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# Intravarietal diversity analysis of a Western Ghat *Mangifera indica* L. variety 'Kottoorkonam' using ISSR markers

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**Abstract**

Mangifera indica L. is the most popular and widely cultivated fruit crop in the tropics. According to historical data, mango trees are intimately connected with Indian culture and folklore since 4000 years ago. The tropical rain forests occurring on the slopes of the Western Ghats region of Kerala state is rich in natural varieties of *M. indica*. Most of the common varieties under cultivation have been locally named in accordance with their taste, quality and place of distribution. A preliminary investigation conducted in the Thiruvananthapuram district of the Kerala state of India on the availability and variability of mango varieties have identified 'Kottoorkonam' as the dominant variety with high yielding capacity, high disease resistance and good taste of fruits. The present paper reports for the first time the genetic diversity and differentiation analysis of the same using ISSR markers indicating 16.32% polymorphism. The study has brought out information on the diversity of indigenous mango varieties and it will be a landmark in the field of restoration of wild germplasm of mangoes. Also it reveals that ISSR markers are useful not only for varietal identification, but also in future mango breeding programmes to maximize genetic variability among the mango cultivars.

**Key words**: Mangifera indica L., 'Kottoorkonam' variety, genetic diversity, ISSR analysis

**Introduction**

Mango (*Mangifera indica* L.) is one of the most important fruit crops of the Anacardiaceae family which originated as an allopolyploid from Eastern India, Assam and Burma [1]. It occurs as a domesticated or wild entity in the complex biotic community of the ecosystem and has been undoubtedly under cultivation for more than 4000 years in Eastern India and Burma [2]. Now it is cultivated pantropically throughout the world and is one of the most popular fruits in the tropics [3]. In India, mangoes are cultivated in an area of 2.31 million ha with an annual production of 12.75 million tons. Indian mangoes occupy 45.59% of the total world's mango area and contribute 35.70% to the mango production globally. Moreover India is the largest mango producer in the world [4]. The introduction of mango in other parts of the world is comparatively recent. Thousands of natural varieties of mango have been observed in India and their origin is probably from the Assam-Burma-Thailand region [5], where truly wild mango trees belonging to both *Mangifera indica* and *M. sylvatica* have been recorded [6]. Kerala region coming under the Western Ghats is rich in wild varieties of mangoes [7]. They are consumed from time immemorial as delicacies for different purposes and occasions and were named according to their taste and quality [8]. However, they are drastically depleted from the wild due to various reasons. The influx of cultivation of popular horticulture varieties of mango in these areas has made the local varieties unpopular and many of them become rare and they are facing severe threat of extinction. Urbanization of Kerala villages and construction of houses in farm lands have also led to drastic depletion of these local varieties of mangoes. Another observation is that the destruction of sacred groves, extensive use of wood of mango trees for construction purposes as well as the Hindu ritual ceremonies with mango wood, led many of the local varieties to become either endangered or rare [9]. Commercial exploitation of local varieties especially for pickles and fruits is one of the major financial gains of the local people of the district. However, several natural varieties are depleting and have reached the verge of extinction due to various reasons like monoculture plantation crops and other anthropogenic activities for various domestic purposes.

Crop diversity is well represented as developed cultivars, landraces or as folk varieties in different ecogeographical regions of India in mangoes. Allopolyploidy, cross pollination and wide range of agro-climatic conditions prevailing in this country contributes to the enormous genetic diversity in them. Over 40 centuries of its cultivation and domestication, the wide genetic diversity of the plant has been fixed in many varieties. The present day cultivars are mainly seedling selections and are maintained through clonal propagation [10]. India has over 1000 mango cultivars and represents the biggest mango gene-pool in the world [11].

We have conducted an investigation in the Thiruvananthapuram district in Kerala State of India on the availability and variability of mango varieties and have identified 15 nos. of local varieties of mangoes with clear cut characteristics. Among this group 'Kottoorkonam' variety from the originating location Kottoor landmarked in the foot hills of Southern Western Ghats of Kerala region is earmarked as the dominant one further selected for genetic variability study and differentiation analysis using ISSR markers. In an attempt to study genomic polymorphism within the 'Kottoorkonam'

cultivar from several orchards and stocks, we have carried out a set of experiments using ISSR primers. The work presented here focussed on validation of ISSR markers, their polymorphic potential and assessment of intravarietal polymorphism among 38 accessions of *M. indica* variety 'Kottoorkonam', as a fundamental base for mango breeding programs and conservation.

