Journal of Agricultural Technology 2010 Vol. 6(3): 559-571
Available online http://www.ijat-rmutto.com
ISSN 1686-9141

Opimization of DNA isolation and PCR protocol for RAPD analysis of *Mangifera indica* L.

Jena, R.C.[*], Samal, K.C. and Das, B.K.

Biotechnology cum Commercial Tissue Culture Centre, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Orissa, India.

Jena, R.C., Samal, K.C. and Das, B.K. (2010). Opimization of DNA isolation and PCR protocol for RAPD analysis of *Mangifera indica* L. Journal of Agricultural Technology 6(3): 559-571.

Genetic analysis of plants relies on high yields of pure DNA samples. Here we present the optimization of DNA isolation and Polymerase chain reaction (PCR) conditions for Random amplified polymorphic DNA (RAPD) analysis of a major fruit crop of Orissa, Mango. The leaf of mango contains high level of polysaccharides, polyphenols and secondary metabolites. The extracted DNA from these cultivars when subjected to PCR is often problematic, especially when mature tissues are used for DNA extraction. In order to overcome these problems a protocol has been developed, availing on a high salt concentration and on the combination of Polyvinylpyrrolidone (PVP) and Hexadecyltrimethylammonium bromide (CTAB) in the extraction buffer in a order to prevent the solubilization of polysaccharides and polyphenols during the DNA extraction method. It also involves successive long term chloroform: Isoamylalcohol extractions,an long term RNAse treatment with all steps carried out at Room temperature (RT). Using this method, DNA was extracted from mangoes of more than 66 mango cultivars including young leaves, old leaves, frosted old leaves and withered old leaves. The yield of DNA ranged from 1-2 µg/µl per gram of the leaf sample/tissue and the purity ratio was between 1.7-1.8 indicating minimal levels of contaminating metabolites. The technique is ideal for isolation of DNA from different plant species/cultivars and the isolated DNA were used for RAPD analysis. The optimization of RAPD protocol was based on the use of 50 ng of template DNA, higher concentration of $MgCl_2$ (2 mM) and lower concentration of primer (0.5µM), Thermus aquaticus (Taq) polymerase (1units) and an annealing temperature of 37^{o}C, which resulted, optimal amplification. In all PCR reactions Reproducible amplifiable products were observed. Thus the results indicate that the optimized protocol for DNA isolation and PCR was applicable to plant cultivars/species belonging to different genera and this process is suitable for further work on diversity analysis. Ferthermore here we used suitable DNA isolation protocol for RAPD analysis to study the genetic variation in the future in Anacardiaceae species.

Key words: Mango, *Mangifera*, polysacchrides, phenols, PCR amplification, DNA isolation, RAPD

[*]Corresponding author: R. C. Jena; e-mail: jena_ramchandra@yahoo.co.in

Introduction

Mango is an important member of the family Anacardiaceae and belongs to genus Mangifera order sapindales. It is believed to have originated in Indo-Burma region (Popenoe, 1927; Mukherjee, 1951; Decandolle, 1904). Mango is the most popular fruit among millions of people in India and is also National fruit of India. It is also the most important fruit of Orissa grown from time immemorial. More than 1000 varieties of mango are available in Orissa (Rao and Parida, 1988). The production of mango in India is 11 million tones, which accounts for 57 % of total world's production (Negi, 2000). Mango is rich in vitamins as well as beta carotene, minerals and antioxidants etc. and also contains an enzyme with stomach soothing properties. The other use of mango includes the seed fat that contains high stearic acid. The bark contains Mangiferine used for tanning and also employed against rheumatism and diphtheria. The medicinal use of mango include protection against cancer, serves as astringents in case of diarrhea, chronic dysentery, chronic urethritis, lowering blood cholesterol level etc. The resinous gum from trunk is applied on cracks in the skin of the feet and on seabies and is helpful in case of syphilis. Fat is administered in case of stomatitis. These excellent uses also bring a name to mango as "King of fruit". The medicinal use has attracted global attention which prompted us to conduct preliminary studies on genetic diversity in Magnifera. At present several mango cultivars have many synonyms in different regions which make identification difficult. Differentiation of cultivars through morphological features is inefficient and inaccurate. This problem is further compounded by perennial nature of crops. These complications can be overcome by identification of genotype by various types of DNA based molecular techniques to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR) based methods and sequencing based methods. PCR based methods involves *in vitro* amplification of Loci or particular DNA sequences with the help of specific or arbitrary oligonucleotide primers and the thermostable DNA polymerase enzyme. PCR based techniques where random primers are used include random amplified polymorphic DNA (RAPD).

