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Development of an efficient protocol for genomic DNA extraction from mango (*Mangifera indica*)

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Abstract. *Majumder DAN, Hassan L, Rahim MA, Kabir MA. 2011. Development of an efficient protocol for genomic DNA extraction from mango (*Mangifera indica*). Nusantara Bioscience 3: 105-111.* A simple and efficient method for genomic DNA extraction from woody fruit crops containing high polysaccharide levels has been described here. In the present study, three kinds of plant DNA extraction protocols were studied and the target was to establish the water-saturated ether (WSE) with 1.25 M NaCl method as the most efficient protocol for removing the highly concentrated polysaccharides from genomic DNA of woody fruit crops. This method involves the modified CTAB or SDS procedure employing a purification step to remove polysaccharides using the WSE method. Precipitation with an equal volume of isopropanol caused a DNA pellet to form. After being washed with 70% ethyl alcohol, the pellet became easily dissolved in TE buffer. Using these three methods, DNA was extracted from samples of 60 mango genotypes, including young, mature, old, frosted old and withered old leaves. Compared with the three studied DNA extraction protocols of mango, it was found that the WSE method with NaCl had the highest value of average percentage (85.44%) in DNA content of the mango genotypes. The average yield of DNA ranged from 5.05 µg/µL to11.28 µg/µL. DNA was suitable for PCR and RAPD analyses and long-term storage for further use.

Keywords: DNA extraction, fruit crops, polysaccharides, RAPD, water-saturated ether.

Abbreviations: CTAB: hexadecyltrimethylammonium bromide; RAPD: Random Amplified Polymorphic DNA; RFLP: Restriction Fragment Length Polymorphism; SSR: Simple Sequence Repeats; RT: Room temperature; WSE: Water: saturated ether.

Abstrak. *Majumder DAN, Hassan L, Rahim MA, Kabir MA. 2011. Pengembangan protokol ekstraksi DNA genom mangga (*Mangifera indica*) yang efisien. Nusantara Bioscience 3: 105-111.* Sebuah metode sederhana dan efisien untuk ekstraksi DNA genom tanaman buah berkayu yang mengandung banyak polisakarida telah dilakukan. Dalam penelitian ini, tiga protokol ekstraksi DNA tumbuhan dipelajari; dan tujuannya adalah menetapkan metoda ether jenuh air (WSE) dengan NaCl 1.25 M sebagai protokol yang paling efisien dalam mengeluarkan polisakarida yang sangat melimpah pada DNA genom tanaman buah berkayu. Penelitian ini mencakup CTAB yang dimodifikasi dan prosedur SDS sebagai langkah pemurnian untuk menghilangkan polisakarida, serta penggunaan metode WSE. Presipitasi dengan isopropanol yang sama volumenya menyebabkan pelet DNA terbentuk. Setelah dicuci dengan etil alkohol 70%, pelet menjadi mudah larut dalam buffer TE. Menggunakan tiga metode di atas, DNA diekstraksi dari sampel 60 genotipe mangga, termasuk daun muda, daun dewasa, daun tua, daun kering-beku dan daun kering. Perbandingan tiga protokol ekstraksi DNA mangga, menunjukkan bahwa metode WSE dengan NaCl menghasilkan nilai persentase rata-rata (85,44%) kandungan DNA genotipe mangga yang tertinggi. Hasil rata-rata DNA berkisar antara 5,05 µg/mL hingga 11,28 µg/mL. DNA cocok untuk analisis PCR dan RAPD dan memungkinkan penyimpanan jangka panjang untuk digunakan lebih lanjut.

Kata kunci: ekstraksi DNA, tanaman buah, polisakarida, RAPD, eter jenuh air.