## Materials and methods

### Plant material

A total of 38 accessions of *Mangifera indica* L. variety 'Kottoorkonam' from different altitudes of Thiruvananthapuram district of the Kerala State in India were collected for the present study (Table 1).

**Table 1:** Collection localities of *M. indica* L. variety 'Kottoorkonam' samples

| Pop ID | Location | Altitude (m) | Site co ordinate |
|---|---|---|---|
| 1 | Paripally | 59 | N8° 49.997″ E 76° 41.440″ |
| 2 | JNTBGRI quarters | 90 | N8° 44.852″ E 77° 01.429″ |
| 3 | JNTBGRI junction | 88 | N8° 45.293″ E 77° 01.396″ |
| 4 | Jawahar colony | 85 | N8° 45.290″ E 77° 01.394″ |
| 5 | Ex. Colony | 95 | N8° 46.064″ E 77° 01.542″ |
| 6 | Elavupalam | 93 | N8° 46.152″ E 77° 01.110″ |
| 7 | Challimukku | 101 | N8° 46.094″ E 77° 01.553″ |
| 8 | Edacolony | 100 | N8° 46.112″ E 77° 01.109″ |
| 9 | Kollayil | 92 | N8° 46.110″ E 77° 01.020″ |
| 10 | Thannimmoodu | 91 | N8° 46.110″ E 77° 1.200″ |
| 11 | Ozhukupara | 90 | N8° 46.110″ E 77° 1.560″ |
| 12 | Kalayapuram | 92 | N8° 46.109″ E 77° 1.201″ |
| 13 | Madathara | 114 | N8° 45.951″ E 77° 1.402″ |
| 14 | Chippanchira | 115 | N8° 44.825″ E 77° 1.621″ |
| 15 | Manthuruthy | 114 | N8° 43.674″ E 77° 1.643″ |
| 16 | Agri. farm | 111 | N8° 45.876″ E 77° 1.765″ |
| 17 | Idinjar | 112 | N8° 43.634″ E 77° 1.876″ |
| 18 | Thennoor | 106 | N8° 44.776″ E 77° 1.681″ |
| 19 | Kallar | 114 | N8° 44.761″ E 77° 1.711″ |
| 20 | Vithura | 115 | N8° 44. 654″ E 77° 1.862″ |
| 21 | Karimancode | 116 | N8° 44.109″ E 77° 1.765″ |
| 22 | Pappanamcode | 83 | N8° 44.094″ E 77° 1.935″ |
| 23 | Peringammala | 86 | N8° 44.163″ E 77° 1.112″ |
| 24 | Poovar | 60 | N8° 18.519″ E 77° 6.631″ |
| 25 | Oorambu | 64 | N8° 18.685″ E 77° 7.570″ |
| 26 | Chenkavila | 62 | N8° 18.630″ E 77° 7.112″ |
| 27 | Parasala | 66 | N8° 18.540″ E 77° 7.630″ |
| 28 | Vellarada | 60 | N8° 18.132″ E 77° 7.410″ |

| | | | |
|---|---|---|---|
| 29 | Karakonam | 95 | N8° 23.341" E 77° 10.281" |
| 30 | Panachammoodu | 123 | N8° 25.314" E 77° 11.815" |
| 31 | Aryanadu | 132 | N8° 26.986" E 77° 11.666" |
| 32 | Kallikkadu | 130 | N8° 36.521" E 77° 11.696" |
| 33 | Kuttichal | 66 | N8° 32.619" E 77° 7.137" |
| 34 | Kottoor | 70 | N8° 32.619" E 77° 7.034" |
| 35 | Nedumangadu | 92 | N8° 45.212" E 77° 1.313" |
| 36 | Kallara | 91 | N8° 43.216" E 77° 1.543" |
| 37 | Neyyattinkara | 67 | N8° 18.410" E 77° 7.260" |
| 38 | Balaramapuram | 68 | N8° 17.160" E 77° 6.257" |

**Preparation of Genomic DNA using CTAB**

Total genomic DNA from the young leaves was isolated following modified cetyl trimethyl ammonium bromide (CTAB) method [12]. After ethanol precipitation DNA was resuspended in 0.1$cm^3$ of 1xTE buffer (pH 8.0) and was quantified spectrophotometrically by taking the absorbance at 260 nm. ISSR assay was carried out in 0.025 $cm^3$ reaction mixture containing 0.2 mM dNTP's, 10 mM Tris-HCL, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 1.0 U Taq DNA polymerase (Finzymes, Helsinki, Finland), 15 pmol primers kit 'C' (`IDT, Coralville, USA) and 50 ng of genomic DNA. Amplification was performed in a thermal cycler (Veriti Thermal cycler, Applied Biosystems). After the initial cycle of 2 min at 93 °C, 2 min at 50 to 55 °C and 2 min at 72 °C, 39 cycles of 1min at 93 °C, 1min at 50 to 55 °C and 1 min at 72 °C were performed. The last cycle comprised 10 min extension at 72 °C. Reaction mixture wherein template DNA replaced by distilled water was used as negative control. Amplified products where resolved in 1.40% agarose gel (1xTBE) followed by EtBr staining.

