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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 17 (1): 56-61, 2014
ISSN 1028-8880 / DOI: 10.3923/pjbs.2014.56.61
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Assessment of Genetic Diversity and Relationships among Egyptian Mango (*Mangifera indica* L.) Cultivers Grown in Suez Canal and Sinai Region Using RAPD Markers

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Abstract: DNA-based RAPD (Random Amplification of Polymorphic DNA) markers have been used extensively to study genetic diversity and relationships in a number of fruit crops. In this study, 10 (7 commercial mango cultivars and 3 accessions) mango genotypes traditionally grown in Suez Canal and Sinai region of Egypt, were selected to assess genetic diversity and relatedness. Total genomic DNA was extracted and subjected to RAPD analysis using 30 arbitrary 10-mer primers. Of these, eleven primers were selected which gave 92 clear and bright fragments. A total of 72 polymorphic RAPD bands were detected out of 92 bands, generating 78% polymorphisms. The mean PIC values scores for all loci were of 0.85. This reflects a high level of discriminatory power of a marker and most of these primers produced unique band pattern for each cultivar. A dendrogram based on Nei's Genetic distance co-efficient implied a moderate degree of genetic diversity among the cultivars used for experimentation, with some differences. The hybrid which had derived from cultivar as female parent was placed together. In the cluster, the cultivars and accessions formed separate groups according to bearing habit and type of embryo and the members in each group were very closely linked. Cluster analysis clearly showed two main groups, the first consisting of indigenous to the Delta of Egypt cultivars and the second consisting of indigenous to the Suez Canal and Sinai region. From the analysis of results, it appears the majority of mango cultivars originated from a local mango genepool and were domesticated later. The results indicated the potential of RAPD markers for the identification and management of mango germplasm for breeding purposes.

Key words: RAPDs, molecular markers, diversity, *Mangifera indica*, Genetic relationships

INTRODUCTION

Mango (*Mangifera indica* L.), is an important member of the family Anacardiaceae. A tropical canopy tree, originated in India and Burma, but is now cultivated pan-tropically, Total world production of mango was 24,420,116 metric tons in 1999 (FAO, 2000), mainly concentrated in India which produced half that total. Mangoes are grown in 85 countries and 63 countries produce more than 1,000 metric tons per year. In the sixth century, Phoenicians and Arabs spread the crop from India to East-Africa where it has been cultivated ever since. In Egypt, mango is grown throughout the Nile Valley, in the Nile Delta and in the areas around El Faiyum. In 2009, an estimated 534,434 metric tons of mango were harvested (Egyptian Ministry of Agriculture). Egyptian mangos are grown on irrigated lands, most of which are sandy and not overly fertile. The soils are alkaline, with pH running from 7.0 to 8.0 as a rule. Many plantings are on "new" or reclaimed lands in the Nile delta north of Cairo and eastward toward Ismailia and the Sinai. Some of the early plantings were cultivated in the 1920s, but most were made more recently. Most plantings are over 10 years old. Some of the newest plantings are under drip irrigation, but the vast majority are irrigated by flooding at intervals of 10 to 20 days (Elkhoreiby, 1997).

Mango cultivars are commonly divided into two groups based on their mode of reproduction from seeds and their origin; monoembryonic (indian types) and polyembryonic (Indo Chinese types). Monoembryonic mango seeds contain a single zygotic embryo. While polyembryonic seeds contains one zygotic embryo and several vegetative or nucleolar embryos and the plants developing from them are clone of the mother plant (Crane *et al*., 1997; Iyer and Degani, 1997).

Mango is considered to be difficult plant species to handle in breeding programmes because of certain inherent characteristics including: a long juvenile phase of trees that can range from a minimum of 3-5 years to a maximum of 7-10 years; In addition, there has been widespread hybridization of character in mango. Over the thousands years of its cultivation and domestication, the wide genetic diversity of the plant has been fixed in many varieties. There is a considerable confusion in

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nomenclature of mango cultivars because many clonally propagated mango cultivars have unique local and regional names. polyembryony in some cultivars and the large acreage required for a meaningful assessment of hybrids. On the positive side, the wide range of available genetic variation and the ease with which a selected hybrid can be vegetatively propagated makes mango breeding an attractive method of crop improvement (Knight and Sanford, 1998).

