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# Molecular Diversity Analysis of Kalanamak Rice Genotypes Using Microsatellite Markers

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**ABSTRACT**

*Rice is the predominant staple feeding more than half of the world population. Genetic diversity analysis is highly essential for employing suitable diverse genotypes as parents in target oriented hybridisation programmes. The diversity analysis was conducted in 35 recombinant Kalanamak rice genotypes using 30 microsatellite markers. A total of 38 alleles were detected among the 35 rice genotypes of rice exhibiting 89.47% polymorphism. The overall size of amplified products ranged from 90 to 400 bp and the number of alleles per locus varied from 1-3. The genotypes were clustered into six clusters. SSR primers namely, RM 159 and RM 264 generated higher levels of polymorphism and can be used for rice profiling in future.*

***Key words:*** *Kalanamak rice, Diversity analysis, Microsatellite markers, Clusters*

## INTRODUCTION

Rice is the most important staple of more than half of the world's population feeding about 3.5 billion people worldwide[9]. Rice is produced in all continents except Antarctica and the major share in world production is contributed from Asia. In most of the developing world, rice cultivation is indispensable for food security and sustainable agriculture. The genus *Oryza* belongs to the tribe *Oryzeae* of the family Poaceae and sub family *Oryzoideae*. *Oryza* has two cultivated species, *Oryza sativa* and *Oryza glaberrima*. Although the parental species are native to Asia (*Oryza sativa)* and certain parts of Africa (*Oryza glaberrima)*, centuries of trade and exploitation have made rice commonplace in many cultures worldwide.

India, being one of the original centres of rice cultivation is the second largest producer and consumer of rice in the world with a production of 138.5mt[7]. Basmati rice, the queen of rice is cultivated over an area of 58.06lakh hectares in India and the major basmati growing states are Haryana, Punjab, Uttar Pradesh, Uttarakhand, Himachal Pradesh, Jammu and Kashmirand Delhi[2].

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The total basmati rice production of India has stagnated at 1.5million tonnes in the past decade due to the narrow adaptability of the scented rice grown in few parts of Haryana, Punjab and Uttarakhand only. In India, more attention was given to basmati rice leaving the various indigenous scented rice varieties behind. India has several basmati and non basmati rice varieties and landraces of which Kalanamak rice from eastern Uttar Pradesh and Bihar is a prominent non basmati variety. Kalanamak derives its name from the black husk (*kala*) and a mild salty (*namak*) taste[14]. It is well known for its taste and aroma and is offered as a gift to honour the guests in olden times. Kalanamak, the indigenous aromatic rice variety is found to offer a great advantage as an export commodity over other basmati rice varieties and can be greatly beneficial for farmers of Eastern Uttar Pradesh and Tarai area of Bihar[3]. Kalanamak rice variety was awarded Geographical Indication (GI) tag in 2013.

As a major cereal crop, rice is one of the most diversified crop species due to its adaptation to a wide range of geographical, ecological and climatic regions. The rice germplasm serves as reservoir of valuable genes for many important issues challenging the rice production and expansion of acreage. Determination of available and exploitable genetic diversity in the germplasm is the foremost step in any crop improvement programme and there are several ways for estimation of diversity in germplasm, such as evaluation of phenotypic variation, biochemical and DNA polymorphisms. However, both phenotypic and biochemical characterizations stands unreliable because they are environmentally challenged, labour demanding and numerically limited. On the contrary, DNA-based molecular markers are ubiquitous, repeatable, stable and highly reliable. Among the several classes of available DNA markers, microsatellite or simple sequence repeat (SSR) markers are considered the most suitable due to their multiallelic nature, high reproducibility, co dominant inheritance, abundance and extensive genome coverage. A large number of SSR markers have been developed and mapped in rice[12], which vary in the degree of polymorphism depending on their position in the coding or non coding segments, nature of their repeat motifs and the genomewide abundance. Therefore, an ideal set of SSR markers providing genomewide coverage will facilitate an unbiased assay of genetic diversity which in turn will provide a robust, unambiguous molecular description of rice cultivars. Such a diversity analysis help to assess the genetic distance between the genotypes and the divergent parent could be used in the different rice improvement programmes either to generate new diversity through hybridisation or through direct selection of outstanding genotypes. Hence the present study was planned to estimate the genetic diversity of kalanamak rice genotypes using microsatellite markers.

## MATERIAL AND METHODS

### Plant material

The study was carried out with 35 recombinant Kalanamak rice accessions (Table 1) at G .B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand.