INTRODUCTION

Several tropical or subtropical fruit crops like *Mangifera indica*, *Citrus* spp. and others are perennial woody plants. In those crops, the polysaccharide contents, even in young tissues are higher than those of field crops. Isolation of high-quality DNA is essential for molecular research. Polysaccharide contamination is a common problem in the DNA extraction of higher plant. DNA samples of higher plants often contain melicera colloidal hyalosome, which is almost insolvable in water or TE buffer, and inhibits enzyme reactions (Fang et al. 1992; Porebski et al. 1997; Schlink and Reski 2002), and hinder the downstream work in molecular biology research. DNA samples are also unstable for long term storage (Lodhi et al. 1994; Sharma et al. 2002). Several plant DNA extraction protocols for removing polysaccharides have been reported (Porebski et al. 1997; Schlink and Reski 2002). Moreover, some woody fruit crops like mango (*Mangifera indica* L.) citrus (*Citrus* spp.), litchi (*Litchi chinensis* S.), custard apple (*Annona squamosa* L.), guava (*Psidium guajava* L.), banana (*Musa* spp.), pomegranate

(*Punica granatum* L.), jujube (*Ziziphus mauritiana* M.), papaya (*Carica papaya* L.) and pineapple (*Ananas comosus* L.) also contain high polysaccharide levels, the protocols could only be used on vigorous tissue (Luro et al. 1995; Porebski et al. 1997) and the quality of DNA isolated was not high enough to use in PCR, RAPD, RFLP, and SSR analyses. In the present study, a modified protocol was applied to utilize the water-saturated ether and 1.25-1.3 M NaCl. Residual phenols and most polysaccharides were removed and DNA was precipitated selectively in the presence of high salt (Fang et al. 1992; Moller et al. 1992).

The target of the present study was to establish the water-saturated ether with 1.25-1.3 M NaCl method as an efficient protocol for removing the high concentration polysaccharides from the genomic DNA of woody fruit crops.

MATERIALS AND METHODS

Plant materials

Sixty mango genotypes including landraces, as well as exotic and cultivated varieties, were used as the plant materials for genomic DNA extraction. Leaves were collected at different developmental stages (i.e. young, recently matured, old, frosted and withered).

Isolation of genomic DNA

Total genomic DNA was isolated from the mango leaves following three different methods: Sodium Dodecyl Sulphate (SDS) method, CTAB (Hexadecyltrimethylammonium bromide) method, and water–saturated ether and 1.25 M NaCl method.

SDS method

Reagents:

a) Extraction buffer: 50 m M Tris-HCl, 25mM EDTA (Ethylenediaminetetraacetic Acid) and 300 mM NaCl, pH=8.0 and 1% SDS (sodium dodecy1 sulfate)

b) Phenol: chloroform: isoamy1 alcohol (P: C: I): 25:24:1, equilibrated to pH near 8.0

c) TE buffer: Tris-HCl 10mM, 1mM EDTA, pH=8.0

d) Sodium acetate (3M), pH=5.2

e) Absolute ethanol (100%)

f) Ethanol (70%)

Protocol of genomic DNA isolation:

Genomic DNA was isolated from fully expanded young, recently matured, old, frosted, and withered leaves following Doyle and Doyle (1990) method with a few modifications. Approximately 300 mg of clean leaf tissue was cut into small pieces and poured into Eppendorf tube. The tissue was grounded with 800 µL extraction buffer, vortexed for 20 seconds and incubated at 65ºC for 5 minutes in a hot water bath. The extract was centrifuged for 10 minutes at 14000 rpm to allow precipitation of the cell debris. About 600 µL of upper aqueous phase was transferred to another tube; about 600 µL of phenol: chloroform: isoamy1 alcohol (v: v: v= 25:24:1) was added to it and mixed gently. Then the solution was centrifuged for 10 minutes at 14000 rpm. The upper aqueous layer was carefully transferred to another Eppendorf tube without disturbing the lower portion. For precipitation of DNA, about 800 µL of absolute alcohol (100%) was added to the aqueous solution and centrifuged for 3 minutes at 14000 rpm to form pellet. After discarding the liquid completely, the DNA solution was reprecipitated by adding 400 µL of 70% ethanol with 20 µL 3 M sodium acetate and again pelleted by centrifuging for 3 minutes at 14000 rpm. Then the liquid was removed completely, the pellet was air-dried and resuspended in 50 µL of TE buffer and samples were stored at -20ºC for use.