In the Suez Canal and Sinai region, breeding attempts are always in progress for creating better cultivars that can be overcome the previously mentioned obstacles, using DNA markers to characterize the genetic relationships within such germplasm diversity of mango currently available for cultivation in Egypt might allow the implementation of a more efficient breeding program.

Polymerase Chain Reaction (PCR) technology had led to the development of several novel genetic assays based on selective DNA amplification. The protocol is also relatively quick and easy to perform. In this context, RAPD (Williams *et al*., 1990; Welsh and McClelland, 1990) has been employed extensively estimation of genetic variability within plant taxonomic groups and apply it genetic maps (Williams *et al*., 1990; Dunemann *et al*., 1994; Paillard *et al*., 1996). Moreover, the RAPD technique is fundamental in developing specific sequence-characterized amplified region (SCAR) markers for use in the assisted selection of crops (Marieschi *et al*., 2010; Wu *et al*., 2010). There are numerous examples of the application of molecular markers in the analysis of the genetic diversity of mango (Viruel *et al*., 2005; Pandit *et al*., 2007; Santos *et al*., 2008; Singh and Bhat, 2009; Galvez-Lopeza *et al*., 2009; Singh *et al*., 2009).

The aim of the present study is to use RAPD markers to analyze the genetic variability and relationships among mango cultivars and accessions thus contributing to the maintenance and multiplication of this important collection and the improvement of mango culture in the Suez Canal and Sinai region.

MATERIALS AND METHODS

Plant material: The plant material for the present study consists of a ten cultivars: Sedeek, Succary momtaz, Barbary, Alfonse, Ewais, Zebda, Succary Abiad and three unknown accessions were marked by Sinai-1, Sinai-2, Sinai-3. All of cultivars and accessions obtained from orchard of Dr. Mansour Greish, new Meet Abu El-Kome, East bank of El-Morra lakes, Suez Canal Region, Sinai. Young leaves from each of the 10 mango genotypes were collected from the orchard and washed thoroughly using double distilled water. The leaves were wrapped in moist tissue paper and were refrigerated at -20°C when not subjected to immediate use.

Total genomic DNA extraction: Fresh leaf tissue (0.5 g) was ground in liquid Nitrogen to form a thin powder which was transferred into 5 mL of freshly prepared, preheated (60°C) Cetylhexadecyl-trimethyl ammonium Bromide (CTAB) buffer in a tube followed by addition of 0.2% (v/v) β-mercaptoethanol and 2% (v/v) Polyvinylpyrrolidone (PVP). The tube was incubated in a water bath held at 60°C for 45 min with intermittent swirling. 2/3 volume of chloroform: isoamyl alcohol (24:1 v/v) was added to the tube which was tilted several times and centrifuged for 10 min at 2208 g. This extraction step was repeated twice. 2/3 volume of ice-cold isopropanol was added to the supernatant and the tube was incubated at -20°C overnight. The tube was centrifuged for 30 min at 5652 g (40°C). The supernatant was discarded and the DNA pellet was washed with 70% ethanol followed by centrifugation for 2 min at 10000 rpm to resettle the pellet. The alcohol was poured off and the DNA pellet was allowed to dry and thereafter it was dissolved in 1 mL of TE buffer. The DNA was purified by incubation with RNase followed by a phenol treatment.

DNA amplification: RAPD reactions were carried out in a DNA Thermocycler (Perkin-Elmer 2400). Negative controls comprised all PCR components excluding the template. Each 25 μL reaction volume comprised 100 ng of template DNA, 1X PCR Buffer (10 mM Tris HCl pH 8.8; 50 mM KCl), 4 mM $MgCl_2$, 0.2 mM dNTP, 0.5 μM of single primer, 1 U of *Taq* DNA polymerase. The thermal profile set comprised a denaturation step of 5 min at 94°C, followed by 38 cycles of 30 sec at 94°C, 30 sec at 35°C, an extension at 72°C for 2 min and a final extension at 72°C for 10 min. 30 Operon primers were used to screen for polymorphism. PCR products were electrophoresed on 1.5% (w/v) Agarose gels using 0.5x TBE Buffer at 115 V and stained with ethidium bromide (0.5 μg mL^{-1}) for visualization and photographing under UV light.