### Isolation of genomic DNA

Fresh and healthy leaves from each rice genotype were collected and DNA was extracted following CTAB method of Doyle and Doyle[6], with slight modifications. The DNA was quantified using 0.8% agarose gel.

### SSR Marker Assay

The strains were genotyped using 30 SSR primers (Table 2). PCR was performed in a 25 µl reaction with 2.5 µl of 10X buffer, 2.0 µl of dNTPs, 1µl of primer (100ng/µl), 0.3 µl of Taq polymerase and 1µl of template DNA (20ng/µL). The PCR protocol comprised of the initial denaturation of 94°C for 5 min. This was followed by repeat of 39 cycles of denaturation at 94°C for 1 min, annealing at 46-51°C for 2 min and extension at 72°C for 2 min followed by final extension at 72°C for 10 min and stored at 4°C. Amplification product and loading dye were mixed in 10:1 ratio and fractioned on 2.5% agarose gel.

Electrophoresis was performed for 2.5h with constant voltage of 80 V.

**SSR Data Scoring and Analysis**

Gel photographs were used to score the data manually and independently for analysis. Scoring of gels on 0-1 pattern was done for further analysis. Presence of amplified products was scored as 1 and its absence as '0' for all the genotypes and primer combinations (Fig.1). These data matrix were entered into NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System Programme). The data were analyzed using SIMQUAL (Similarity for qualitative data) which generate Jaccard's similarity coefficients. These similarity coefficients were used to construct dendrogram using the unweighted pair-group method with Arithmetic average using (UPGMA) NTSYS program. The same programme was used for the coordinate analysis. PowerMarker software was used to calculate the PIC values.

## RESULTS AND DISCUSSION

An overall comparison of the markers and genotypes involved in genetic diversity analysis revealed that the markers could be distinguished between different genotypes. Four primers (RM 19, RM 346, RM 547 and RM 20) were found to be monomorphic. Two unique bands were observed for primer RM 264. A total of 38 loci were amplified that exhibited 89.47% polymorphism.

**Number of alleles per locus**

Using 30 microsatellite markers, a total of 38 alleles were detected among the 35 rice genotypes of rice. The overall size of amplified products ranged from 90 to 400 bp. The number of alleles per locus varied from 1-3 (Table 3). The average number of alleles per locus was 1.23, with a range of one (RM 154,RM 528, RM 338, RM 226, RM 307, RM 25, RM 215, RM 217, RM 519, RM 19, RM 346, RM 547, RM 10, RM 8225, RM 8226, RM 340, RM 229, RM 287, RM 168, RM 104, RM 413, RM 228, RM 428, RM 151, RM 259, RM 20 and RM 264) to as many as 3(RM 264, RM 159). Similar result for the highest number of alleles by RM 264 has been reported by Kumar *et al.*[10]. In agreement with Cho *et al.*[4], loci with di-nucleotide repeat motifs tended to detect a greater number of alleles. Siwach *et al.*[15], reported contrary results where the tri nucleotide repeats had greater number of alleles. No results (-) were obtained from two SSR primers namely RM 229 and RM 413.

**Allele size range**

The size variation between the smallest and the largest allele at a given locus was correlated with the number of alleles per locus. Thus, RM 8225 had the smallest allele size range (90 bp) and had only one allele per locus, while RM 159 had the largest allele size range ( 400 bp) and a total of three alleles. This pattern of correlation was also observed by Jain *et al.*[8].

**Null alleles**

A genotype was assigned a null allele for an SSR locus whenever an amplification product(s) was not detected for the particular genotype x marker combination. The loci harboring the highest frequency of null alleles were RM 264 (nulls detected in 34 genotypes) followed by RM 104 (nulls detected in 21 genotypes) and RM 159 (nulls detected in 21 genotypes).Out of the 30 SSR loci, RM 229 and RM 413 had null alleles for all the genotypes.

**Rare alleles**

An allele that was observed in only one or two of the 35 genotypes was considered rare. A total of two rare alleles were observed and these were present in only one genotype each namely RKN-34.These rare alleles were observed at RM 264 locus. In general, markers detecting a greater number of alleles per locus detected more rare alleles. Marker RM 264 detected the greatest number of alleles and 66 % of the alleles were observed in fewer than two genotypes each. In this respect, our results are comparable to published results of the study done by Jain *et al.*[8].

