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Diverse genetic bases of Indian polyembryonic and monoembryonic mango (*Mangifera indica* L) cultivars

Mango is one of the most important fruit crops in India. It is known to have been cultivated in India since the last 4000 years. Almost all cultivars belong to the species *Mangifiera indica*, family Anacardiaceae. *M. indica* is native to India and occurs abundantly in forests and cultivated areas. Hence, it is difficult to differentiate true wild forms from cultivated ones. The commercially grown cultivars have arisen through seedling selections made for different fruit characters like colour, taste, flavour, size, etc. Later, these cultivars have been vegetatively propagated and cultivated in a wide area[1]. Mango cultivars are classified into two groups: monembryonic type or Indian type and polyembryonic type or Indo-Chinese type[2]. In India, majority of the cultivated types are monoembryonic. Surprisingly, polyembryonic types were grown only in southern India, especially in coastal parts of Kerala, Karnataka and Goa.

Emergence of multiple seedlings from a single seed is referred to as polyembryony. This was observed in 59 families, 158 genera and 239 species[3]. In mango, Sachar and Chopra[4] observed nucellar embryos in nineteen mango varieties. Using crosses of polyembryonic and monoembryonic cultivars and their segregating hybrids, Arnon *et al.*[5] demonstrated that polyembryony in mango is controlled by a single dominant gene. Polyembryonic and monoembryonic types are intercrossable, and cultivation of polyembryonic varieties is confined to the west coast adjacent to the Western Ghats, which is one of the hot spots of biodiversity. Keeping this in view, the present study was carried out to examine whether these two classes have a common or different genetic base using Random Amplified Polymorphic DNA (RAPD) and chloroplast DNA Restriction Fragment Length Polymorphism (RFLP) analysis.

In this study, ten polyembryonic and monoembryonic cultivars each traditionally grown in the west coast of southern India were used to determine the genetic relatedness among them using RAPD markers (Table 1). DNA isolation and RAPD analysis were carried out as described by Ravishankar *et al.*[6]. We have employed 19 random primers which amplified 153 polymorphic and 33 monomorphic markers.

Eight mango cultivars from each of these groups were used for chloroplast DNA RFLP analysis. The primers ORF 106-rbcL and GIF-GI460 were used to amplify chloroplast-specific DNA fragments[7,8]. They amplified around 3.1 kb and 1.5 kb fragments respectively. ORF 106-rbcL products were restriction-digested with six enzymes, *Eco*RI, *Bam*HI, *Xba*I, *Xho*I, *Taq*I *and Pst*I. Digested PCR fragments were separated on 2% agarose. Except *Pst*I, no other enzyme digested PCR-amplified fragments. *Pst*I digestion produced similar bands. Therefore, data from restriction digestion of ORF-rbcL fragment were not used for statistical analysis. For GIF-G1460 products, 15 enzymes were used for restriction digestion. *Bam*HI, *Hae*III, *Hind*III, *Hinf*I, *Msp*I, *Pst*I, *Taq*I and *Xba*I were able to digest amplified DNA. Restriction enzymes *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Kpn*I, *Pvu*II, and *Xho*I did not digest GIF-G1460 amplified fragments. Bands on agarose gel were scored as in the case of RAPD. Data from RAPD markers and chloroplast DNA RFLP markers were used for cluster analysis and principal component analysis (PCA), separately[9]. For cluster analysis, squared Euclidian distances were calculated for pair-wise differences among cultivars and based on minimum variance algorithm, the dendrogram was constructed[10].