RAPD profile analysis: The screened primers that gave bands were used to amplify the DNA of all the 10 mango varieties. Each genotype was characterized by its banding pattern (Fig. 1, 2), using the DNA hyperladder 2 (Bioline) as base pair ladder. The RAPD markers as viewed from the gels after electrophoresis and staining were converted into a matrix of binary data, where the presence of the band corresponded to value 1 and the absence to value 0. primer. The scores obtained using all the primers in the RAPD analysis were then pooled to

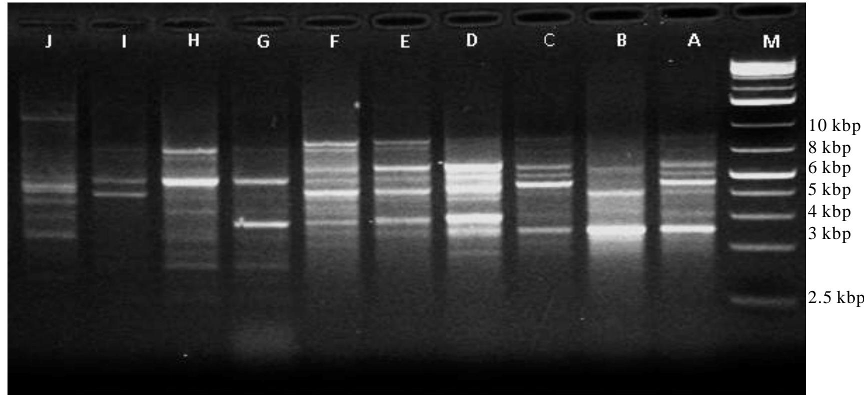

Fig. 1: RAPD profiles of 10 mango germplasm using Primer Z-10. M: Molecular, weight marker (10K bp DNA ladder in right side), A: Sedeeq, B: Succary momtaz, C: Barbary, D: Alfouns, E: Sinai-1, F: Sinai-2, g: Sinai-3, H: Ewiss, I: Zebda, J: Succary Abied

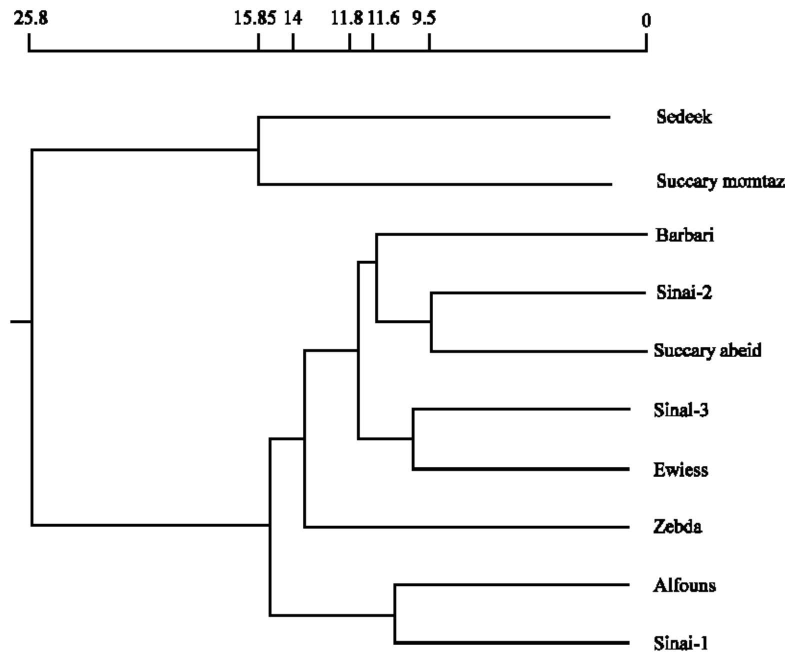

Fig. 2: Association among *Mangifera indica* genotypes revealed by UPGMA cluster analysis of Nei's Genetic distance coefficients calculated from RAPD data of 92 amplification products generated by eleven primers

create a single data matrix and used to estimate polymorphic loci, gene diversity, genetic distance (D) among populations and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram based on Nei (1972) using a computer program, POPGENE (Version 1.31) (Francis *et al*., 1999).

Genetic diversity was calculated at each locus for allelic Polymorphism Information Content (PIC), based on allelic frequencies among all 10 genotypes analyzed. The PIC values were estimated by determining the frequency of alleles per locus using the following formula:

$$PIC = 1-\sum x_i^2$$

where, xi is the relative frequency of the ith allele of the RAPD loci. Markers were classified as informative when PIC was = 0.5.