**Polymorphism of SSR markers**

The alleles revealed by SSR markers showed a high degree of polymorphism; with as many as 26 primers produced 100% polymorphic bands. A total of 37 bands were obtained from

30 SSR primers, of which all were polymorphic, with an average of 1.23 bands per primer.

**PIC values**

The level of polymorphism among the 35 genotypes was evaluated by calculating PIC values for each of the 30 SSR loci. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all of the SSR loci tested. PIC values varied widely among the loci and ranged from 0.0 (RM 19, RM 346, RM 547, RM 229, RM 413 and RM 20) to 0.3748( RM 428 and RM 259) followed by RM 168(0.3698), RM 167 (0.3673), RM 104( 0.364), RM 159(0.364) and RM 8225(0.357) respectively. According to Akkaya and Buyukunal[1], high PIC value can be attributed to the informative markers. Higher PIC values were observed with SSR primer RM 428 and RM 259(0.3748).This observed pattern was consistent with the findings of Lapitan *et al.*[11], The lowest PCI value (0.06) was observed for RM 264.The average value was 0.18 per locus. Primer RM 19, RM 346, RM 547, RM 20, RM 229 and RM 413 had PIC value of 0.Similar results were obtained by Kumar *et al.*[10].

Markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate of a marker at a specific locus[5]. The mean PIC value observed in this study was lower than the PIC value of 0.56 as recorded by Ravi *et al.*[13], with rice cultivars, landraces and wild relatives. This indicated that the genotypes used in the present study were not much diverse.

**Relationship among rice genotypes using SSR markers**

Based on the SSR marker data the Jaccard's similarity coefficients were estimated between pair of lines and presented in Table 4 .The similarity coefficients were found to vary from 0.550 to 0.975. The highest value for genetic similarity (0.975) was found between RKN 33 and RKN 31.RKN 15 and RKN 9, RKN 14 and RKN 22 were associated with each other with a similarity coefficient 0.55. All the loci amplified by the primer pair were polymorphic which varied in size from <100 to 400.

**Genetic diversity among the genotypes**

The objective of the experiment was to estimate the level of genetic diversity among the rice genotypes using SSR markers. The UPGMA (Unweighted Pair Method with Arithmetic Mean) dendogram was constructed using Jaccard's similarity coefficients based on SSR marker data generated on 35 genotypes (Fig. 2) UPGMA ordered the populations of 35 genotypes into six clusters. Cluster 1 consisted of eighteen genotypes namely RKN 1, RKN 2, RKN 3, RKN 7, RKN 19, RKN 30, RKN 31, RKN 33, RKN 35, RKN 29, RKN 32, RKN 26, RKN 10, RKN 5, RKN 18, RKN 15, RKN 17 and RKN 22 and related to members of cluster 2 with a similarity coefficient 0.02. cluster 2 consisted of eleven genotypes namely RKN4, RKN 6, RKN 21, RKN 23, RKN 24, RKN 8, RKN 11, RKN 12, RKN 16, RKN 9 and RKN 13. Cluster 3 comprised of two genotypes namely, RKN 25 and RKN 34. Cluster 2 and 3 had a similarity coefficient of 0.05. Cluster 4 consisted of only two genotypes namely, RKN 27 and RKN 28 and was related to cluster 3 with a similarity coefficient 1.4. Cluster V and cluster VI had one genotype each namely RKN 20 and RKN 14 respectively. The dendrogram resulting from UPGMA analysis revealed allelic richness of 6 clusters for various sizes.

The identified markers are highly informative for genetic studies and are extremely useful in distinguishing the polymorphism rate at a specific locus in rice. SSR primers namely, RM159 and RM264, generated higher levels of polymorphism and can be used to differentiate rice genotypes[10]. Hence, from the present study, it can be proved that SSR markers can detect high polymorphism and are very useful in studying variation among different genotypes. Varietal profiling based on SSR markers will be more reliable as compared to other markers, since SSR markers detect finer levels of variations among closely related lines. Therefore, it can be concluded that the genetic diversity at

functional regions of the genome, should be targeted and utilized in heterotic rice breeding programmes, for predicting hybrid performance.

