reaction was carried out in a thermal cycler Eppendorf AG 22331 Hamburg machine.

Agarose gel electrophoresis:

The amplified products of PCR reaction was separated by electrophoresis in 1% Agarose gel containing ethidium bromide in 1X TAE buffer and constant voltage of 60 V for 25-30 min.

Data analysis:

Based on the banding pattern obtained, the polymorphism percentage was calculated with different primers. The amplified product for each primer was scored as 1 for presence of the band and 0 for the absence of the band to find out genetic of red colour in rice.

Table 1: List of selected SSR markers used for grain quality trait

Sr. No	Primer Name	Rice Chromosome	Forward Primer	No. of Bases	Reverse Primer	No. of Bases	GC (%)
1	RM 26	5	GAGTCGACGAGCGGCAGA	18	CTGCGAGCGACGGTAACA	18	63.89
2	RM 102	1	AACTTTCCCACCACCACCGCGG	22	AGCAGCAGCAAGCCAGCAAGCG	22	63.64
3	RM 154	2	ACCCTCTCCGCCTCGCCTCCTC	22	CTCCTCCTCCTGCGACCGCTCC	22	72.73
4	RM 166	2	GGTCCTGGGTCAATAATTGGGTTACC	26	TTGCTGCATGATCCTAAACCGG	22	50.00
5	RM 167	11	GATCCAGCGTGAGGAACACGT	21	AGTCCGACCACAAGGTGCGTTGTC	24	57.78
6	RM 174	2	AGCGACGCCAAGACAAGTCGGG	22	TCCACGTCGATCGACACGACGG	22	63.64
7	RM 206	11	CCCATGCGTTTAACTATTCT	20	CGTTCCATCGATCCGTATGG	20	47.50
8	RM 215	9	CAAAATGGAGCAGCAAGAGC	20	TGAGCACCTCCTTCTCTGTAG	21	51.22
9	RM 231	3	CCAGATTATTTCCTGAGGTC	20	CACTTGCATAGTTCTGCATTG	21	43.90
10	RM 234	7	ACAGTATCCAAGGCCCTGG	19	CACGTGAGACAAAGACGGAG	20	56.41
11	RM 251	3	GAATGGCAATGGCGCTAG	18	ATGCGGTTCAAGATTCGATC	20	50.00
12	RM 253	6	TCCTTCAAGAGTGCAAAACC	20	GCATTGTCATGTCGAAGCC	19	48.72
13	RM 484	10	TCTCCCTCCTCACCATTGTC	20	TGCTGCCCTCTCTCTCTCTC	20	57.50

Table 2: Master mixture for PCR analysis (20 µl tube^{-1})

Sr. No	Components		Quantity (µl)
1	Taq Buffer	:	2.5
2	$MgCl_2$	:	0.5
3	dNTP's	:	1.0
4	Primer (F)	:	1.0
5	Primer (R)	:	1.0
6	DNA	:	1.0
7	Water	:	12.5
8	Taq polymarase	:	0.5
	Total	**:**	**20.0**

RESULT AND DISCUSSION

The technical efficiency and multiplex potential of SSRs makes them preferable for many forms of high throughput mapping, genetic analysis and marker assisted plant improvement strategies. The fact that SSR markers are co-dominant, multi-allelic and can be reliably used to analyze both indica and japonica germplasm, as well as groups of AA genome *Oryza* species Chen *et al.*[5], makes them attractive as genetic markers and facilitates the integration of results from independent studies. Thus, the availability of a high-density SSR map is valuable as a public resource for studies aiming to interpret the functional significance of the rapidly emerging rice genome sequence information.

In polymorphic chain reaction (PCR) technique, DNA is amplified *In-vitro* in a series of polymerization cycles consisting of three temperature dependent steps: DNA denaturation, primer template annealing and DNA synthesis by thermostable DNA polymerase. The purity and yield of reaction product depends on several parameters, one of which is annealing temperature (Ta). If the Ta is too low, non-specific DNA fragments are amplified, causing the appearance of multiple bands on agarose gels. If the Ta is too high, the yield of the desired product, and sometimes the purity is reduced due to poor annealing of primers.