**Genetic data analysis**

Amplification with each arbitrary primer was repeated 3 times and those primers that produced reproducible and consistent bands were selected for data generation. Reproducible ISSR products were scored against the presence or absence of a fragment. Dice coefficient of similarity defined as 2a/2a+u, where 'a' is the number of positive matches and 'u' the number of non-matches was computed using the WINDIST software [13]. The scored binary matrix was analyzed using the WINBOOT software [13]. Intragenetic variation was analyzed for various parameters. The genotype and allelic frequency data were used to compute the genetic diversity indices i.e. observed number of alleles (na), expected number of alleles (ne), Shannon index of genetic diversity (I) and nei's gene diversity (h) at the population level using the statistical package POPGENE 1.3 [14]. The sampling populations were assumed to be in Hardy–Weinberg equilibrium implying that the population is at random mating. Based on the above assumption, the bands were scored and estimation of heterozygosity (Ht) was done according to the formula: $Ht = 1-\Sigma pi^{-2}$ where pi is the frequency of the $i^{th}$ allele in the population.

## Results and Discussion

### ISSR profiling

There are different methods such as morphological, biochemical and molecular markers to characterize intravarietal heterogeneity. Several procedures have been employed by several authors for the identification and characterization of intracultivar heterogeneity of mango based on morphological [15, 16, 17, 18], biochemical [16] and genetic traits [19, 20, 21, 22]. However, the disadvantages of morphological characterizations are their low polymorphism, heritability and sensitivity to changes in environmental conditions. In addition to using morphological descriptors, molecular markers analysis techniques are increasingly used for characterisation of intravarietal variability of mango including isozymes [16], randomly amplified polymorphic DNA-RAPDs [19, 20], inter simple sequence repeats-ISSRs [23, 22]. The study presented here has assessed the intravarietal heterogeneity of 'Kottoorkonam' cultivar of mango available at orchards grown by the farmers through polymerase chain reaction (PCR) based inter simple sequence repeat (ISSR) marker system.

The genomic DNA isolated was used as template for ISSR assays which was carried out with 10 primers of 17–18 bp, of which some were anchored at the 3' end for increased specificity. A total of 49 products were obtained and 8 products were polymorphic, thereby showing 16.32% polymorphism (Table 2). The number of products generated by these arbitrary primers was found to range from 2 to 7 with primer 845 giving the maximum (7) and primer 847 giving the minimum number (2) of amplicons (Fig.1). The similarity matrix developed using the WINDIST software showed that similarity index ranged from 0.95 to 0.98 with mean value of 0.97 suggesting low levels of genetic variability in the species. Nei's gene diversity at population level (h), Shannon index (I) and expected number of alleles calculated to estimate genetic variation levels using POPGENE program indicated relatively low level of genetic diversity with h=0.07 and I= 0.12 (Table 3). The mean genetic diversity based on Nei (1987) [24] statistical analysis also supported the other data. In contrast highest genetic dissimilarity coefficient of 0.50 was observed among 31 accessions of 'Beneshan' cultivated throughout the Andrapradesh state [25]. Similar level of genetic dissimilarity coefficient of 0.45 among 25 accessions of 'Rosa' cultivar of mango was reported [20] using RAPD markers which confer that they are not pure clones and it is possible to breed this cultivar. However absolutely low level of genetic dissimilarity coefficient of 0.05 was noted [19] among 15 accessions of 'Kensington Pride', a polyembryonic cultivar of mango using 10 RAPD markers concluding the purity of clones thereby substantiating our findings.

The mean value of heterozygosity (Ht) observed in the various accessions of *M. indica* variety 'Kottoorkonam' was found to be 0.07; the mean value of average heterozygosity was 0.04. The heterozygosity values and degree of genetic differentiation (Gst) is shown in Table 3. The other diversity measures also indicated a similar result. The gene flow (Nm) value of 0.50 among all accessions was calculated on the assumption that the accession under study follows the inland model [26] which predicts a simple relationship between the number of migrants an accession receives per generation and Fst. Fst =(1/4Nm+1) from which Nm was

derived as Nm = (1-Fst)/4Fst. The GST value obtained from the POPGENE analysis was substituted for Fst and derived the rate of gene flow [27].