**Table 1: List of advanced recombinant kalanamak genotypes (RKN) used for diversity analysis**

| S. No | Genotypes | S. No | Genotypes |
|---|---|---|---|
| 1 | RKN- 1 | 19 | RKN -19 |
| 2 | RKN -2 | 20 | RKN -21 |
| 3 | RKN -3 | 21 | RKN -22 |
| 4 | RKN -4 | 22 | RKN -23 |
| 5 | RKN -5 | 23 | RKN -24 |
| 6 | RKN -6 | 24 | RKN -25 |
| 7 | RKN -7 | 25 | RKN -26 |
| 8 | RKN -8 | 26 | RKN -27 |
| 9 | RKN -9 | 27 | RKN -28 |
| 10 | RKN -10 | 28 | RKN -29 |
| 11 | RKN -11 | 29 | RKN -29 |
| 12 | RKN -12 | 30 | RKN -30 |
| 13 | RKN -13 | 31 | RKN -31 |
| 14 | RKN -14 | 32 | RKN -32 |
| 15 | RKN -15 | 33 | RKN -33 |
| 16 | RKN -16 | 34 | RKN -34 |
| 17 | RKN -17 | 35 | RKN -35 |
| 18. | RKN -18 | | |

*RKN – genetically developed advanced recombinant kalanamak genotypes through hybridization followed by selection.

**Table 2: List of SSR markers used for molecular diversity studies**

| S. No | SSR primer | Chromo-some | Sequence | |
|---|---|---|---|---|
| | | | Forward primer | Reverse primer |
| 1 | RM 154 | 2 | ACCCTCTCCGCCTCGCCTCCTC | CTCCTCCTCCTGCGACCGCTCC |
| 2 | RM 159 | 5 | GGGGCACTGGCAAGGGTGAAGG | GCTTGTGCTTCTCTCTCTCTCTCTCTC |
| 3 | RM 528 | 6 | GGCATCCAATTTTACCCCTC | AAATGGAGCATGGAGGTCAC |
| 4 | RM 338 | 9 | CACAGGAGCAGGAGAAGAGC | GGCAAACCGATCACTCAGTC |
| 5 | RM 226 | 1 | AGCTAAGGTCTGGGAGAAACC | AAGTAGGATGGGGCACAAGCTC |
| 6 | RM 307 | 4 | GTACTACCGACCTACCGTTCAC | CTGCTATGCATGAACTGCTC |
| 7 | RM 25 | 8 | GGAAAGAATGATCTTTTCATGG | CTACCATCAAAACCAATGTTC |
| 8 | RM 215 | 9 | CAAAATGGAGCAGCAAGAGC | TGAGCACCTCCTTCTCTGTAG |
| 9 | RM 277 | 12 | CGGTCAAATCATCACCTGAC | CAAGGCTTGCAAGGGAAG |
| 10 | RM 247 | 12 | TAG TGC CGA TCG ATG TAA CG | CAT ATG GTT TTG ACA AAG CG |
| 11 | RM 264 | | GTTGCGTCCTACTGCTACTTC | GATCCGTGTCGATGATTAGC |
| 12 | RM 167 | 11 | GATCCAGCGTGAGGAACACGT | AGTCCGACCACAAGGTGCGTTGTC |
| 13 | RM 519 | 12 | AGAGAGCCCCTAAATTTCCG | AGGTACGCTCACCTGTGGAC |
| 14 | RM 19 | 4 | CAAAAACAGAGCAGATGAC | CTCAAGATGGACGCCAAGA |
| 15 | RM 346 | 6 | CGAGAGAGCCCATAACTACG | ACAAGACGACGAGCAGGAGGGAC |
| 16 | RM 547 | 8 | TAGGTTGGCAGACCTTTT | GTCAAGATCATTCTCGTAGCG |
| 17 | RM 10 | 6 | TTGTCAAGAGGAGGCATCG | CAGAATGGGAAATGGGTCC |
| 18 | RM 8225 | 6 | ATGCGTGTTCAGAAATTAGG | TTGTTGTATACCTCATCGACAG |
| 19 | RM 8226 | 6 | TTAGGATACGGCTTCTAGGC | CGTAATTGTTGCATATGGTG |
| 20 | RM 340 | 6 | GGTAAATGGACAATCCTATGGC | GACAAATATAAGGGCAGTGTGC |
| 21 | RM 229 | 11 | CACTCACACGAACGACTGAC | CGCAGGTTCTTGTGAAATGT |
| 22 | RM 287 | 11 | TTCCCTGTTAAGAGAGAAATC | GTGTATTTGGTGAAAGCAAC |
| 23 | RM 168 | 3 | TGCTGCTTGCCTGCTTCCTTT | GAAACGAATCAATCCACGGC |
| 24 | RM 104 | 1 | GGAAGAGGAGAGAAAGATGTGTGTCG | TCAACAGACACACCGCCACCGC |
| 25 | RM 413 | 5 | GGCGATTCTTGGATGAAGAG | TCCCCACCAATCTTGTCTTC |
| 26 | RM 228 | 10 | CTGGCCATTAGTCCTTGG | GCTTGCGGCTCTGCTTAC |
| 27 | RM 428 | 1 | AACAGATGGCATCGTCTTCC | CGCTGCATCCACTACTGTTG |
| 28 | RM 151 | 1 | GGCTGCTCATCAGCTGCATGCG | TCGGCAGTGGTAGAGTTTGATCTGC |
| 29 | RM 259 | 1 | TGGAGTTTGAGAGGAGGG | CTTGTTGCATGGTGCCATGT |
| 30 | RM 20 | 1 | ATCTTGTCCCTGCAGGTCAT | GAAACAGAGGCACATTTCATTG |