In the present investigation all thirteen primers linked to the red kernel colour trait of rice were screened for their specific banding pattern based on their Tm value and GC content. It has been observed that most of the primers yielded expected products at 55 ^{0}C annealing temperature (Plate. III) i.e. (RM 26, RM 206, RM 215, RM 231, RM 234, RM 251, and RM 484). The lowest annealing temperature recorded in RM 235 (54.1^{0}C). The highest annealing temperature was recorded by primer RM 174 (67.0^{0}C) (Table. 3) which is confirmed by the earlier work reported by Rychlik *et al.*[12], Dograr and Akkaya[6], and McCouch *et al.*[10].

Progress in rice breeding for biotic and abiotic tolerance, constitutes the identification of the major locus conferring a tolerant gene at different growth stages. With the recent development in the field of molecular marker analysis, it is now feasible to analyze both the simple inherited traits and the quantitative traits and then identifying the individual genes controlling biotic and abiotic tolerance which could facilitate selection in rice for this low heritable trait. Molecular marker could be used to tag QTL and evaluate their contributions to the phenotype by selecting for favorable allele at those loci in marker assisted selection (MAS) scheme aimed to accelerate genetic advancement in rice which is faster, more efficient and cost-efficient than conventional screening under field conditions. Like that with the recent development in the field of molecular marker analysis, it is now feasible to analyze both the simple inherited traits and the quantitative traits and then identifying the individual genes controlling red kernel colour which could facilitate selection in rice for this quality heritable trait.

In present study, a total of 13 SSR markers were used for parental validation i.e. Karjat-2 (Recipient) and Munga (Donor) in order to detect the polymorphism between them. Among these 11 markers shown monomorphism and only two were shown polymorphism i.e. RM 206 and RM 251. The primer RM 206 was amplified at 127 bp in Karjat-2 and 167 bp in Munga while the primer RM 251 was amplified at 145 bp in Karjat-2 and 124 bp in Munga,(Table. 4) (Plate. I.) therefore these two primers could be utilized for the foreground selection. It indicates that QTL associated with kernel colour was detected through only RM 206 and RM 251. The results of the present investigation are similarly with the earlier results obtained by Jewel *et al.*[9], Aliyu *et al.*[1], Siva kumar *et al.*[14], Chattopadhyay *et al.*[4], and Awasthi and Lal[3]. observed maximum polymorphism with informative primers.

Identifying hybrids in the F_1 generation can be difficult because the F_1 may not be readily distinguishable from the parents, especially in the greenhouse where plants cannot grow to full size due to limited space. In the field, it is often possible to distinguish F_2 plants by segregation for morphological traits. Identification of hybrids can be performed through use of DNA markers. Codominant markers are preferable because they produce different alleles (markers) for each parent, and F_1 hybrids will possess an allele from each parent. Of the major DNA marker types, restriction fragment length polymorphism (RFLP) and simple sequence repeat (SSR) markers are usually codominant. The SSR-based markers require smaller quantities of DNA than do RFLP-based markers[11], and analysis by SSR markers is quicker and does not involve the use of radioisotopes.

In present investigation DNA was extracted from leaf tissues of F_1 and their parents and polymorphic SSR markers were amplified by compairing the resulting banding patterns of those of the parents most of putative hybrids were shown (Plate. II) to be true hybrids on the basis of possessing the marker allele form their male parent. These pure hybrids were grown for further studies. This result was in accordance with Gomez *et al.*[8], Salgotra *et al*[13].

In present investigation it was also revealed that the F_2 population of both crosses was screened using selected two SSR markers RM 206 and RM 251 (Plate. IV & V). Out of 100 individual plants 81 and 19 were found to exhibit red and white kernel colour, respectively and these individuals was segregated in chi square ratio which was fitted with 3:1 ratio for kernel colour trait. The results of the present investigation are similarly with the earlier result obtained by Ashkani *et al.*[2].