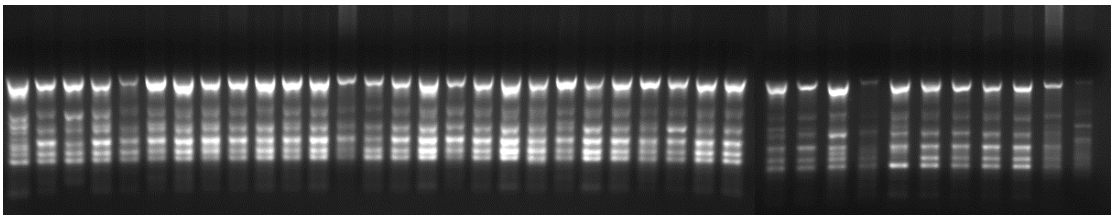

**Figure 1:** Representative ISSR Gel Profile of different accessions of *M. indica* L. Variety 'Kottoorkonam' with primer 824

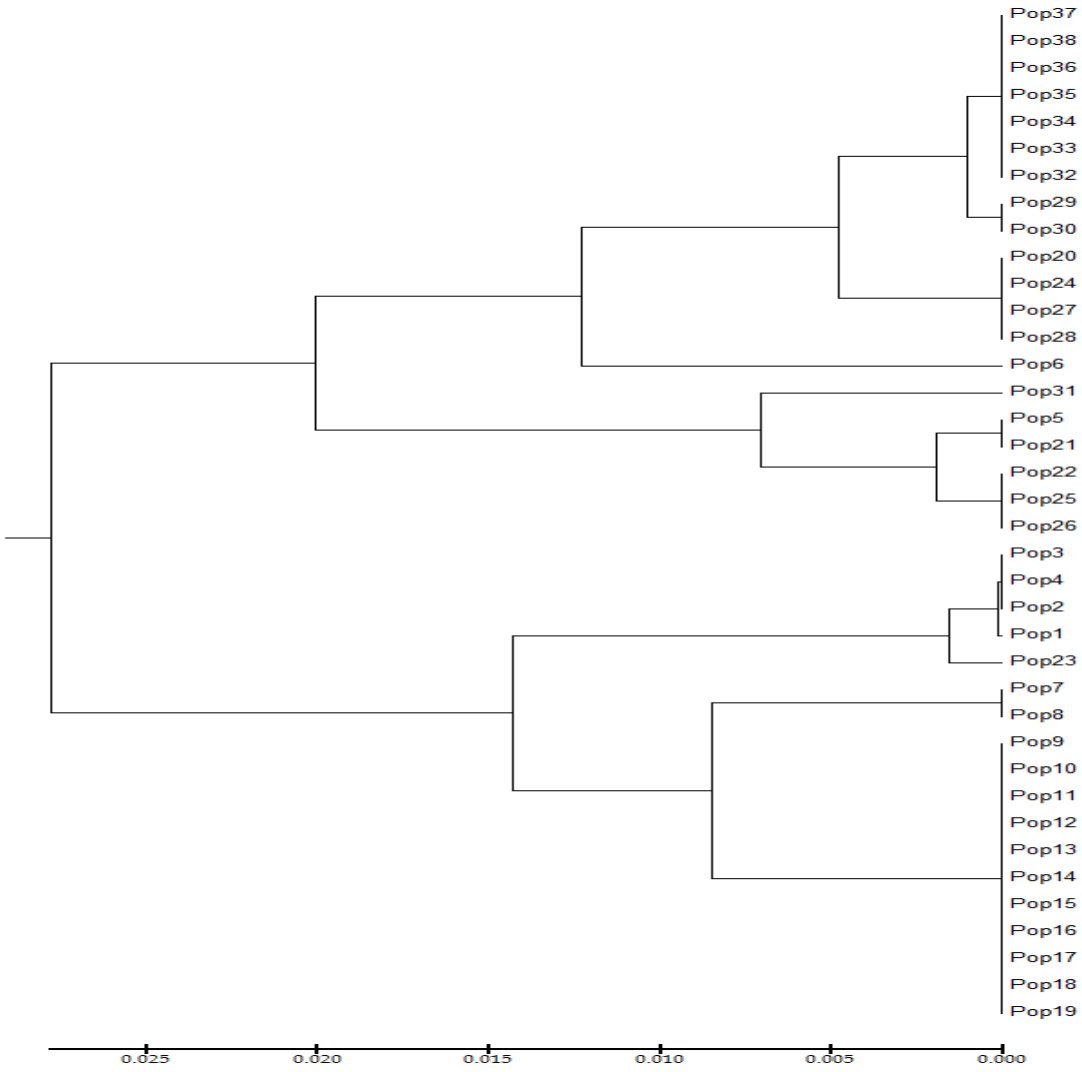


**Figure 2:** Phenogram based on UPGMA analysis of *M. indica* L. variety 'Kottoorkonam'

**Table 2.** List of primers and their sequence used for ISSR analysis of *M. indica* L. variety 'Kottoorkonam'

| Sl.No | Primer | Sequence of primers (5'→3') | No. of Bands | No. of Polymorphic bands |
|---|---|---|---|---|
| 1 | 815 | CTCTCTCTCTCTCTCTG | 5 | 0 |
| 2 | 835 | AGAGAGAGAGAGAGAGYC* | 5 | 4 |
| 3 | 841 | GAGAGAGAGAGAGAGAYC* | 6 | 0 |
| 4 | 844 | CTCTCTCTCTCTCTCTRC** | 3 | 0 |
| 5 | 845 | CTCTCTCTCTCTCTCTRG** | 7 | 0 |
| 6 | 848 | CACACACACACACACARG** | 5 | 0 |
| 7 | 824 | TCTCTCTCTCTCTCTCG | 6 | 1 |
| 8 | 808 | AGAGAGAGAGAGAGAGC | 5 | 0 |
| 9 | 843 | CTCTCTCTCTCTCTCTRA** | 5 | 3 |
| 10 | 847 | CACACACACACACACARC | 2 | 0 |
| **Total** | | | **49** | **8 (16.32%)** |

**Table 3.** Summary of genetic diversity data in *M. indica*. L. variety 'Kottoorkonam'

| Sl. No | Diversity Indices | Value |
|---|---|---|
| 1 | Observed number of alleles (na) | 1.30 |
| 2 | Effective number of alleles (ne) | 1.12 |
| 3 | Nei's gene diversity (h) | 0.07 |
| 4 | Shannon's Information index ( I ) | 0.12 |
| 5 | Ht | 0.07 |
| 6 | Hs | 0.04 |
| 7 | Gst | 0.46 |
| 8 | estimate of gene flow (Nm) | 0.59 |
| 9 | No. of Polymorphic loci (P) | 8 |
| 10 | Percentage Polymorphism (% P) | **16.32%** |

**Cluster analysis**

The samples of *M. indica* L. variety 'Kottoorkonam' grouped into two main clusters. Cluster I has two major groups comprising of accessions from southern part of Thiruvananthapuram district including the originating location of this variety, Kottoor. Cluster II has two groups comprising of accessions from northern parts of Thiruvananthapuram district (Fig.2). Here, a suggested explanation for this division is that these accessions probably had common genomic components that