

Genetic Diversity Study, by Using RAPD Techniques Among the Selected Natural Varieties of *Mangifera Indica* L. Occurring at Nedumangadu Taluk of the Thiruvananthapuram District in Kerala State of India.

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Abstract: *Mango cultivation is one of the most important fruit crops of India occupying about 60% of the total area under fruit crop plantation. The natural varieties or the wild forms found in Andaman's, Khasi hills, Assam, Sikkim, certain parts of U.P. and along the Western Ghats are still lacking proper studies. In Kerala several well known varieties once popular among the previous generations, are either vanishing or being replaced by new high yielding horticultural varieties. During the past decades, the advent of high yielding horticultural varieties of mangoes has already been replaced by several natural varieties from homestead and farmyards of several villages of Kerala state. As a part of urbanization, widening of village roads also has destroyed several mango trees forever and no efforts have been carried out so far, to replace the lost trees. The above facts on these species in Kerala scenario highlight urgent necessity of conservation of local varieties. In this context Nedumangadu taluk of Thiruvananthapuram district has been selected to carry out the survey, collections and genetic variability studies of the natural varieties of mangoes.*

1. INTRODUCTION

Mango is the popular and widely cultivated fruit in India. It has been cultivated in India for the last 4000 years and is intimately connected with Indian culture and folklore [1]. Thousands of natural variants of mango have been observed in India and their origin is probably from the Assam-Burma-Thailand region [2, 3]. Where truly wild mango trees. Mango is by far the most important fruit crop of India occupying about 60% of the total area under fruit. [4]. the introduction of Mango in other parts of the world is comparatively recent. It is now cultivated throughout the tropics as a cash crop. The cultivated varieties are limited in number and they are widely propagated by grafting and

budding while thousands of natural varieties are ignored and unnoticed. In India, the natural varieties or the wild forms occur in Andaman's, Khasi hills, Assam, Sikkim, certain parts of U. P. and along the Western Ghats [5]. A large number of known varieties of mango are popular among Keralites [6]. The low land of Kerala is best suited for the growth of mango trees and it is a common avenue and homestead one.

The Kerala region, coming under the Western Ghats is rich in wild varieties of mangoes [5]. They are consumed from time immemorial as delicacies for different purposes and occasions. They were named according to their taste and quality. [7]. they are alarmingly 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 have become rare and they are facing severe threat of extinction from their habitat. Urbanization of Kerala villages and construction of houses in farm lands have led to drastic depletion of these "unwanted" mangoes. It is also observed that the destruction of sacred groves, extensive use of the wood of the mango tree for construction purposes as well as the Hindu ritual ceremonies with mango wood, led many of the local varieties become either endangered or rare. As a resourceful genetic source, a study of the genetic variants of *Mangifera indica* L. is urgently needed. They are not only rich source of edible fruits but also used for medicinal purposes. Mango, "the king of fruits" has got a unique position due to its nutritional quality, taste and consumers preference. Though mango is a very common fruit to Kerala very limited work has been done for its genetic improvement. The objectives of the present investigations are to find out the genetic variability and pattern of variation among the collected germplasm of mangoes.

2. MATERIALS AND METHODS

2.1 Area of study

Nedumangadu Taluk is located at the foothills of Agastyar hills of the Southern Western Ghats located between 8°31' and 8°51' about an area of 549 sq. kilometer. This is the hilliest Taluk in the district covering 12098 acres. Being under forest. Agastyarkoodam, one of The 15 micro endemic centers in India and the highest peak in Nedumangadu taluk is endowed with rich diversity of several mango varieties. Commercial exploitation of local varieties, especially for pickles and fruit is one of major financial gains of the local people of the Nedumangadu taluk.

2.1.1 Survey of local varieties of Mango

An intrinsic and extensive survey has been conducted throughout the Nedumangadu Taluk on the existing local varieties of mango. A preliminary investigation was conducted in the Taluk on the availability and variability of mango varieties. 15 local varieties of mango were identified with clear cut characteristics from this region. The varieties are 1) Kottoor konam, 2) Vellari, 3) Chambavarikka, 4) Karpooram, 5) Pandi, 6) Thali, 7) Moovadan, 8) Gomavu, 9)

Collected from identified individual plants in appropriate time. (The leaves were transported to the laboratory under moist condition.) The leaf samples were used for the isolation of the high molecular weight plant DNA, for the isolation, standard laboratory equipments and reagents were used. Quantification of isolated genomic DNA was done by using a UF recording spectrophotometer of

Chaviri, 10) Mylapoo, 11) Panchara, 12) Pulichi, 13) Kilichundan, 14) Nattumavu, 15) Njettukuzhian.

2.1.2 Genetic variability studies

The RAPD (Randomly Amplified Polymorphic DNA) technique [8]. has been widely used in plants for the genetic variability studies. RAPDs are DNA fragments amplified by the polymerase chain reaction (PCR) using short (generally 10bp) synthetic primers of random sequence. These Oligonucleotides serve as both forward and reverse primer and usually are able to amplify fragments from 3-10 genomic sites simultaneously. Amplified fragments (within the 0.5- 5 kb range) are separated by gel electrophoresis and polymorphisms are detected as the presence or absence of bands of particular size. These polymorphisms are considered to be primarily due to variation in the primer annealing sites. RAPDs have been used for many purposes, ranging from studies at the individual level (eg. Genetic identity) to studies involving closely related species. Due to their very high genomic abundance, RAPDs have also been applied in gene mapping studies [9].

