Development of Barcodes for Identification of Zygotic and Nucellar Seedlings in Polyembryonic Varieties of Mango (*Mangifera indica* L.)

Nesara Begane*, M.R. Dinesh, Amrita Thokchom and K.V. Ravishankar

1Central Agricultural University, College of Horticulture & Forestry, Pasighat, Arunachal Pradesh, India
2Division of Fruit Crops, 3Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru-89, India

*Corresponding author

**ABSTRACT**

Study on the seedling progenies of three polyembryonic varieties was carried out to differentiate zygotic and nucellar seedlings through molecular characterization. The fingerprinting showed variation across the varieties of selected seedling progenies. The variety Peach exhibited 100% zygotic seedlings among the varieties screened. The variety Nekkare was found to be 36.84% zygotic and minimum number of zygotic seedlings (10.52%) was observed in Bappakkai. In breeding program as it is difficult to identify hybrid progenies of zygotic origin and identification of zygotic seedlings from nucellar is vital for a hybridization programme, wherein polyembryonic varieties are used as one of the parents. Hence, molecular markers are vital in identifying the seedlings in order to characterize the seedling progenies and parents by developing the barcodes of polyembryonic mango varieties to utilize in crop improvement.

**Keywords**
Mango, Polyembryony, SSR, Barcode, Zygotic, Nucellar

**Article Info**

- **Accepted:** 04 February 2019
- **Available Online:** 10 March 2019

**Introduction**

The mango (*Mangifera indica* L.) regarded as one of the choicest fruits of the world, belongs to the family Anacardiaceae. It is considered to be the ‘king of fruits’, owing to its captivating flavour, delicious taste, irresistible sweetness and attractive aroma. It is believed to be originated in the Indo-Burma region (De Candolle, 1904 and Mukherjee, 1951). Its origin is traced back to 4000 years (De Candolle, 1884) and in India they are being associated with agriculture and civilization from time immemorial.

Traditional mango cultivars from a particular geographical region are genetically very similar (Ravishankar et al., 2000). Depending on the mode of reproduction of seeds mango can be classified into two groups *viz.*, monoembryonic and polyembryonic. Despite the intercrossability of mono and polyembryonic types and their wild occurrence, diverse genetic base is observed.
for these types (Ravishankar et al., 2004). The nucellar embryos can be used for raising ‘true-to-type’ seedlings and the uniformity of seedlings is beneficial. Polymenopony is one of the impediments since the outcome of hybridization is the development of zygotic recombinants. The identification of resultant hybrid progenies of zygotic origin from that of nucellar embryo is difficult from a cross when one of the parents or both the parents used is a polymenoponic variety. The number of seedlings that a polymenoponic variety generates varies from variety to variety and from region to region (Juliano, 1937).

Polyenproonic genotypes like 13-1 in mango possess most of superior traits such as dwarf stature and tolerance to salt (Schmutz and Ludders, 1993); Gomera-1 tolerant to salt stress (Martinez et al., 1999); Nekkare and Olour tolerant to salt (Pandey et al., 2014). In these cases polymenopony is advantageous in clonal propagation, fixing of heterosis and restoration of vigour. However, they proved to be impediment in the breeding program as it is difficult to identify hybrid progenies of zygotic origin. Identification of zygotic seedlings from nucellar is vital for a hybridization programme, wherein polymenoponic varieties are used as one of the parents. Markers are vital in identifying the seedlings. In order to characterize the seedling progenies and parents an effort was made to develop the barcodes.

**Materials and Methods**

Fully matured and ripened fruits of the polymenoponic varieties namely, Nekkare, Bappakkai and Peach were collected from the mango field genebank of Indian Institute of Horticultural Research (IIHR) and stones were extracted from fully ripened fruits. Collected stones from fully ripen fruits were sown in polybags. Timely plant protection measures were taken for these half sib seedlings to maintain them in healthy condition. Recently matured leaf samples of both parents and offspring’s were used for extracting DNA.