**Table 3: Polymorphism Information Content (PIC) of SSR loci across various varieties analyzed in the investigation**

| S. No | SSR primer | Total Number of alleles | Number of polymorphic alleles | Number of monomorphic alleles | % polymorphism | Size range of alleles | PIC value |
|---|---|---|---|---|---|---|---|
| 1. | RM 154 | 1 | 1 | 0 | 100% | 200 | 0.14 |
| 2 | RM 159 | 3 | 3 | 0 | 100% | 150-400 | 0.364 |
| 3 | RM 528 | 1 | 1 | 0 | 100% | 320 | 0.101 |
| 4 | RM 338 | 1 | 1 | 0 | 100% | 200 | 0.214 |
| 5 | RM 226 | 1 | 1 | 0 | 100% | 190 | 0.268 |
| 6 | RM 307 | 1 | 1 | 0 | 100% | 140 | 0.290 |
| 7 | RM 25 | 1 | 1 | 0 | 100% | 120 | 0.182 |
| 8 | RM 215 | 1 | 1 | 0 | 100% | 120 | 0.101 |
| 9 | RM 277 | 1 | 1 | 0 | 100% | 150 | 0.101 |
| 10 | RM 247 | 2 | 2 | 0 | 100% | 200 | 0.09 |
| 11 | RM 264 | 3 | 3 | 0 | 100% | 190-300 | 0.06 |
| 12 | RM 167 | 2 | 2 | 0 | 100% | 130-150 | 0.367 |
| 13 | RM 519 | 1 | 1 | 0 | 100% | 110 | 0.214 |
| 14 | RM 19 | 1 | 0 | 1 | 0% | 200 | 0 |
| 15 | RM 346 | 1 | 0 | 1 | 0% | 120 | 0 |
| 16 | RM 547 | 1 | 0 | 1 | 0% | 225 | 0 |
| 17 | RM 10 | 1 | 1 | 0 | 100% | 150 | 0.209 |
| 18 | RM 8225 | 1 | 1 | 0 | 100% | 90 | 0.357 |
| 19 | RM 8226 | 1 | 1 | 0 | 100% | 290 | 0.243 |
| 20 | RM 340 | 1 | 1 | 0 | 100% | 170 | 0.268 |
| 21 | RM 229 | 0 | 0 | 0 | 0% | 0 | 0 |
| 22 | RM 287 | 1 | 1 | 0 | 100% | 100 | 0.182 |
| 23 | RM 168 | 1 | 1 | 0 | 100% | 100 | 0.369 |
| 24 | RM 104 | 1 | 1 | 0 | 100% | 125 | 0.364 |
| 25 | RM 413 | 0 | 0 | 0 | 0% | 0 | 0 |
| 26 | RM 228 | 1 | 1 | 0 | 100% | 100 | 0.180 |
| 27 | RM 428 | 1 | 1 | 0 | 100% | 270 | 0.374 |
| 28 | RM 151 | 2 | 2 | 0 | 100% | 250-300 | 0.182 |
| 29 | RM 259 | 1 | 1 | 0 | 100% | 200 | 0.374 |
| 30 | RM 20 | 1 | 0 | 1 | 0% | 200 | 0 |