CTAB method

Reagents:

a) Extraction buffer: 100m M Tris-HCl, 20 mM EDTA (ethylenediaminetetraacetic acid) and 1.4M NaCl, pH=8.0 and 2% CATB (wv^{-1} hexadecyltrimethylammonium bromide), 2% ($vv\text{-}^{1}$) 2-mercaptoethanol, 1% PVP (polyvinyl-pyrrolidone) equilibrated to pH near 8.0

b) 20% SDS (Sodium Dodecyl Sulphate)

c) Chloroform: isoamy1 alcohol (C:I): 24:1(v/v), equilibrated to pH near 8.0

d) TE buffer: Tris-HCl 10mM, 1mM EDTA, pH=8.0

e) Sodium acetate (3M), pH=5.2

f) Absolute ethanol (100%)

g) Ethanol (70%)

Protocol of genomic DNA isolation:

The CTAB method as described by Saghai-Maroof et al. (1984) with few modifications was followed for DNA isolation. Healthy leaves of each genotype were taken and washed with distilled water to avoid any spore of microorganisms and wiped dry with paper towels; approximately 300 mg of leaf tissue was cut into small pieces (as small as possible to facilitate grinding) and grounded using pre-cooled (-20ºC) mortar and pastel and poured into a 2 mL Eppendorf tube. 670 µL extraction buffer and 50 µL SDS (20%) were added with the grinding tissue for digestion and mixed well. The samples were then vortexed for 20 seconds for proper mixing and incubated at 65ºC for 10 minutes in hot a water bath. 100 µL NaCl and 100 µL CTAB were added and mixed well. The samples were again incubated at the same temperature for approximately 10 minutes. 900 µL chloroform (chloroform: isoamy1 alcohol: 24:1, v/v) was added and mixed well by shaking. Then to allow the precipitation of cell debris the extract was centrifuged for 10 minutes at 14000 rpm with a microcentrifuge. About 600 µL of upper aqueous phase was transferred to another tube, and then about 600 µL of ice-cooled isopropanal was added to it and mixed gently. At this stage, DNA became visible as white strands by flicking the tube several times with fingerings. The solution was centrifuged for 10 minutes at 14000 rpm. The supernatant was decanted and pellets were washed with adding 70% ethanol (200 µL), and centrifuged for 5 minutes at 1400 rpm. Then the liquid was removed completely, the pellet was air-dried and re-suspended in 50 µL of TE buffer. Finally, the DNA samples were stored at -20ºC.

Water-saturated ether and 1.25M NaCl method

Reagents:

a) Liquid nitrogen

b) Extraction buffer: 100 mM Tris-HCl (pH 8), 1.5 mM NaCl, 50 mM EDTA (pH 8), 0.5% 2-mercaptoethanol, 4 % (w/v) CTAB (added just before use), 1% PVPP (polyvinyl poly-pyrrolidone) 0.5% 2- mercaptoethanol

c) Chloroform-isoamyl alcohol (24:1)

d) Phenol-chloroform-isoamyl alcohol (25:24:1)

e) TE buffer (pH 8): 10 mM Tris-HCl, 1 mM EDTA

f) 10 mg/mL RNase A (free of DNase)

g) Water-saturated ether

h) Ethanol

i) 5 M NaCl

j) 70% ethanol

Protocol of genomic DNA isolation:

Total genomic DNA was extracted using the hexadecyltrimethylammonium bromide (CTAB) method as described by Saghai-Maroof et al. (1984) by employing a purification step to remove polysaccharides utilizing water-saturated ether and 1.25 M NaCl (Cheng et al. 2003). Fresh leaves (300mg) were grounded to a fine powder in liquid nitrogen, followed by the addition of 900µL extraction buffer (CTAB 2x 1.4 M NaCl, 20 mM EDTA, 100nM Tris-HCl pH 8.0, polyvinylpolypyrrolidone and 0.2% 2-mercaptoethanol), which was preheated to 65^0C. The mixture was incubated at 65^0C for one hour with intermittent gentle vortexing. The homogenate was cooled to room temperature and 600 µL chloroform: isoamyl alcohol (24:1) solution was added and mixed well. The mixture was then centrifuged at 10000 rpm for 20 munites at 4^0C and the supernatant was collected. After that, 20µL of 5 M NaCl (final concentration of 1.25-1.3 M) and 60 µL water-saturated ether were added with the top aqueous solution and mixed well by using gentle inversion and then centrifuged at 10000rpm for 10 minutes at 4^0C. The top ether layer was discarded and the bottom aqueous layer was poured from the slot into a new Eppendorf tube. Equal volume (approximately 150 µL) cold isopropanol (-20^0C) was added with the DNA solution to precipitate the DNA. The mixture was frozen at-20^0C for 30 minutes to accentuate the precipitation of DNA. Then it was spun at 8000rpm for 20 minutes at 4^0C to pellet the DNA and washed with 70% alcohol. After having been washed, dried and treated with RNase (10µg/ ml), the DNA pellet was dissolved in 50 µL of TE (Tris- HCl 10ml and EDTA 1mM pH8.0) buffer and stored in -20^0C.