Isolating high quality DNA is essential for molecular research. .Mango leaf contains exceptionally high amounts of polysaccharides, polyphenols, tannins, secondary metabolites such as alkaloids, fluvanoids and phenols, and terpenes which interfere with DNA isolation procedure. The problem encountered due to these compounds include coisolation of highly viscous polysaccharides degradation of DNA due to endonuclease, inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with enzymatic reactions. Moreover, the contaminating RNA that precipitates along

with DNA causes suppression of PCR amplification and other many problems (Pikkart and Villeponteau, 1993; Padmalatha and Prasad, 2006), interference with DNA amplification involving random primers e.g. RAPD analysis (Padmalatha and Prasad, 2006) and improper priming of DNA templates during thermal cycle sequencing.By using one DNA isolation protocol the optimal DNA yield may not possible from different plant taxa. For example, some closely related species of the same genus require different isolation protocols. So we have to standardize an efficient protocol for DNA isolation and optimization of the PCR condition. Various protocols for DNA extraction have been successfully applied to many plant species (Dellaporta *et al*., 1983; Tai and Tranksley, 1990; Ziegenhagen *et al*., 1993; Porebski *et al.*, 1997; Sharma *et al*., 2002; Risterucci *et al*., 2000; Wang and Taylor, 1993; Ziegenhagen and Scholz, 1998; Saghai Maroof *et al*., 1984; Doyle and Doyle, 1987). We have tested previously mentioned DNA isolation protocols in some woody fruit crops that contain high polysaccharide level such as crops of mango and citrus species but these methods resulted in DNA with lot of impurities and not very suitable for RAPD analysis. Therefore, we report here a total genomic DNA isolation protocol derived from a method originally developed for other plants (Doyle and Doyle, 1987). Modification were made to minimize phenols, polysaccharide co-isolation and to simplify the procedure for processing of large number of DNA samples, Using polyvinylpyrolidone (PVP) and high salt concentration (Fang *et al.*, 1992; Moller *et al.*, 1992). DNA extraction from plant is preferentially performed from young tissues due to the lower content of polysaccharides, Polyphenols and other secondary metabolites which co-precipitate with DNA in the extraction procedure, inhibit DNA digestion and PCR (Zhang and Mostewart, 2000), presumably by irreversible interactions with DNA (Dabo *et al.*, 1993).

The use of primers, Taq polymerase, quantity of DNA, dNTPs, and the reaction volume to standardize thre protocol for RAPD is proved to be inexpensive.The protocol optimized for RAPD proved to be inexpensive with relation to Thus the protocol derived for both RAPDs and genomic DNA isolation, is genus independent, efficient, inexpensive, simple, and yields pure DNA amplifiable by PCR as indicated by the results of the RAPD technique. The DNA which is isolated by the present protocol is suitable for further downstream applications.

Materials and methods

Plant material

The sixty Mango cultivars (*Mangifera indica* L.) were used in this study. The leaf samples of sixty six Mango cultivars were collected from orchard of

OUAT, Bhubaneswar, Orissa. One gram of young leaves were harvested fresh for DNA isolation.

Reagents/chemicals used

An extraction buffer consisting of 2% CTAB (w/v), NaCl 1.5 M, Tris HCl pH 8.0 (100 mM) and EDTA pH 8.0 (20 mM), PVP (2%) ,β-mercaptoethanol 2% (V/v). was prepared. ribonuclease A (10 mg/ml), chloroform: Isoamylalcohol (24:1) v/v/v), Ethanol (70%, 100%), Sodium acetate (3M) solution (pH 8.0), and TE buffer (Tris HCl, 10 mM ,pH 8.0 , 1mM EDTA; pH 8.0) are the additional solutions required.