**Table 4: Jaccards Similarity Coefficient between rice genotypes**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2 | 0.95 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3 | 0.90 | 0.90 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4 | 0.72 | 0.72 | 0.82 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 5 | 0.87 | 0.87 | 0.92 | 0.85 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 6 | 0.70 | 0.65 | 0.75 | 0.87 | 0.77 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | 0.85 | 0.85 | 0.85 | 0.67 | 0.82 | 0.60 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | 0.80 | 0.80 | 0.85 | 0.87 | 0.87 | 0.80 | 0.75 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 9 | 0.65 | 0.60 | 0.70 | 0.77 | 0.72 | 0.85 | 0.65 | 0.75 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 10. | 0.95 | 0.95 | 0.95 | 0.77 | 0.92 | 0.70 | 0.90 | 0.85 | 0.65 | 1 | | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | 0.70 | 0.70 | 0.75 | 0.77 | 0.77 | 0.85 | 0.65 | 0.90 | 0.75 | 0.75 | 1 | | | | | | | | | | | | | | | | | | | | | | | | |
| 12. | 0.82 | 0.82 | 0.82 | 0.80 | 0.85 | 0.82 | 0.77 | 0.87 | 0.77 | 0.87 | 0.87 | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| 13. | 0.60 | 0.65 | 0.70 | 0.72 | 0.72 | 0.75 | 0.75 | 0.75 | 0.80 | 0.65 | 0.75 | 0.77 | 1 | | | | | | | | | | | | | | | | | | | | | | |
| 14. | 0.75 | 0.70 | 0.70 | 0.62 | 0.72 | 0.75 | 0.70 | 0.65 | 0.65 | 0.75 | 0.70 | 0.72 | 0.60 | 1.0 | | | | | | | | | | | | | | | | | | | | | |
| 15 | 0.85 | 0.85 | 0.85 | 0.72 | 0.82 | 0.70 | 0.85 | 0.75 | 0.55 | 0.90 | 0.70 | 0.77 | 0.65 | 0.75 | 1.0 | | | | | | | | | | | | | | | | | | | | |
| 16 | 0.77 | 0.77 | 0.82 | 0.80 | 0.80 | 0.87 | 0.72 | 0.87 | 0.77 | 0.82 | 0.92 | 0.95 | 0.77 | 0.72 | 0.77 | 1 | | | | | | | | | | | | | | | | | | | |
| 17 | 0.82 | 0.82 | 0.87 | 0.75 | 0.85 | 0.67 | 0.77 | 0.72 | 0.62 | 0.87 | 0.62 | 0.75 | 0.62 | 0.62 | 0.82 | 0.70 | 1 | | | | | | | | | | | | | | | | | | |
| 18 | 0.87 | 0.92 | 0.87 | 0.70 | 0.85 | 0.67 | 0.82 | 0.77 | 0.62 | 0.92 | 0.72 | 0.85 | 0.72 | 0.72 | 0.87 | 0.80 | 0.85 | 1.0 | | | | | | | | | | | | | | | | | |
| 19 | 0.80 | 0.85 | 0.85 | 0.72 | 0.82 | 0.65 | 0.80 | 0.80 | 0.60 | 0.85 | 0.70 | 0.72 | 0.75 | 0.65 | 0.85 | 0.72 | 0.77 | 0.87 | 1 | | | | | | | | | | | | | | | | |
| 20 | 0.67 | 0.67 | 0.67 | 0.75 | 0.70 | 0.72 | 0.57 | 0.82 | 0.62 | 0.67 | 0.82 | 0.70 | 0.67 | 0.57 | 0.67 | 0.75 | 0.60 | 0.70 | 0.72 | 1 | | | | | | | | | | | | | | | |
| 21 | 0.70 | 0.70 | 0.80 | 0.87 | 0.82 | 0.90 | 0.65 | 0.85 | 0.80 | 0.75 | 0.85 | 0.87 | 0.85 | 0.70 | 0.75 | 0.87 | 0.72 | 0.77 | 0.75 | 0.72 | 1 | | | | | | | | | | | | | | |
| 22 | 0.75 | 0.75 | 0.80 | 0.82 | 0.77 | 0.75 | 0.70 | 0.85 | 0.70 | 0.80 | 0.75 | 0.82 | 0.70 | 0.55 | 0.75 | 0.82 | 0.87 | 0.77 | 0.75 | 0.72 | 0.80 | 1 | | | | | | | | | | | | | |