The genomic DNA was extracted from leaf samples by using CTAB (cetyl trimethylammonium bromide) method (Ravishankar et al., 2000). PCR reaction was performed in a 10µl reaction volume containing 10X complete buffer, 25 mM MgCl₂, 1mM dNTP’s, 0.3 µM primers, 0.5 U of Taq DNA polymerase (Homemade Taq) and 20ng template DNA in Biometra thermal cycler. Optimised reaction conditions for analysis were followed so as to get repeatable results. The amplified PCR products were then separated in 1.5% Agarose gel and viewed under UV light gel documentation system (UVi PRO, UK). The SSR profiling was carried out according to Ravishankar et al., (2015). Samples were separated on an automatic 96-capillary automated DNA sequencer (ABI 3730 DNA Analyzer, Applied Biosystems, USA) at ICRISAT facility, Hyderabad, India. Generated raw data was analyzed and compiled using Peak Scanner v1.0 software (Applied Biosystems) to determine allele sizes. The results obtained were used for developing barcodes. Total of eight SSR markers developed by Ravishankar et al., (2011) were used for developing barcodes. The details of the SSR markers used in this study are given in Table 1. Barcoding uses short genetic sequence from standard part of genome. It was done for both parents and half sibs using ‘Barcode of life database’ (BOLD, maintained by University of Guelph).

**Results and Discussion**

Eight SSR markers were used to develop the barcode. The details of the barcode generated for half sibs and their parents are presented in Figure 1. In the variety Peach none of the seedling progenies were observed to be similar to that of the maternal parent, whereas in the
variety Bappakkai 52.63 % progenies were similar to that of maternal parent and in the variety Nekkare 10.52 % progenies were similar to that of maternal parent.

DNA fingerprinting techniques using SSR widely used for cultivar identification in a wide range of species due to their high heritability and sufficient polymorphism to discriminate genotypes (Jeffreys et al., 1985; Karp et al., 1998). SSR markers are widely used for their multiallelic and codominant inheritance nature and the fact that they are highly suitable for high throughout PCR based platforms (Powel et al., 1996; Zietkiewicz et al., 1994). It was assumed that SSRs were primarily associated with noncoding DNA, but it has now become clear that they are also abundant in the single and low copy fraction of the genome (Yi et al., 2006; Bindler et al., 2007). In a highly heterozygous crop viz., mango where nomenclature ambiguity is one of the main hindrances in crop improvement (Vasugi et al., 2013), DNA fingerprinting can be a very handy tool for individual identification of cultivars or rootstock for different horticultural purpose, such as breeder’s right, identification of pollen parents and determination of genetic relatedness (Lavi et al., 1993). The potential of SSR markers in fingerprinting is well established in mango (Viruel et al., 2005; Shareefa, 2008).

Validation of parentage by comparing the characteristics of the parents and hybrid progenies would help in the future breeding programmes. One of the very important conclusions that emerge out from this study also is which are all the varieties that can contribute to the progenies for certain desirable traits can be better explored for crop improvement programme.

In this study eight SSR markers were used to develop barcode for polyembryonic varieties and their half sibs. Half sibs of Peach exhibited 100% disimilarity from their maternal parent. Whereas in Bappakkai (10.52 %) progeny differed from their maternal pattern and 21.05 % of plantlets were considered doubtful as they differed with only one primer. In the variety Nekkare (36.84 %) differed from their maternal parent and 52.63 % were doubtful as they differed with one primer. This variation in different varieties might be due to heterozygosity existing in the variety and variation in per cent of nucellar seedlings. SSR allele size values generated in different laboratories are known to differ by 1 to 4 base pairs due to different analytical and rounding methods (This et al., 2004). As such laboratory specific deviations tend to be systematic, they will cause a minor shift in the position of the size bars, but leave the overall barcode unchanged (Kanupriya et al., 2011).

**Table 1.** Details of 8 SSR markers used in development of barcode

| Locus | Repeat motif | H<sub>O</sub> | H<sub>e</sub> | PIC  | F(Null) |
|-------|--------------|--------------|--------------|------|---------|
| MiIIHR17 | (GT)13GAGTG(GA)10 | 0.050 | 0.510 | 0.470 | +0.8258 |
| MiIIHR18 | (GT)12 | 0.000 | 0.782 | 0.744 | +1.0000 |
| MiIIHR 23 | (GA)17 GG(GA)6 | 0.017 | 0.728 | 0.693 | +0.9541 |
| MiIIHR 26 | (GA)14 GGA(GAA)2 | 0.000 | 0.757 | 0.718 | +1.0000 |
| MiIIHR 30 | (CT)13 | 0.044 | 0.762 | 0.713 | +0.8910 |
| MiIIHR 31 | (GAC)6 | 0.024 | 0.885 | 0.862 | +0.9469 |
| MiIIHR 34 | (GGT)9 (GAT)5 | 0.389 | 0.876 | 0.855 | +0.3847 |
| MiIIHR 36 | (TC)17 | 0.000 | 0.845 | 0.818 | +1.0000 |