DNA isolation protocol

Freshly harvested young and tender or old leaf samples (1 g) were ground in liquid Nitrogen using a pre-chilled mortar and pestle. The ground powder was quickly transferred into a clear autoclaved 50 ml centrifuge tube and then 10 ml of pre-warmed (60^{o}C) extraction buffer was added and shaken gently to form a slurry. The tubes were incubated at 60^{o}C in circulating water bath for one hour with intermittent shaking for every 10 minutes with occasional inversion and cold to normal temperature. An equal volume of chloroform: isomyl alcohol (24:1) was added and mixed properly by inverting the tubes 20-25 times to form an emulsion and centrifuged at 12000 rpm for 20 minutes at RT to separate the phases (long term mixing of samples in chloroform: isomylalcohol approximately for 30 minutes, will help in removal of pigments and formation of brownish colour in DNA sample can be omitted). The supernatant was carefully decanted and transferred to a new tube and the second chloroform: isoamyl alcohol (24:1) extraction performed for the cloudy nature of aqueous phase due to presence of PVP.

Again the supernatant was carefully decanted and transferred to a new tube and was precipitated with two volume of prechilled (-20^{o}C) 95 % ethanol and sodium acetate (final concentration 0.3 M), and gently mixed by inverting up and down (10 minutes) to produce fibrous DNA and incubated at -20^{o}C for a minimum of one hour. The samples were centrifuged at speed 10,000 rpm for 15 minutes. Pour off the supernatant and the pellet was washed twice to thrice with 70 % ethanol. Decanted the supernatant and air dried DNA pellet at RT until the whitish pellet turned to transparent and resuspended in 300 µl of TE Buffer and 6µl of RNAase (10 µg/µl) was added incubated at 37^{o}C for two hour (RNAase treatment helped achieving in proper genomic DNA). To this 600 µl of Ice chilled ethanol and 10 ml of 3M sodium acetate was added and incubated at -20^{o}C for one hour to re-precipitate DNA. The solution was centrifuged at 10,000 rpm for 15

minutes; DNA pellet was dried at 37^{o}C and resuspended in 300 µl of Tris-EDTA (TE) buffer. All the centrifugation steps were carried out at RT to avoid precipitation with CTAB, DNA degradation and to obtain good quality of DNA.

Quantification of extracted DNA and purity checking

The yield of DNA per gram of leaf tissue isolated was measured using a UV spectrophotometer at 260 nm. The purity of DNA was determined by estimating the ratio of absorbance at 260 nm to that of 280 nm. DNA concentration and purity was also determined by running the samples on 0.8 %. Agarose gel depending on the intensities of band when compared with lambda DNA marker (used to determine the concentration). The nucleic acid concentration was estimated following (Sambrook *et al*., 1989).

Optimization of RAPD reaction

For the optimization of RAPD reaction using DNA extracted from 60 different mango cultivars, Oligonucleotide primers from RPI-C series (Genei, Bangalore) and also OPA and OPD series (Operon, Technologies Inc. Almeda CA, USA) were used for amplification to standardize the PCR conditions. The reactions were carried out in a DNA thermocycler (Bio Red). Reactions without DNA were used as negative controls. Each 25 µl reaction volume contained about 1 x PCR Buffer (10mM Tris HCL pH 8.3;50mM KCL), 2mM $MgCl_2$, 200 µM dNTP mix, 1U of Taq DNA polymerase(Genei,Bangalore) 0.5µM of single primer (Genei, Bangalore and Operon Technologies Inc. Almeda CA, USA), 50 ng of temptate DNA. The thermocycler was programmed for an initial denaturation step of 3 min at 94^{o}C, followed by 44 cycles of 1 min at 94^{o}C, 60s at 37^{o}C, extension was carried out at 72^{o}C for 2 minutes and final extension at 72^{o}C for 7 minutes and at last the hold temperature was of 4^{o}C. PCR products were electrophoresed on 1.5% (W/V) aganose gels, in 1x Tris borate-EDTA (TBE) Buffer at 70 V for four hour and then stained with elthidium bromide (1.0 µg/ml). Gels with amplification fragments were visualized and photographed under UV light. Medium range DNA Ruller was used as molecular marker (Bangalore Genei, Bangalore, India) to know the size of the fragments.for each experiment the reproducibility of the amplification products was tested twice using similar reaction conditions at different times.

Results

Extraction of genomic DNA from the leaf of 66 varieties of mango (*Mangifera indica)* (Table-1) was carried out using modified CTAB DNA isolation protocol (Doyle and Doyle, 1987). The isolated DNA was of high quality as it showed a reading in between 1.7 to 1.8 after calculating the ratio of absorbance 260/280 nm (Fig. 1). The optimization of RAPD-PCR reaction parameters for different mango cultivars was shown in Table-2. The DNA yield found ranged from 1 to 2 ng /µl. DNA isolated by this method was amplified by RAPD-PCR using random decamer primers (Fig. 2, 3, 4a, 4b, 5a, 5b).