Table 3: Standardization of annealing temperature for SSR markers

Sr. No	Primer Name	GC (%)	Melting Temp. (Tm^0)	Temp. range (^0C)						Annealing Temp. (^0C)
				1	2	3	4	5	6	
1	RM 26	63.89	F 59.8 R 57.8	54.2	55.0	57.4	59.7	61.6	63.9	55.0
2	RM 102	63.64	F 64.7 R 65.7	59.9	60.7	62.7	65.4	67.9	69.6	60.7
3	RM 154	72.73	F 67.2 R 65.8	60.2	61.0	64.1	66.9	68.7	70.6	61.0
4	RM 166	50.00	F 59.2 R 57.8	54.6	56.4	58.9	61.0	63.7	64.6	61.0
5	RM 167	57.78	F 59.5 R 63.4	55.2	56.7	59.1	61.7	63.9	64.9	61.7
6	RM 174	63.64	F 64.1 R 63.1	59.9	60.7	62.7	65.4	67.0	69.6	67.0
7	RM 206	47.50	F 50.2 R 55.3	50.4	53.2	55.0	57.9	59.1	60.3	55.0
8	RM 215	51.22	F 54.9 R 55.7	49.3	51.8	53.7	55.0	57.6	59.4	55.0
9	RM 231	43.90	F 50.0 R 52.3	45.7	47.5	49.1	51.9	53.3	55.0	55.0
10	RM 234	56.41	F 56.8 R 55.5	50.6	52.2	54.1	55.0	58.3	60.7	55.0
11	RM 251	50.00	F 54.3 R 53.0	49.9	50.1	53.3	55.0	57.8	59.6	55.0
12	RM 253	48.72	F 53.1 R 54.5	49.4	51.6	54.1	55.9	57.2	59.5	54.1
13	RM 484	57.50	F 56.1 R 58.1	50.7	52.9	53.1	55.0	57.6	60.1	55.0

Table 4: Parental validation using SSR markers

Sr. No.	Primer names	Observed bands	Size of Amplification (bp)	
			Karjat-2	Munga
1	RM 26	Monomorphic	110	110
2	RM 102	Monomorphic	549	549
3	RM 154	Monomorphic	172	172
4	RM 166	Monomorphic	322	322
5	RM 167	Monomorphic	135	135
6	RM 174	Monomorphic	208	208
7	RM 206	Polymorphic	127	167
8	RM 215	Monomorphic	142	142
9	RM 231	Monomorphic	76	76
10	RM 234	Monomorphic	230	230
11	RM 251	Polymorphic	145	124
12	RM 253	Monomorphic	143	143
13	RM 484	Monomorphic	348	348