RESULTS AND DISCUSSION

In this study, the analysis of the prescreening data using 10 selected mango cultivars and accessions and 30 Operon random ten-base long, single stranded primers were screened. At least one product was amplified in all the primers screened. The selected eleven primers generated right and reproducible amplified products which detected polymorphism among the cultivars and accession used. These primers amplified 92 RAPD marker bands. Of these 72 bands were polymorphic and generating 78 % polymorphism. The number of RAPD bands detected by each primer depends on primer, sequence and the extent of variation in specific genotype. Therefore, the number of bands varied in different cultivars. The number of bands varied from 6 (for OPG_10, OPB_20 and OPA_15) to 14 (for OPB_12) with an average of 8.36 bands per primer, of sizes ranging from 200bp to 5000 bp (Table 1). All 92 bands were either monomorphic or polymorphic were considered for the precise calculation of genetic diversity.

The basic protocol described by Williams *et al.* (1990) for PCR was optimized for high quality amplification and intense repeatable banding patterns. However, a reduction in the amplification of fainter bands was noticed with large changes in template DNA concentrations, while too much DNA produced a smear effect which emphasized the importance of quantification of the DNA for clear amplification. The PVP was used in extraction solution because it has been shown to improve PCR in DNA samples extracted from tissues rich in polyphenolics and polysaccharides (Khanuja *et al*., 1999; Horne *et al*., 2004). Examples of amplification patterns obtained by RAPD in different cultivars and accession were shown in Fig. 1. Kumar *et al.* (2001) used only 10 primers which produced reproducible bands and therefore they could distinguish between the mango cultivars used with similarity coefficient of 27%. In our study, out of 30 screened RAPD primers, 11 produced polymorphisms and all cultivars and accessions are distinguishable by unique RAPD profiles generated by these oligonucleotide 10-mer primers. These results not agree with reports by Ravishankar *et al*. (2000) and agree with reports by Shiran *et al*. (2007) used high number of primers and were able to discriminate all the genotypes in their studies. From the 72 polymorphic bands, 14 bands (19.4%) bands are putative species-specific markers. These data indicated that RAPD markers were efficient to discriminate and assessment of genetic diversity in mango. For each of the 11 RAPD random primers, PIC values ranged between 0.81 and 0.91 (Table 1). The mean PIC scores for all loci were of 0.85. The mean PIC scores were greater than 0.85 for 45.4% of the RAPD primers. The PIC value provides an estimate of the discriminatory power of a marker by taking into account not only the number of alleles at a locus but also the relative frequencies of these alleles.

Mango, with chromosome number 2n = 40 has a DNA content around 439 million bases (Arumuganathan and Earle, 1991). Analysis of diversity based on 92 PCR fragments amounted to saturating the genome on an average of a marker per 300 million bases which is sufficient to give a realistic representation of the genetic make-up of the plant and make meaningful statements about diversity/relationships of mango cultivars. Although, there could be gaps of 10 to 15 million bases unrepresented in the genome.

A representative of the PCR amplification product of 10 cultivars and accession is shown in Fig. 1 which yielded sufficient polymorphisms to distinguish between cultivars. UPGMA cluster analysis using Nei's coefficient of genetic distance of 10 cultivars revealed sufficient diversity. Bases on 92 fragments obtained, all cultivars and accession were grouped into two main clusters (Fig. 2). The dendrogram revealed a maximum genetic distance of 71.5 % between Sedeek and Succary Abiad cultivars and the minimum genetic distance was around 19.1 % between Succary Abiad and hybrid Sinai-2, also between Ewais and hybrid Sinai- 3. Every single cultivars or hybrid could be identified using eleven primers. The produced tree was consistent with known genetic relationships. Cultivars which formed distinct groups had similar bearing habit and the same embryo type. Hybrids

Table 1: Degree of polymorphism and information content for 11 RAPD random primers along with their sequences applied to 10 mango genotypes