Discussion

Mango (*Mangifera indica* L) is the most popular fruit among million of people in the Orient particularly India, and is rightly titled as the "king of fruits" because of its wide adaptability, high nutritive value, richness in variety, delicious taste, excellent flavour etc. The performance of varities is found to vary under different climatic conditions (Singh, 1978). Differentiation of cultivers through morphological features is inefficient and inaccurate. As presently several mango cultivers have synonyms in different regions, which make identification are difficult, many of these complications of a phenotype based assay can be over come through DNA fingerprinting. Many researches on DNA based molecular markers are in progress in various research institutions all over the world. For authentication of plant species these techniques have been used widely. DNA extraction was standardized by modifying some of the steps in original CTAB DNA isolation protocol (Doyle and Doyle, 1987). Different stages of leaves i.e. tender, young, old are used for DNA isolation. Samples frozen in liquid nitrogen and preserved at -20^{o}C for several weeks can also be used. The procedure Presented here resulted in extracting, high quality, low polysaccharide genomic DNA from 66 different mango cultivars belonging to (*Mangifera indica* L.)

Tannins, terpenes and resins considered as secondary metabolites are also difficult to separate from DNA (Ziegenhangen and Scholz, 1998). Certain polysaccharides are known to inhibit RAPD reactions. They distort the results in many analytical applications and therefore, lead to wrong interpretations (Kotchoni *et al*., 2003). Polysaccharide co-precipitationis avoided by adding a selective precipitant of nucleic acids, i.e., centyltrimethyl Ammonium bromide (CTAB) to keep polysaccharides in solution through SDS (Dellaporta *et al.,* 1983).

Table 2. Optimization of the RAPD-PCR reaction parameters for different mango cultivars.

PCR condition	Range(Tested)	Optimum conditions	Inference
DNA concentration (ng)	10, 20, 40, 45, 50, 55, 60, 70, 90, 130, 150, 170, 190	50 ng	At higher concentration the presence of smear and with lower concentration absence of amplification effected the repeatability.
Magnesium chloride (mm)	0.5, 1, 2, 3 and 4	2 mM	Non specificity and yield of product increases excess or lower conc.
Deoxynucleotide triphosphates (dNTPs µM)	50,100,150,200, 250,300.	200 µM	Reduction of free Mg^{2+} occurs at increased concentration, interfering with the enzyme.
Primer concentration (µm)	0.2, 0.4, 0.5, 0.8, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0	0.5 µM	Primer dimer formation and absence of amplification occurs at higher and lower concentrations respectively.
Taq polymerase (units)	0.5, 1.0, 2.0, 3.0	1.0 U	Proper amplification can not be shown at lower concentration.specificity decreased at higher concentration.
Initial denaturation time interval (minute) at 94°C	1,2, 3, 4 and 5	94°C for 3 minutes	Reduction in amplification,loss of Taq polymerase activity and lack of reproducibility happened at higher/lower time intervals(from optimum).
Annealing temperature (°c)/Time intervals (seconds)	30, 35, 37, 40, 45, 50, 55 and 60 30,50,60,80,100,120, 160	37°C for 60 seconds	Difference in specificity found at higher/lower annealing temperature (from optimum).
Reaction volume (µl)	20, 25, 30, 35 and 40	25	Influences cost of PCR ingredients
Number of cycles	30, 35, 40, 42, 43,44, 45, 48, 50 and 60	44	Lower/Higher cycles (from optimum) effects the amplification

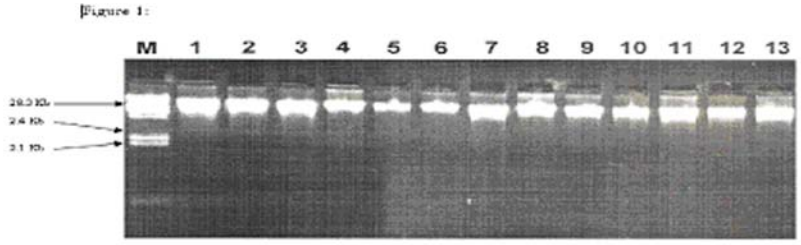

Fig. 1. Genomic DNA were loaded in 0.8% agarose gel and separated by electrophoresis for 90 min at 100 volt., Lane M: 1 Kb ladder size standard marker, Lane 1-15: genomic DNA isolated from different mango cultivars. The numbers on the top of the lanes correspond the genotypes of mango is given in Table-1.