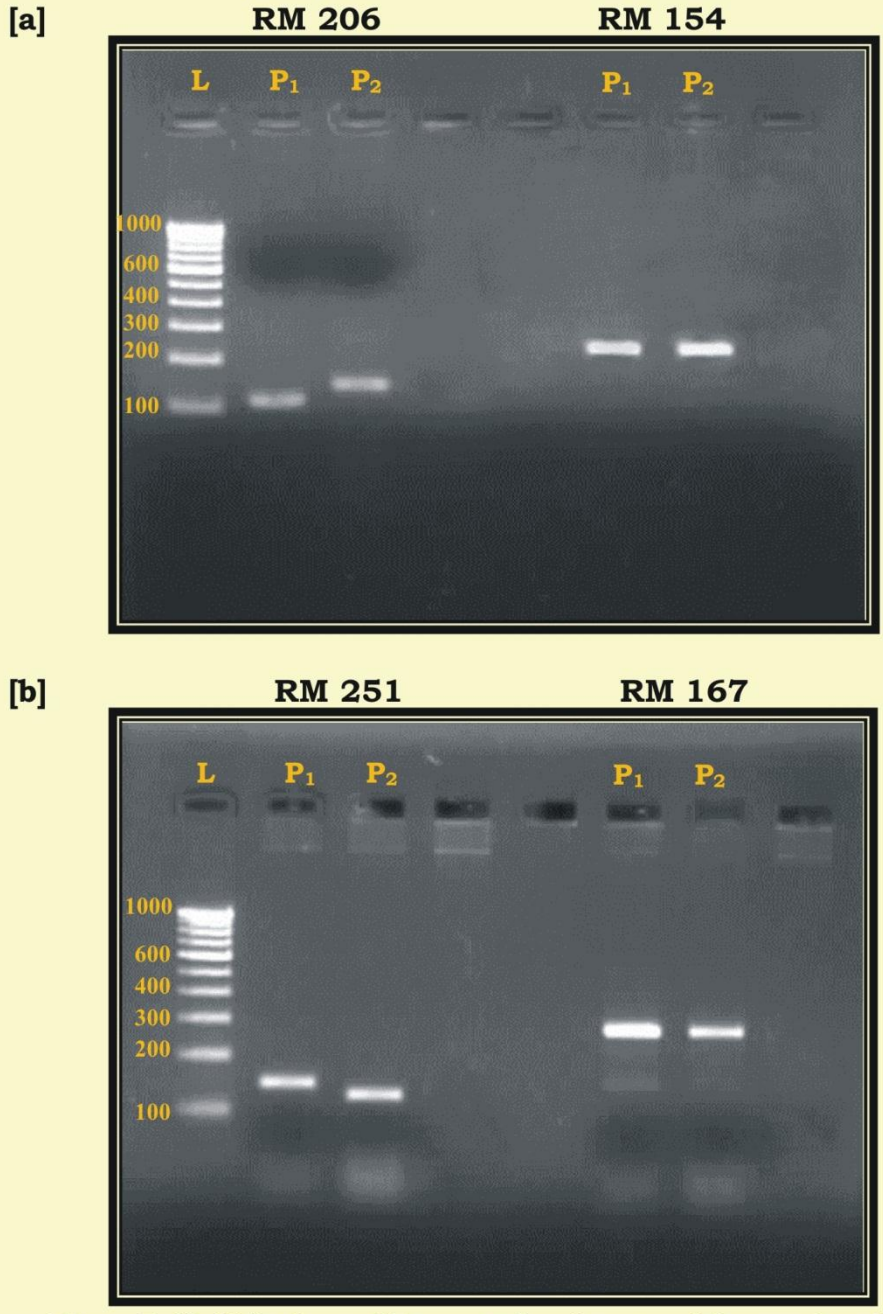


Plate I. Validation of Parental polymorphism using SSR markers

L	:	100 bp Ladder
P_1	:	Karjat-2
P_2	:	Munga

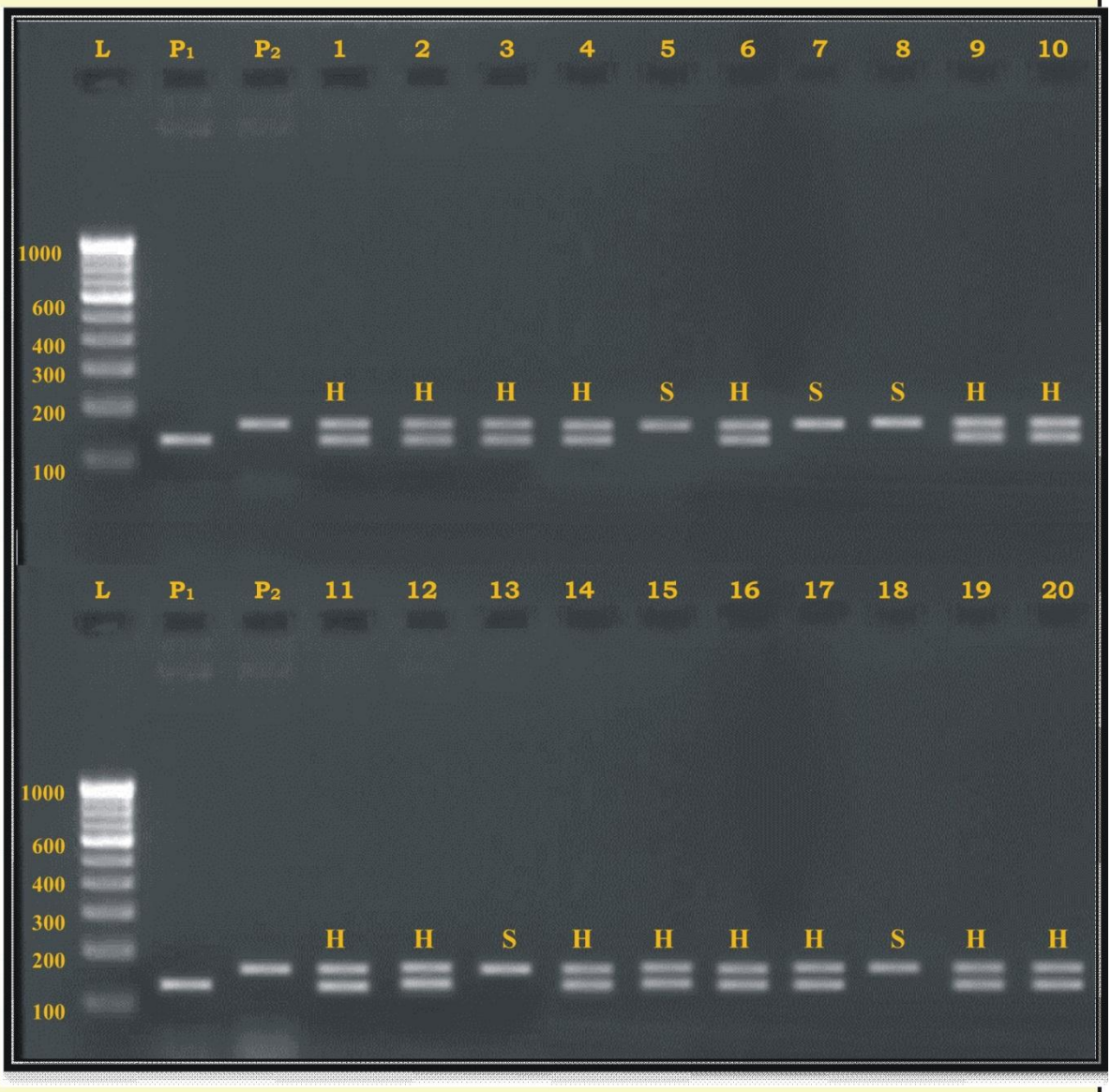
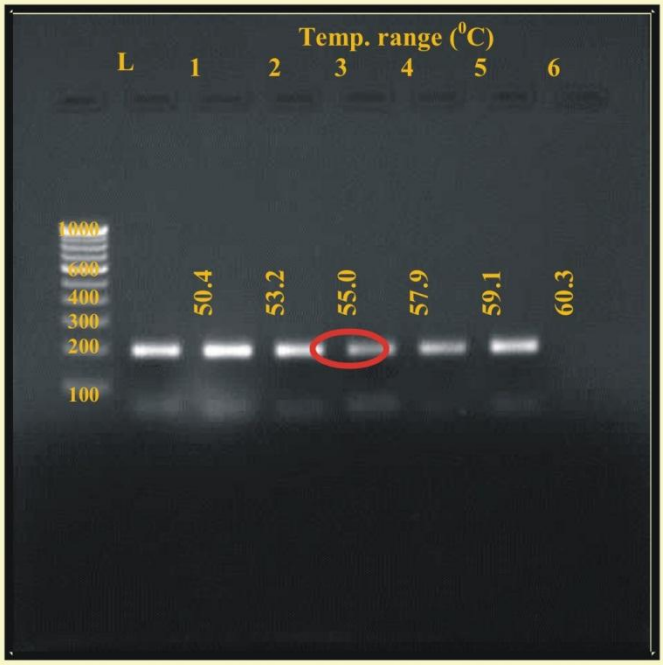


Plate II. Confirmation of F_1 population using RM 206 SSR marker

L	:	100 bp Ladder
P_1	:	Karjat-2 (male parent)
P_2	:	Munga (female parent)
1 - 20	:	F_1Samples of both the crosses
H	:	True Hybrid
S	:	Self

[a]



[b]