Primer name	sequence	Total bands	Poymorphic bands	Monomorphic bands	Polymorphism (%)	Pic value
OPG_1	CTACGGAGGA	7	4	3	57.14	0.85
OPG_10	AGGGCCGTCT	6	2	4	33.33	0.82
OPB_15	GGAGGGTGTT	8	7	1	87.5	0.87
OPB_1	GTTTCGCTCC	7	5	2	71.42	0.84
OPB_20	GGACCCTTAC	6	6	0	100.00	0.81
OPB_12	CCTTGACGCA	14	12	2	85.71	0.91
OPC_12	TGTCATCCCC	9	6	3	66.66	0.87
OPF_14	TGCTGCAGGT	10	8	2	80.00	0.88
OPZ_10	CCGACAAAC	8	8	0	100.00	0.84
OPA_15	TTCCGAACCC	6	4	2	66.66	0.81
OPQ_5	AGTGCAGCCA	11	10	1	90.9	0.87
Sum/Mean		92	72	20	0.78	0.85

were uniformly distributed across the dendrogram. The hybrids which derived from one of the cultivar were placed closely. For instance, Sinai- 2 hybrid which has Succary Abiad cultivar as female parent and is regular bearer was placed close to Succary Abiad, also a regular bearer. Sinai- 1, a progeny of Alphonso and Barbary was placed next to Alphonso. The case was similar with Sinai-3 and Ewais cultivar, where the latter is a parent of the former, both of which are a superior chance seedling, regular bearer and polyembryonic were also placed together.

In the dendrogram generated based on Nei's Genetic distance coefficient (data not shown), Sedeek and Succary momtaz cultivars formed a distinct cluster and both of them are polyembryonic. These two cultivars were closely related and showed similarity (68 %). These two cultivars are indigenous to the Delta of Egypt as a superior chance seedling. These cultivars are grown widely in Suez Canal and Sinai region.

In the second main cluster, it can be seen that Ewais, Succary Abiad, Barbary cultivars, Sinai-2 and Sinai-3 accessions which originate from the same node (Fig. 2) are indigenous to the Suez Canal region and all of them are polyembryonic and regular bearers. However, Zebda cultivar which is widely grown in Delta of Egypt was found to be closely related to cultivars from the Suez Canal region. This cultivar has some unique morphological characteristics, for instance growth habit, bearing habit, time of flowering, time of maturity, fruit weight, leaf width and fruit color compared to the rest genotypes in the same cluster. This may be justified its position in the dendrogram, justifying our results. This cultivar is a superior chance seedling planted by some of the farmers in Suez Canal region, there is a possibility that this cultivar was brought from Delta of Egypt to Suez Canal and Sinai region.

Thus there seems to be a clear segregation of mango cultivars mainly depending on their geographical location. Using RAPD markers (Schnell *et al*., 1995) were able to cluster maternal half sibs together in mango, indicated the usefulness of RAPD markers for a study of genetic relationships.

Since the cultivars and accessions used here are seedling selections, the results from this study show that the cultivars might have evolved from the existing mango gene pool in that geographical location. These cultivars were selected by local people and later domesticated by cultivation in large areas. Egyptian mango cultivars and accessions used in this study were classified into two groups, Egyptian Delta and Suez Canal region. The dendrogram analysis also showed two group of Egyptian mango (Fig. 1). However, a few cultivars like Zebda were also introduced by travelers who collected them from other parts of the country. Apart from common RAPD markers observed in the cluster, some of the markers were closely associated with bearing habit and embryo type. Cultivars from the same location and hybrids which had one of their parents, were placed close in a cluster. The selection and hybridization programmes in mango can be affected based on the clustering (Kumar *et al*., 2001). RAPD analysis revealed a high degree of genetic diversity among the cultivars and accessions examined in this study which can contribute to the crop development of mango. RAPD analysis can also used for detected gene flow between species. Furthermore, this techniques is less restricting than other molecular techniques like RFLPs (Restriction Fragment Length Polymorphism), as no hybridization and no use of radioisotopes is required and it is therefore more convenient for use in research centers in developing countries.

ACKNOWLEDGMENTS

We thank Prof. Mansour Greishe for permitting us to bring the required young leaves samples from his private orchard in Sinai and for his kind help and guidance during sampling and cultivars identification.

REFERENCES

Arumuganathan, K. and E.D. Earle, 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep., 9: 208-218.

Crane, J.H., I.E. Bally, R.V. Mosqueda-Vazquez and E. Tomer, 1997. Crop Production. In: The Mango: Botany, Production and Uses, Litz, R.E. (Ed.). CAB International, Wallingford, UK., pp: 203-256.

Dunemann, F., R. Kahnau and H. Schmidt, 1994. Genetic relationships in Malus evaluated by RAPD fingerprinting of cultivars and wild species. Plant Breed., 113: 150-159.