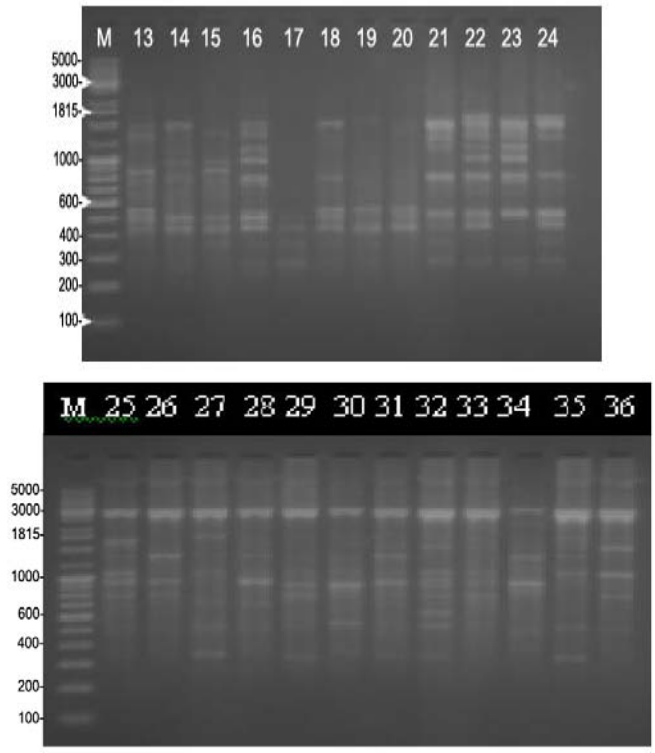

Fig. 2. and 3. RAPD-PCR amplification product of different Mango germplasm by using Genei,Bangalore primer RPI- C Series,RPI-9(5'-ACCGCCTATG-3') and RPI-2(5'-AACGCGTCGG-3') indicated in figure 2 and 3 respecttively. The amplification product were fractioned in a 1.5% agaroge gel.
Lane M: Represents Molecular Marker (Medium Range DNA ruller), Lane 13-36: The numbers on the top of the lanes correspond the genotypes of mango as given in table-1.

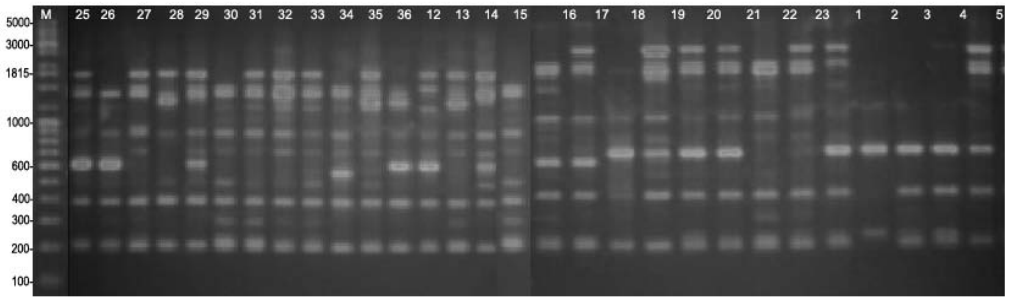

Fig. 4. RAPD-PCR amplification product of different Mango germplasm by using Genei, Bangalore primer RPI- C Series, RPI-9 (5'-ACCGCCTATG-3'). The amplification product were fractioned in a 1.5% agaroge gel.
Lane M: Represents Molecular Marker (Medium Range DNA ruller), Lane 30-58: The numbers on the top of the lanes correspond the genotypes of mango as given in table-1.