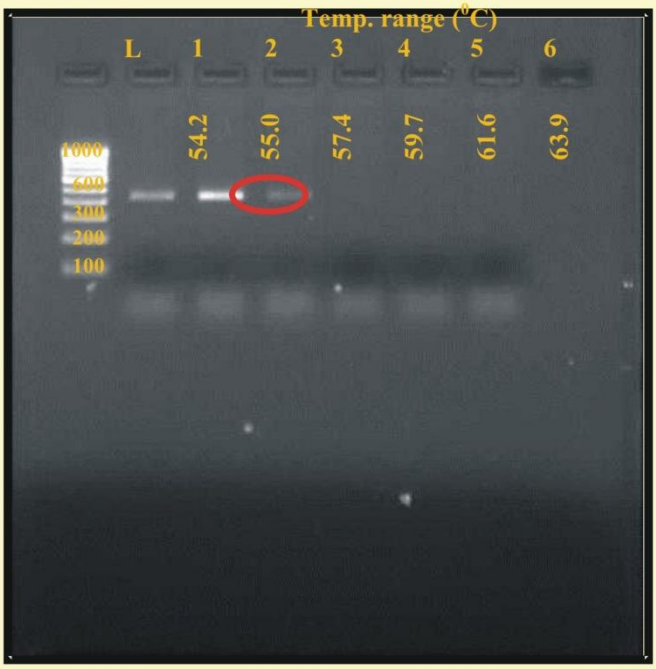


Plate III. Standardization of annealing temperature for SSR markers

[a] RM 206 and [b] RM 26

L	:	100 bp Ladder
1 to 6	:	Temperature range

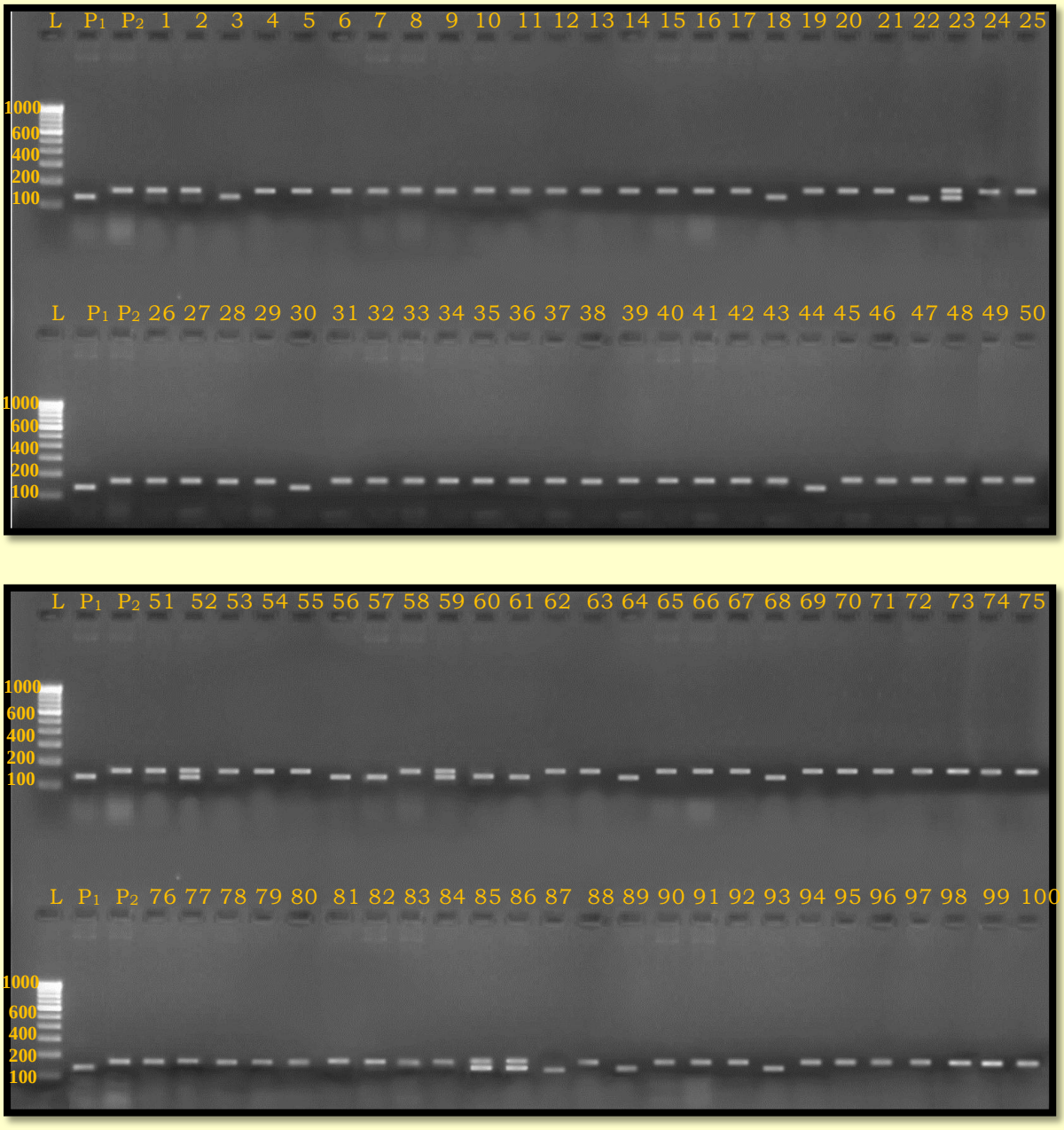


Plate IV. F_2 Population screening using RM 206 SSR marker

L	:	100 bp Ladder
P_1	:	Karjat-2
P_2	:	Munga
1-100	:	F_2 Samples

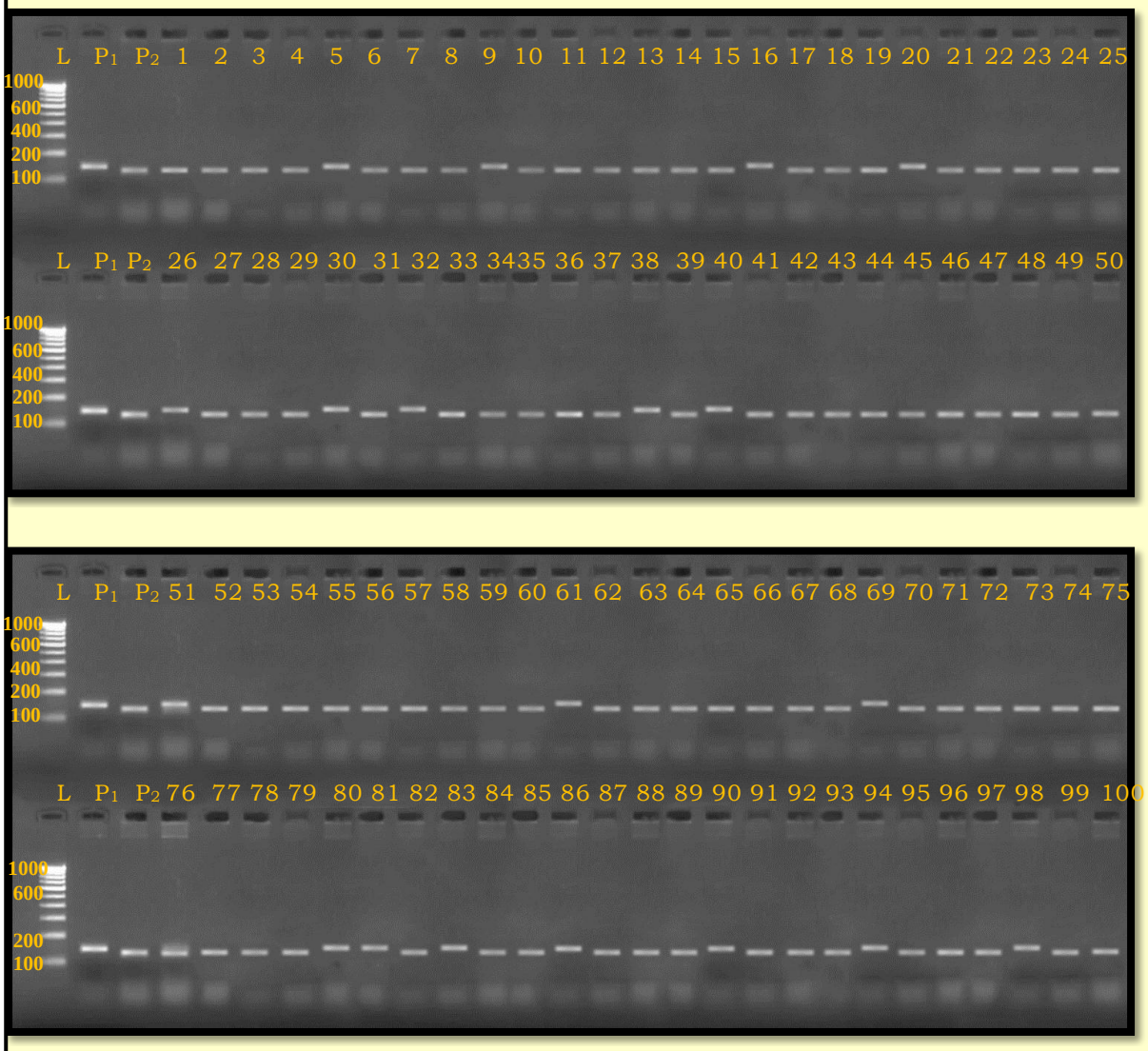


Plate V. F_2 Population screening using RM 251 SSR marker

L	:	100 bp Ladder
P_1	:	Karjat-2
P_2	:	Munga
1-100	:	F_2 Samples

REFERENCES

1. Aliyu, R., Adamu, A. K., Muazu, S. and Alonge, S. O., Tagging and Validation of SSR markers to Salinity Tolerance QTLs in Rice (*Oryza* spp). *International Conference on Biology, Environment and Chemistry Press*, Singapore **1:** 328-332 (2011).
2. Ashkani, S., Rafii, M. Y., Sariah, M., Siti Nor Akmar, A., I. Rusli, H. Abdul Rahim and M. A. Latif., Analysis of simple sequence repeat markers linked with blast disease resistance genes in a segregating population of rice (*Oryza sativa*). *Genet. and Mol. Res*., **10(3):** 1345-1355 (2011).
3. Awasthi, S. and Lal, J. P., Validation of SSR markers associated with drought tolerant QTLs in rice (*Oryza sativa* L.). *International Journal of Scientific Footprints*., **2(3):** 99-113 (2014).