For the present genetic variability studies, young leaves of all selected varieties of mangoes were SHIMADZU. Make. The genomic DNA isolated was used for PCR analysis. A total of 17 random primers were used for the estimation of inter varietals variation of *Mangifera indica* L. The samples were electrophoresed on 1.2% agarose gel to detect amplification. The gel was documented using the UV exposed gel documentation system.

3. RESULTS AND DISCUSSIONS

List of primers and its sequence used for RAPD analysis

Sl. No.	Primer sequence	No. of bands produced	No. of polymorphic bands
1	C 62 GTG AGG CGT C	2	1
2	C 63 GGG GGT CTT T	9	8
3	C 65 GAT GAC CGC C	16	15
4	C 66 GAACGGACTC	12	11
5	C67 GTC CCG ACG A	7	5
6	C68 TGG ACC GGT G	4	4
7	C69 CTC ACC GTC C	14	13
8	C71 AAA GCT GCGG	11	7
9	C72 TGTCATCCCC	2	1
10	C73 AAG CCT CGT C	5	4
11	C74 TGC GTG CTT G	15	14
12	C75 GACGGATCAG	12	10
13	C76 CACACTCCAG	14	9
14	C77 TTCCCCCAG	15	13
15	C78 TGAGTGGGTG	8	5
16	C79 GTTCCCAGCC	3	1

17	C80	ACTTCGCCAC	2	1
			151	122

A total of 17 random primers were used for the estimation of intervarietal variation in *Mangifera*. Out of 151 products generated, 122 were found to be polymorphic (81% polymorphism). On an average, the primers generated 8.8 products and 7.2 polymorphism per primer. The number of products generated by these arbitrary 10-mer primers was found to range from 2-16 with primer C 65 giving the maximum (15) and primer C 62, C 72, C 79, and C 80 giving the minimum (1) number of amplicons. The number of amplicons so generated can be arbitrarily grouped under two classes of bands from sl. nos. 2 to 10 and from sl. nos. 11 to 17 + sl. no. 1 respectively. Lower number of bands was generated by primers – C62, C63, C67, C68, C72, C73, C78, C79 and C80 while higher number of products was observed by primers C65, C66, C69, C71, C74, C75, C76 and C77. Although primer like C68 produced 100% polymorphism, not a single primer could reveal 100% monomorphism across the accessions.

The coefficient of similarity value in terms of genetic relatedness obtained from RAPD data analysis was found to range from 0.58 to 0.88 with mean value of GS= 0.77, thus suggesting high levels of intervarietal variation. The phenogram obtained using cluster analysis following the standard statistical procedure of UPGMA (WINBOOT) grouped 7 out of 15 varieties under a single cluster and the rest are non clustered or loose. The Cluster has two groups wherein varieties 1, 2, 3, & 4 are under group I while varieties 8, 9 and 10 are under the group II. Though varieties 12 and 14 shares the same group with high gene similarity value of 0.83 and varieties 5 and 7 with similar GS value groups fall in the 2nd group, as they are not grouped under the same cluster but as separate Non cluster groups, thus suggesting that they are

As diverse as other varieties like 6, 11, 13 and 15 which constitute the Loose groups.

3.1 Cluster analysis

The RAPD experiments were carried out among 15 varieties of *Mangifera indica* L. Varieties *Moovandan*, *Kilichundan*, *Pandi*, *Njettukuzhyan*, *Chamba varikka*, *Chaviri* and *Gomavu* can be clustered together under two groups which include *Moovandan*, *Kilichundan*, *Pandi*, *Njettukuzhian* and *Chamba varikka*, *Chaviri*, *Gomavu* respectively. The remaining varieties cannot be

grouped into clusters with common characteristic features. However, similarities have been noted in four varieties of two groups of *Panchara*, *Thali* and *Karpooram*, *Vellari* respectively. The remaining varieties *Nattumavu*, *Pulichchi*, *Mylapoo*, *Kottoor konam* are having no similarities and hence they are unique

1. Cluster Groups: Moovandan, Kilichundan, Pandi, Njettukuzhian, Chamba varikka, Chaviri and Gomavu.

Group 1 – Moovandan, Kilichundan, Pandi and Njettukuzhian

Group 11 – Chamba varikka, Chaviri, Gomavu

11. Non Cluster Groups: Panchara, Thali, Karpooram, Vellari

Group 1 – Panchara, Thali

Group 11- Karpooram, Vellari

111. Loose Groups: Nattumavu, Pulichchi, Mylapoo, Kottoorkonam

4. CONCLUSIONS

In conclusion, the study is a pioneer work to assess the rate of depletion of indigenous varieties of mangoes from Nedumangadu taluk which is a major supplier of native mangoes to the nearby towns and cities. The study also revealed local information on various uses of particular varieties of mangoes. The genetic variability studies conducted by using 17 random primers are also new to the field and area of study. The study has revealed the high diversity of indigenous mango varieties of the taluk. The study hopes to extend similar work on more varieties and places, which will be highly useful for gathering indigenous knowledge and diversity among natural varieties of mangoes.

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