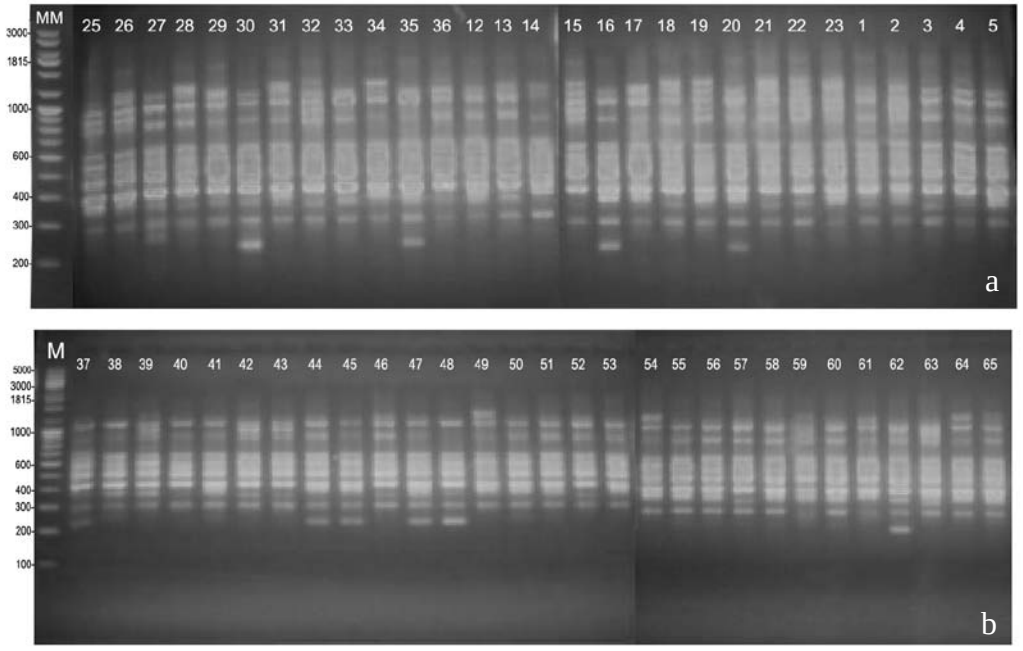

Fig. 5a. and 5b. RAPD-PCR amplification product of different Mango germplasm using operon primer OPA-1 (5'- TTC GAG CCA -3'). The amplification product were fractioned in a 1.5% agaroge gel.
Lane M: Represents Molecular Marker (Low Range DNA Ruller Plus), Lane MM:Represents Molecular Marker (Medium Range DNA Ruller), Lane 1-58: The numbers on the top of the lanes correspond the genotypes of mango as given in Table 1.

The presence of polyphenols, which are powerful oxidizing agents present in many plant species, can reduce the yield and purity by binding covalently with the extracted DNA making it useless of most research applications (Katterman and Shattuck, 1983; Peterson *et al.*, 1997, Porebski *et al.*, 1997; Padmalatha and Prasad, 2006). Mixing of PVP along with CTAB may bind to the polyphenolic compounds by forming a complex with hydrogen bonds and may help in removal of impurities to some extent. The removal of chlorophyll and other colouring substances such as pigments, dyes, etc.occured by the help of Long term chloroform: isoamyl alcohol treatment. Many DNA isolation procedure also yield large amounts of RNA, especially 18S and 25S rRNA (Doyle and Doyle, 1987). The yield of PCR reduction can be possible by large amounts of RNA in the sample. A prolonged 2 hour RNase treatment degraded RNA into small ribbonucleosides that donot contaminate DNA preparation and yielded RNA Free pure DNA. To avoid the DNA degradation and precipitation for some