(Source: Ravishankar et al., 2011)

H<sub>O</sub> – Observed heterozygosity  
H<sub>e</sub> – Expected heterozygosity  
PIC – Polymorphic Information Content  
F(Null) – Frequency of null allele
**Fig.1** Barcode developed for polyembryonic varieties and their half sibs [numericals (1,2,3) indicates individual stones and alphabets (a,b,c) indicates number of seedlings emerged from a single stone]

| Peach (Maternal parent) | Bappakkai (Maternal parent) | Nekkare (Maternal parent) |
|--------------------------|-------------------------------|---------------------------|
| P1a                      | B1a                           | N1a                       |
| P1b                      | B1b                           | N1b                       |
| P2a                      | B1c                           | N2a                       |
| P2b                      | B2a                           | N2b                       |
| P3                       | B2b                           | N2c                       |
| P4                       | B2c                           | N3a                       |
| P5                       | B3a                           | N3b                       |
| P6a                      | B3b                           | N3c                       |
| P6b                      | B3c                           | N4a                       |
| P7                       | B4a                           | N4b                       |
|                          | B4b                           | N5a                       |
|                          | B4c                           | N5b                       |
|                          | B7a                           | N5c                       |
|                          | B7b                           | N6a                       |
|                          | B7c                           | N6b                       |
|                          | B8a                           | N7a                       |
|                          | B8b                           | N7b                       |
|                          | B9a                           | N7c                       |
|                          | B9b                           | N7d                       |

Polyembryony on mango is considered a genetic feature, although it is not yet known if it is a product of a recessive or dominant single gene (Sturrock, 1968; Aron et al., 1998). On the other hand, nucellar plantlets are those which develop very well and become the most vigorous in diameter and height. In this study opposite results were obtained.

On comparison of their allelic data with female parent showed that zygotic seedlings might be the vigorous one.

These results were in comparison with the findings of Cordeiro et al., (2006).
Acknowledgement

The authors wish to thank the Division of Fruit crops and Division of Biotechnology, Indian Institute of Horticultural Research, Bengaluru, for providing facilities to conduct this research. We also wish to express our gratitude for the staff of College of Horticulture, UHS, Bengaluru, for their constant support.

References

Aron, Y., Czosnek, H., Gazit, S., and Degani, C. 1998. Polyembryony in mango (Mangifera indica L.) is controlled by a single dominant gene. Hort. Sci., 33: 1241-1242.

Bindler, G., Van Der Hoeven, R., Gunduz, I., and Plieske, J. 2007. A microsatellite marker based linkage map of tobacco. Theor. Appl. Genet. 114: 341-349.

Cordeiro, M.C.R., Pinto, A.C.Q., Ramos, V.H.V., Faleiro, F.G., and Fraga, L.M.S. 2006. Identification of plantlet genetic origin in polyembryonic mango (Mangifera indica, L.) cv. Rosinha seeds using RAPD markers. Rev. Bras. Frutic., 28: 454-457.

De Candolle, A., 1884. Origin of cultivated plants. Kegan Paul, Trench, London.

De Candolle, A., 1904. Origin of cultivated plants. Kegan Paul, Trench, London.

Jeffreys, A.J., Wilson, V., and Thien, S.L. 1985. Hypervariable minisatellite regions in human DNA. Nature, 314: 67–73. Juliano, J.B., 1937. Embryos of carabao Mango, Mangifera indica L. Philippines J. Agric., 25: 749-760.

Kanupriya., Madhavi Latha, P., Aswath, C., Laxman, R., Padmakar, B., Vasugi, C., and Dinesh, M.R. 2011. Cultivar identification and genetic fingerprinting of guava (Psidium guajava) using microsatellite markers. Int. J. Fruit Sci., 11: 184-196.

Karp, A., Issar, P.G., and Ingram, D.S. 1998. Molecular tools for screening biodiversity. Chapman & Hall, London.