| 23 | 0.72 | 0.72 | 0.82 | 0.90 | 0.85 | 0.87 | 0.67 | 0.87 | 0.82 | 0.77 | 0.82 | 0.85 | 0.82 | 0.62 | 0.72 | 0.85 | 0.80 | 0.80 | 0.77 | 0.75 | 0.92 | 0.87 | 1 | | | | | | | | | | | | |
| 24 | 0.67 | 0.67 | 0.77 | 0.85 | 0.80 | 0.92 | 0.62 | 0.82 | 0.82 | 0.72 | 0.87 | 0.85 | 0.82 | 0.67 | 0.72 | 0.90 | 0.75 | 0.75 | 0.72 | 0.75 | 0.92 | 0.82 | 0.95 | 1 | | | | | | | | | | | |
| 25 | 0.67 | 0.67 | 0.77 | 0.80 | 0.75 | 0.77 | 0.67 | 0.77 | 0.72 | 0.72 | 0.77 | 0.85 | 0.77 | 0.57 | 0.67 | 0.85 | 0.65 | 0.70 | 0.72 | 0.70 | 0.82 | 0.72 | 0.80 | 0.80 | 1 | | | | | | | | | | |
| 26 | 0.80 | 0.75 | 0.80 | 0.62 | 0.77 | 0.60 | 0.80 | 0.65 | 0.65 | 0.80 | 0.60 | 0.67 | 0.65 | 0.60 | 0.75 | 0.62 | 0.77 | 0.77 | 0.75 | 0.67 | 0.65 | 0.65 | 0.67 | 0.62 | 0.67 | 1 | | | | | | | | | |
| 27 | 0.57 | 0.57 | 0.62 | 0.75. | 0.70 | 0.72 | 062. | 0.62 | 0.72 | 0.67 | 0.62 | 0.72 | 0.75 | 0.77 | 0.62 | 0.70 | 0.65 | 0.65 | 0.67 | 0.65 | 0.77 | 0.67 | 0.80 | 0.80 | 0.80 | 0.57 | 1 | | | | | | | | |
| 28 | 0.57 | 0.62 | 0.62 | 0.70 | 0.70 | 0.77 | 0.62 | 0.72 | 0.67 | 0.62 | 0.77 | 0.75 | 0.82 | 0.62 | 0.67 | 0.75 | 0.65 | 0.70 | 0.72 | 0.70 | 0.77 | 0.67 | 0.80 | 0.85 | 0.75 | 0.57 | 0.90 | 1 | | | | | | | |
| 29 | .80 | 0.80 | 0.85 | 0.82 | 0.92 | 0.75 | 0.80 | 0.80 | 0.70 | 0.85 | 0.70 | 0.77 | 0.75 | 0.65 | 0.85 | 0.72 | 0.87 | 0.82 | 0.85 | 0.67 | 0.80 | 0.80 | 0.87 | 0.82 | 0.72 | 0.75 | 0.77 | 0.77 | 1 | | | | | | |
| 30 | 0.77 | 0.77 | 0.87 | 0.80 | 0.90 | 0.72 | 0.77 | 0.82 | 0.67 | 0.82 | 0.72 | 0.75 | 0.77 | 0.62 | 0.82 | 0.75 | 0.80 | 0.80 | 0.92 | 0.70 | 0.82 | 0.77 | 0.85 | 0.80 | 0.80 | 0.77 | 0.75 | 0.75 | 0.92 | 1 | | | | | |
| 31 | 0.72 | 0.77 | 0.72 | 0.80 | 0.80 | 0.72 | 0.72 | 0.82 | 0.72 | 0.77 | 0.72 | 0.80 | 0.77 | 0.62 | 0.77 | 0.75 | 0.75 | 0.80 | 0.82 | 0.75 | 0.77 | 0.82 | 0.85 | 0.80 | .70 | 0.62 | 0.75 | 0.80 | 0.87 | 0.80 | 1 | | | | |
| 32 | 0.77 | 0.77 | 0.77 | 0.80 | 0.85 | 0.72 | 0.77 | 0.87 | 0.67 | 0.82 | 0.77 | 0.80 | 0.72 | 0.62 | 0.82 | 0.75 | 0.80 | 0.80 | 0.82 | 0.75 | 0.77 | 0.82 | 0.85 | 0.80 | 0.70 | 0.67 | 0.80 | 0.80 | 0.92 | 0.85 | 0.90 | 1 | | | |
| 33 | 0.75 | 0.80 | .750 | 0.82 | 0.82 | 0.75 | 0.75 | 0.85 | 0.70 | 0.80 | 0.75 | 0.82 | 0.80 | 0.60 | 0.80 | 0.77 | 0.77 | 0.82 | 0.85 | 0.77 | 0.80 | 0.85 | 0.87 | 0.82 | 0.72 | 0.65 | 0.77 | 0.82 | 0.90 | 0.82 | 0.97 | 0.92 | 1 | | |
| 34 | 0.72 | 0.67 | 0.77 | 0.65 | 0.70 | 0.72 | 0.67 | 0.67 | 0.67 | 0.72 | 0.67 | 0.75 | 0.72 | 0.62 | 0.72 | 0.75 | 0.70 | 0.75 | 0.77 | 0.60 | 0.77 | 0.67 | 0.75 | 0.75 | 0.80 | 0.72 | 0.70 | 0.70 | 0.72 | 0.80 | 0.65 | 0.70 | 0.67 | 1 | |
| 35 | 0.67 | 0.72 | 0.72 | 0.80 | 0.80 | 0.72 | 0.67 | 0.77 | 0.67 | 0.72 | 0.72 | 0.75 | 0.77 | 0.57 | 0.72 | 0.70 | 0.75 | 0.75 | 0.77 | 0.75 | 0.77 | 0.77 | 0.85 | 0.80 | 0.70 | 0.67 | 0.80 | 0.80 | 0.87 | 0.80 | 0.90 | 0.85 | 0.92 | 0.65 | 1 |