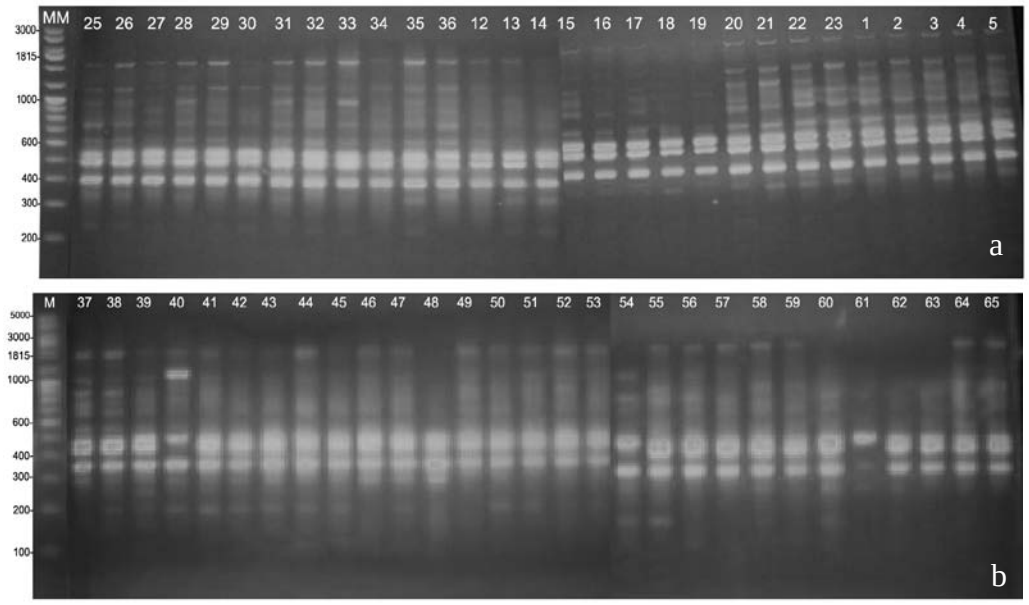

Fig. 6a. and 6b. RAPD-PCR amplification product of different Mango germplasm using operon primer OPA-2 (5'- GTG AGG CGT-3'). The amplification product were fractioned in a 1.5% agaroge gel.
Lane M: Represents Molecular Marker (Low Range DNA Ruller Plus), Lane MM: Represents Molecular Marker (Medium Range DNA Ruller), Lane 1-58: The numbers on the top of the lanes correspond the genotypes of mango as given in Table 1.

extent all the steps were carried out at RT .Additional centrifugation steps, modified speed and time removed large amounts of precipitates like protein and polysaccharides. We found these modified steps necessary to standardize and increase the quality and quantity of genomic DNA. The degree of purity and quantity varies between applications (Padmalatha and Prasad, 2000). Isolation of good quality DNA suitable for analysis from semi-processed or processed botanicals is also a challenge (Li *et al.*, 2003). DNA isolated by this method yielded strong and reliable amplification products showing its compatibility for RAPD-PCR using random decamer primers (Fig. 2, 3, 4a, 4b, 5a, and 5b). The amplified fragments size ranged in between 2000 bp to 200 bp. For RAPDs almost all the tested parameters like the primer, Taq polymerase, dNTPs, magnesium chloride, concentration of template DNA and temperature and time intervals during denaturation, annealing and elongation were optimized which also had an effect on amplification, reproducibility and banding patterns . The optimized conditions for RAPD protocol are given in Table 2. The described conditions in the present work, modified for use in RAPD analysis, consistently

amplified DNA fragments of different mango cultivars belonging to Mangifera *indica* L. which are highly recalcitrant. The optimized DNA isolation and RAPD technique may serve as an efficient tool for further molecular studies. We have recently performed this protocol for genomic DNA isolation from withered old leaves and young leaves of other fruit crops. These samples included Banana (*Musa* spp.), *Citrus* spp. Results prove the reproducibility, reliability and practicality of this protocol. Thus we concluded that present protocol describes a reliable, simple, and consistent DNA isolation method for mango and other common fruit crops.

Acknowledgements

Authors are grateful to Ministry of Agriculture, Government of India for financial support. We are also grateful to the Vice Chancellor and Dean of Research, Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, India for providing laboratory facilities.

References

Dabo, S.M., Mitchell, E.D. and Melcher, U. (1993). A method for the isolation of nuclear DNA from cotton (Gossypium) leaves. Analytical Biochemistry 210: 34-38.

De candolle, A. (1904). Origin of cultivated plants. Kegan Paul, Trench, London.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983). A plant DNA mini-preparation; version2. plant mol Biol Rep. 1: 19-21.

Doyle, J.J. and Doyle, J.L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue.phytochem. Bull.19: 11-15.

Fang, G., Hammar, S. and Grumet, R. (1992). A quick and inexpensive method for removing polysachharides from plant genomic DNA. Bio feedback. 13: 52-54

Katterman, F.R.H. and Shattuck, V.I. (1983). An effective method of DNA isolation from mature leaves of *Gossypium species* that contain large amounts of phenolic terpenoids and tannins. Prep. Biochem. 13: 347-359.

Kotchoni, S.O., Gachomo, E.W., Betiku, E. and Shonukan, O.O. (2003). A home made kit for plasmid DNA mini preparation. African Journal of Biotechnology 2: 88-90.

Li, Y.F., Li, X.Y., Lin, J., Xu, Y., Yan, L., Tang, F. and Chen, F. (2003). Identification of bulb from Fritillaria chirrhosa by PCR with specific primers. Planta medica 69:186-188.

Moller, E.M., Bahnweg, G., Sandermann, H. and Geiger, H.H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies and infected plant tissues. Nucleic Acids Research 22: 6115-6116.