Dendrogram analysis of RAPD and chloroplast DNA RFLP data clearly grouped the cultivars into two based on

Table 1. Polyembryonic and monoembryonic cultivars used in this study

Serial no.	Polyembryonic cultivar	Serial no.	Monoembryonic cultivars
P1	Peach	M1	Rumani
P2	Kurukkan	M2	Raspuri
P3	Mylupilian	M3	Totapuri
P4	Vellaikulumban	M4	GoaMankurd
P5	Muvandam	M5	Alphonso
P6	Bappakai	M6	Xavier
P7	Nekkare	M7	Kadari
P8	Olour	M8	Padari
P9	Chandrakaran	M9	Suvarnarekha
P10	Starch	M10	Banganapalli

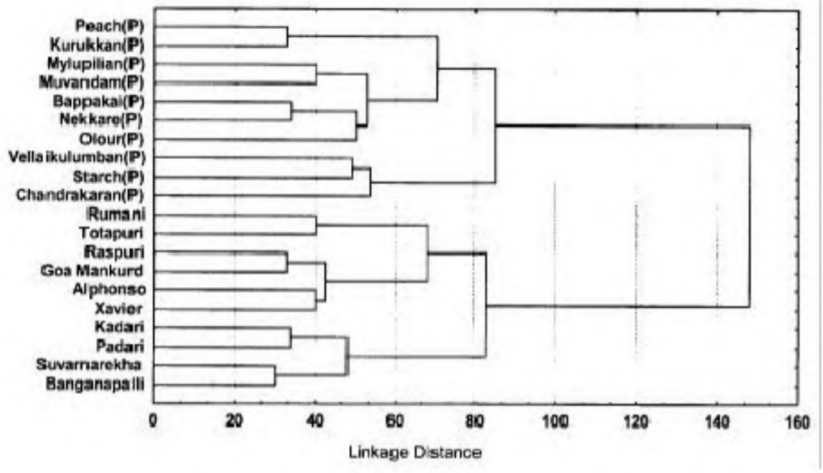

Figure 1. Cluster analysis of RAPD makers generated for polyembryonic and monoembryonic mango cultivars. Suffix (P) denotes polyembryonic cultivars, others are monoembryonic cultivars.

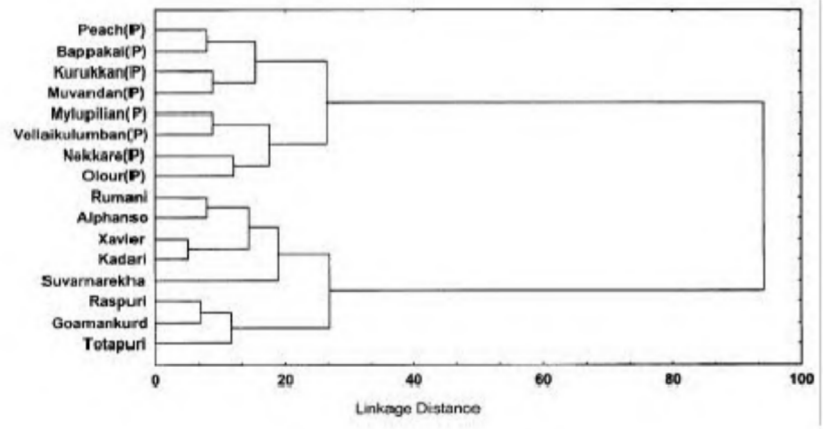

Figure 2. Cluster analysis of chloroplast DNA RFLP markers generated for Indian monoembryonic and polyembryonic mango cultivars. Suffix (P) denotes polyembryonic cultivars.

embryo types, i.e. monoembryonic and polyembryonic (Figures 1 and 2). PCA also confirms these results, where monoembryonic and polyembryonic cultivars were clustered separately (data not shown; Eigenvalue 58.5%). In another study, Lopez-Valenzuela *et al.*[11] were able to differentiate mango cultivars by embryo type and geographical origin using RAPD markers. However, in their study, monoembryonic and polyembryonic types used were from different geographical regions (Mexico, Philippines and Florida). In the present study, we have used seedling selections from the same geographical region, where they have been traditionally grown over the years.

Both the embryonic types are intercrossable and polyembryony is governed by a single dominant gene. Therefore, we have employed RAPD analysis using total genomic DNA and chloroplast DNA RFLP analysis to study the genetic relatedness and lineage among polyembryonic and monoembryonic cultivars. In both the analyses, the grouping of cultivars based on their embryo types indicates that monoembryonic and polyembryonic types of Indian mango cultivars have a different genetic base. These results suggest that the polyembryonic types might have been introduced from other parts of southeast Asia and are unlikely to have originated from India.

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