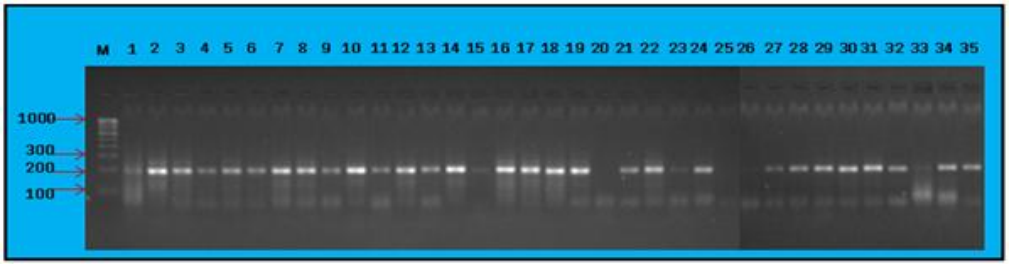


**Figure 1: PCR amplification of advanced recombinant kalanamak rice genotypes by SSR primer RM 154**

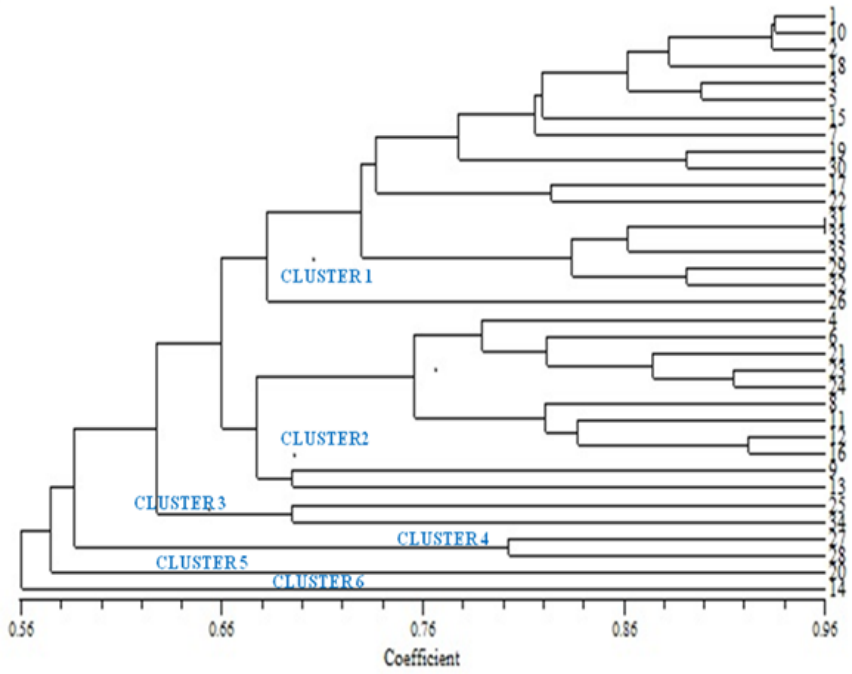


**Figure 2: Dendogram showing the clustering pattern of advanced recombinant kalanamak rice genotypes studied**

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