originated from other mother trees. From the above results, the relationships among various 'Kottoorkonam' accessions could be well resolved by the ISSR markers, strongly verifying their effectiveness in identification of these cultivar accessions. Possibly, this finding implies a potential relation between the genetic markers and 'Kottoorkonam' accessions. Relatively low level of dissimilarity values suggests that

'Kottoorkonam' accession collection represent a genetically less diverse population. The observed low level of polymorphism may be because of the fact that the farmers are not supplied with the most productive and uniform planting material. Although the use of homogenous, well documented material as scion is highly recommended for mango grafting, some of the nurseries are using genetically variable scion from their own mother blocks, while some others are using scion from the farmers' orchards of unknown genetic identity. On the other hand, the evident intracultivar heterogeneity in 'Kottoorkonam' mango is important from breeding point of view. This intracultivar polymorphism would offer good scope for breeding within the cultivar for intracultivar improvement. Here, very few of the accessions can be considered as distinct genotypes exhibiting variation. To generate precise information on the intracultivar heterogeneity within such cultivar, it is essential to test the accessions under replicated trials to compare them against standard commercial table varieties to confirm the distinctiveness and superiority.

## Conclusion

This study has shown that even though the genome of mango is allotetraploid and relatively large, the ISSR markers are capable of individualizing accessions and these have proven to be a valuable tool for identifying intracultivar heterogeneity in mango. The traditional practices are likely to be responsible for the low level of intravarietal polymorphism in the 'Kottoorkonam' variety of *M. indica.*

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