Elkhoreiby, A., 1997. Mango Culture in Egypt: An Overview. In: Mango Subsector Horticultural Assessment Report, Crane, J.H. (Ed.). ATUT/RONCO, Cairo, Egypt.

FAO., 2000. Food and agricultural organization of the United Nations, Rome. Quarterly Bull. Stat., Vol. 1.

Francis, C.Y., R.C. Yang, B.J.B. Timothy, Z.H. Ye and X.M. Judy, 1999. POPGENE Version 1.32, the User-Friendly Shareware for Population Genetic Analysis. University of Alberta, Canada.

Galvez-Lopeza, D., Hernandez-Delgadoa, S., M. Gonzalez-Paza, E.N. Becerra-Leora, M. Salvador-Figueroaa and N. Mayek-Perez, 2009. Genetic analysis of mango landraces from Mexico based on molecular markers. Plant Gen. Res., 7: 244-251.

Horne, E.C., S.P. Kumpatla, K.A. Patterson, M. Gupta and S.A. Thompson, 2004. Improved high-throughput sunflower and cotton genomic DNA extraction and PCR fidelity. Plant Mol. Biol. Rep., 22: 83-84.

Iyer, C.P.A. and C. Degani, 1997. Classical Breeding and Genetics. In: The Mango-Botany, Production and Uses, Litz, R.E. (Ed.). Wallingford Oxon/CAB International, New York, USA., pp: 49-68.

Khanuja, S.P.S., A.K. Shasany, M.P. Darokar and S. Kumar, 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol., Rep., 17: 1-7.

Knight, E.B. and R.E. Sanford, 1998. Mango cultivars evaluation. Technical Report, ATUT, pp: 1-78.

Kumar, N.V.H., P. Narayaswamy, D.T. Prasad, G.K. Mukunda and S.N. Sondur, 2001. Estimation of genetic diversity o f commercial mango cultivars using RAPD markers. J. Hortic. Sci. Biotechnol., 76: 529-533.

Marieschi, M., A. Torelli, F. Poli, A. Bianchi and R. Bruni, 2010. Quality control of commercial Mediterranean oregano: Development of SCAR markers for the detection of the adulterants *Cistus incanus* L., *Rubus caesius* L. and *Rhus coriaria* L. Food Control, 21: 998-1003.

Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.

Paillard, M., P. Lashermes and V. Petrard, 1996. Construction of a molecular linkage map in coffee. Theor. Applied Genet., 93: 41-47.

Pandit, S.S., S. Mitra, A.P. Giri, K.H. Pujari, B.P. Patil, N.D. Jambhale and V.S. Gupta, 2007. Genetic diversity analysis of mango cultivars using inter simple sequence repeat markers. Curr. Sci., 93: 1135-1141.

Ravishankar, K.V., L. Anand and M.R. Dinesh, 2000. Assessment of genetic relatedness among mango cultivars of India using RAPD markers. J. Hortic. Sci. Biotechnol., 75: 198-201.

Santos, C.A.F., F.P.L. Neto, M.A. Rodrigues and J.G. Da Costa, 2008. Similaridade genetica de acessos de mangueira de diferentes origens geograficas avaliadas por marcadores AFLP [Genetic similarity of mango accessions of different geographic origins evaluated with AFLP markers]. Rev. Bras. Frutic., 30: 736-740.

Schnell, R.J., C.M. Ronning and R.J. Jr. Knight, 1995. Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers. Theor. Applied Genet., 90: 269-274.

Shiran, B., N. Amirbakhtiar, S. Kiani, S. Mohammadi, B.E. Sayed-Tabatabaei and H. Moradi, 2007. Molecular characterization and genetic relationship among almond cultivars assessed byRAPD and SSR markers. Sci. Hortic., 111: 280-292.

Singh, S. and K.V. Bhat, 2009. Molecular characterization and analysis of geographical differentiation of Indian mango (*Mangifera indica* L.) germplasm. Acta Hortic., 839: 599-606.

Singh, S., A.B. Gaikwad and J.L. Karihaloo, 2009. Morphological and molecular analysis of intracultivar variation in Indian mango (*Mangifera indica* L) cultivars. Acta Hortic., 829: 205-212.

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

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res., 18: 6531-6535.

Wu, X., H. Li, W. Zhao, L. Fu and H. Paeng *et al*., 2010. SCAR makers and multiplex PCR-based rapid molecular typing of *Lentinula edodes* strains. Curr. Microbiol., 61: 381-389.