4. Chattopadhyay, K., Nath, D., Mohanta, R. L., Bhattacharyya, S., Marndi, B. C., Nayak, A. K., Singh, D. P., Sarkar, R. K. and Singh, O. N., Diversity and validation of microsatellite markers in *Saltol* QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage. *Aus. J. Crop. Sci.*, **8(3):** 356-362 (2014).
5. Chen, X., Cho, Y., and McCouch, S. R., Sequence divergence of rice microsatellites in *Oryza* and other plant species, *Mol. Gen. Genet*., DOI.1007/s00438-002-0739-5 (2002).
6. Dograr, N. and Akkaya, M. S., Optimization of PCR amplification of wheat Simple Sequence Repeat DNA markers. *Turk. J. Biol*., **25:** 153-158 (2001).
7. Doyle, J. J. and Doyle, J. L., Isolation of plant DNA from fresh tissue. *Focus*, **12(1):** 13-15 (1990).
8. Gomez, S. M., Denwar, N. N., Ramasubramanian, T., Simpson, C. E., Burow, G., Burke, J. J., Puppala, N. and Burow, M. D., Identification of peanut hybrids using microsatellite markers and horizontal polyacrylamide gel electrophoresis. *Peanut Science*., **35:** 123–129 (2008).
9. Jewel, Z. A., Patwary, A. K., Maniruzzaman, S., Barua R. and S. N. Begum., Physico-chemical and Genetic Analysis of Aromatic Rice (*Oryza sativa* L.) Germplasm. *The Agriculturists*., **9(1&2):** 82-88 (2011).
10. McCouch, S. R., Teytelman, L., Xu, Y., Maghirang, R., Kono, I., Yano, M. and DeClerck, G., Development and Mapping of 2240 New SSR Markers for Rice (*Oryza sativa* L.) *DNA Research*., **9**: 199–207 (2002).
11. Powell, W., Machray, G. C. and Provan, J., Polymorphism revealed by simple sequence repeats. *Trends Plant Sci*., **1:** 215-222 (1996).
12. Rychlik, W., Spencer, W. J. and Rhoads, R. E., Optimization of the annealing temperature for DNA amplification *in vitro*. *Nucleic Acids Res.*, **18(21):** 6409-6412 (1990).
13. Salgotra, R. K., Gupta, B. B., Reginald, J., Millwood, Balasubramaniam, M. and Stewart, C. N., Introgression of bacterial leaf blight resistance and aroma genes using functional marker-assisted selection in rice (*Oryza sativa* L.). *Euphytica*., **187:** 313–323 (2012).
14. Siva Kumar, G., Aruna Kumari, K., Durga Rani, Ch. V., Sundaram, R. M., Vanisree, S., Md. Jamaloddin and Swathi, G., Study of simple sequence repeat (SSR) polymorphism for biotic stress resistance in elite rice variety JGL 1798. *Afr. J. Biotechnology*., **12(40):** 5833-5838 (2013).