Lavi, U., Cregan, P.B., and Hillel, J. 1993. Application of DNA markers for identification and breeding of fruit trees. Plant Breeding Rev., 16 (In press).

Martinez, R.A., Duran, Z.V.H., and Aguilar, R.J. 1999. Use of brackish irrigation water for subtropical farming production. 17th Congress on Irrigation and Drainage. Special Session ICID-CIID, 1: 61-71.

Mukherjee, S.K. 1951. The origin of mango. Indian J. Genet. Plant Breed., 11: 49–56.

Pandey, P., Dubey, A.K., and Awasthi, O.P. 2014. Effect of salinity stress on growth and nutrient uptake in polyembryonic mango rootstocks. Indian J. Hort., 71: 28–34.

Powel, W., Machray, G., and Provan, J. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci., 1: 215–222.

Ravishankar, K.V., Anand, L. and Dinesh, M.R., 2000. Assessment of genetic relatedness among a few Indian mango cultivars using RAPD markers. J. Hort. Sci. Biotechnol., 75: 198 – 201.

Ravishankar, K.V., Chandrashekar, P., Sreedhara, S.A., Dinesh, M.R., Anand, L., and Saiprasad, G.V.S. 2004. Diverse genetic bases of Indian polyembryonic and monoembryonic mango (Mangifera indica L) cultivars. Curr. Sci., 87: 870 – 871.

Ravishankar, K.V., Mani, B.H., Anand, L., and Dinesh, M.R. 2011. Development of new microsatellite markers from Mango (Mangifera indica) and cross-species amplification. American J. Bot., 98: 96-99.

Ravishankar, K.V., Bommisetty, P., Bajpai, A., Srivastava, N., Mani, B.H., Vasugi, C., Rajan, S., and Dinesh, M. R. 2015.
Genetic diversity and population structure analysis of mango (*Mangifera indica*) cultivars assessed by microsatellite markers. *Trees*, 29: 775–783.

Sachar, R.C., and Chopra, R.N. 1957. A study of endosperm and embryo in *Mangifera*. *Indian J. Agric. Sci.*, 27: 219–228.

Schmutz, U., and Ludders, P. 1993. Physiology of saline stress in one mango (*Mangifera indica* L.) rootstock. *Acta Hort.*, 341: 160-167.

Shareefa, M. 2008. DNA fingerprinting of mango (*Mangifera indica* L.) genotypes using molecular markers. Ph.D. thesis submitted to P.G. School, IARI, New Delhi.

Srivastava, K.C., Rajput, M.S., Singh, N.P., and Lal, B. 1988. Rootstock studies in mango cv Dashehari. *Acta Hort.*, 231: 216-219.

Sturrock, T.T. 1968. Genetics of mango polyembryony. Proceedings of the Florida State Horticultural Societies, 81: 311-314.

This, P., Jung, A., Boccacci, P., Bottego, J., Botta, R., Constantini, L., Crespan, M., Dangl, G.S., Eisenheld, C., Ferreira-Monteiro, F., Grando, S., Ibanez, J., Lacombe, T., Laucou, V., Magalhaes, R., Meredith, C.P., Milani, N., Peterlunger, E., Regner, F., Zulini, L., and Maul, E. 2004. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor. Appl. Genet.*, 109: 1448–1458.

Vasugi, C., Dinesh, M.R., Ravishankar, K.V., and Padmakar, B. 2013. Morphological and molecular characterization—Nomenclature ambiguity in Indian mangoes. *Acta Hort.*, 992: 331-339.

Viruel, M., Escribano, P., Barbieri, M., Ferri, M., and Hormaza, J. 2005. Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Mol. Breed.*, 15:383–393.

Yi, G.B., Lee, J.M., Lee, S., and Choi, D. 2006. Exploitation of pepper EST-SSRs and an SSR-based linkage map. *Theor. Appl. Genet.*, 114: 113-130.

Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, 20: 176–183.

How to cite this article:

Nesara Begane, M.R. Dinesh, Amrita Thokchom and Ravishankar, K.V. 2019. Development of Barcodes for Identification of Zygotic and Nucellar Seedlings in Polyembryonic Varieties of Mango (*Mangifera indica* L.). *Int.J.Curr.Microbiol.App.Sci.* 8(03): 14-19.

doi: [https://doi.org/10.20546/ijemas.2019.803.003](https://doi.org/10.20546/ijemas.2019.803.003)