Mukherjee, S.K. (1951). The origin of mango. Indian journal of Genetics 2: 49.

Negi, S.S. (2000). Mango production in India. Acta Horticulture 509: 69 - 77.

Padmalatha, K. and Prasad, M.N.V. (2006). Optimization of DNA isolation and PCR protocol for RAPD analysis of selected Medicinal and aromatic plants of conservation concern from Peninsular India. African journal of Biotechnology 5(3): 230-234.

Peterson, D.G., Boehm, K.S. and Stack, S.M. (1997). Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersicon esculentum*), a plant containing high levels of polyphenolic compounds. Plant Mol. Biol. Rep. 15: 148-153.

Pikkart, M.J. and Villeponteau, B. (1993). Suppression of PCR amplification by high levels of RNA. Biotechniques 14: 24-25.

Popenoe, W. (1927). Manual of tropical and subtropical fruits. Mcmillan, New York, USA.

Porebski, S., Baily, L.G. and Baum, B.R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Bio Rep. 15: 8-15.

Rao, D.P. and Parida, G.N. (1988). Horticulture in Orissa, Mango special No.14.

Risterucci, A.M.L. and Grivet (2000). A high density linkage map of theobroma cacao L. Theor Appl Genet. 101: 948-955

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. (1984). Ribosomal DNA spacer-length polymerphisim in barley: mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA. 81(24): 8014-8019.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular cloning: a laboratory manual. Cold spring Harbor Laboratory Press, Cold Spring, Harbour, New York,USA.

Sharma, A.D., Gill, P.K. and Singh, P. (2002). DNA isolation from dry and freash samples of polysaccharide-rich plants. Mol Bio Rep. 20: 415a-415f.

Singh, R.N. (1978). Mango indian council of agricultural research, New delhi.pp. 39-55.

Tai, T.H. and Tanksley, S.D. (1990). A rapid and inexpensive method for isolation of total DNA form dehydrated plant tissue.

Wang, Y. and Taylor, D.E. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. Biotechniques 14: 748-750.

Zhang, J. and McSteward, J.D. (2000). Economical and rapid method for extracting catton genomic DNA. Journal of Cotton Science 4: 193-201.

Ziegenhagen, B. and Scholz, F. (1998). Methods for difficult plant species. In: Karp A, Issac PG and Ingram DS (eds). Molecular Tools for screening Biodiversity 2.2 Plants and Animals, Chapman and Hall, London pp. 32-35.

Zioegenhagen, B. and Scholz, F. (1993). A procedure for mini preparation of genomic DNA from needless of silver fir (Abies alba Mill.). Plant Mol Bio Rep. 11: 117-121.

(Received 7 August 2009; accepted 20 February 2010)

Table 1. List of 66 different varieties of *Mangifera indica* used for genomic DNA isolation.

Sl. No.	Name of Variety	Sl. No.	Name of Variety	Sl. No.	Name of Variety	Sl. No.	Name of Variety	Sl. No.	Name of Variety	Sl. No.	Name of Variety
1	Akhurash	12	Pausa Mallika	23	Alphanso	34	Sai Sugandha	45	Hajpuri Langra	56	Jahangir
2	Arka Anmol	13	Banganpalli	24	Chiratpuri	35	Dasahari	46	Amin Tehasil	57	Hatimunda
3	Golab Khas	14	Vanraj	25	Pusa Surya	36	Arka .Nilkiran	47	Sundar pasand	58	baldev
4	Arka Aruna	15	Subarnarekha	26	Pusa Arunia	37	Bilei Mundia	48	Hunkagaga	59	kuanri
5	Arka punit	16	Kesor	27	Ambika	38	Dophasal	49	Lajkuli Bandan	60	Prema sagar
6	Himsagar	17	Langra	28	PKM1	39	Misskanta	50	Khajara	61	Totapari red small
7	Amrapalli	18	Totapari	29	Janardan Pasand	40	Kanchamitha	51	Special Pathansa	62	Anad sagar
8	Mangira	19	Neelam	30	PKM2	41	Jamuna	52	Sabri	63	Kisan bhag
9	Bombay Green	20	Malgua	31	Nilsaha Gujarat	42	Karpura bhag	53	Chausa	64	Milton
10	Fazli	21	Pravasankar	32	Mohammad Vahar	43	Golab bhas	54	Niranjan	65	Bathua
11	Ratna	22	Sindhu	33	Baramasi	44	Dudhia Langra	55	Ganga	66	collector