Notes

(i) With this treatment, polysaccharides were concentrated in the interphase layer while the DNA was still dissolved in the bottom aqueous phase. Most polysaccharides could be removed by discarding the gel-like interphase.

(ii) To prevent contamination of the bottom aqueous layer by the interphase, the mass should be handled carefully

(iii) Ether is highly flammable and can cause drowsiness. All manipulations involving ether should be performed in a well-ventilated fume hood.

(vi) High concentration of NaCl may inhibit enzyme activity; thus, the DNA solution purified by this method should be deposited and washed with 70% ethanol to remove residual salt.

RAPD analysis

The DNA was amplified using the RAPD primers kits A, B, C and E (Operon Technologies, Inc., Boulevard CA, USA), following the protocol of Williams et al. (1990) with a few modifications. The amplification reactions were accomplished using a final volume of 13 µL, containing Tris-HCl 20mM (pH 8.0), KCl 50 mM, $MgCl_2$ 1.5 mM, BSA 1 mg, 300 mM dNTP (dATP, dCTP, dGTP and dTTP), 22.5 ng primer, 0.2 µL Taq DNA polymerase and approximately 10ng genomic DNA. A 50 µL mineral oil was added to this volume after placing the samples into the thermocycler plates. DNA Ladder 100 bp was used as the standard DNA. Amplification reactions were allowed to perform in a DNA thermocycler (MJ Research) for 40 cycles after an initial denaturation at 92^0C for 2 minutes. In each cycle denaturation for 1 minute at 94^0C, annealing for 1minute at 35^0C and elongation by Taq DNA polymerase at 72^0C for 2 minutes was performed with a final extension step at 72^0C for 5 minutes after the 40 cycles. Negative control was used initially to check the fidelity of the PCR reaction. Negative control without template sometimes resulted in nonspecific bands which disappeared after adding the template. For further reactions, negative controls were not used. The amplified DNA fragments were separated by electrophoresis in 1.5% agarose gels in 1xTBE (Tris-borate EDTA, pH 8.0) buffer, stained with 90 µL ethidium bromide. EDTA was used for electrophoresis and for preparing gels. Wells were loaded with 13 µL of reaction volume and 2.5 µL of loading buffer (sucrose and bromo-cresol green dye) together. Electrophoresis was conducted approximately 4 hours at 90 volts, and at the end, the gels were visualized and photographed on an ultraviolet light transilluminator.

RESULTS AND DISCUSSION

Very young leaves were not useful for isolation of DNA as those were burnt due to use of various extraction chemicals or on drying. Similarly, highly matured leaves were not use either as those were highly fibrous and rich in phenols and polysaccharides. The protocol using recently matured leaves resulted in dull white translucent DNA pellets, which were easily dissolved in TE buffer. Prakash et al. (2002) found the similar results from isolating genomic DNA of Psidium guajava L. The purified DNA using the WSE protocol was homogenous and not degraded. It was successfully amplifiable using Taq DNA polymerase.

There were no fragments in the “no template” control while in the “positive control”, the similar pattern of fragments were amplified in every PCR reaction indicating contamination free PCR ingredients and a consistent protocol (Figure 6). The strategy to obtain reproducible fragment profiles of mango DNA involved reactions in which various components of the reaction mixture were varied. Large

changes in concentrations (i.e. in order of magnitude) of template DNA did affect the amplification, too little DNA resulted in either reduced or no amplification of small fragments. As the DNA concentration was increased, the number of fragments appearing on the gels was increased, while too much DNA either produced a smear effect or did not amplify any fragments (Figures 2, 3 and 5).

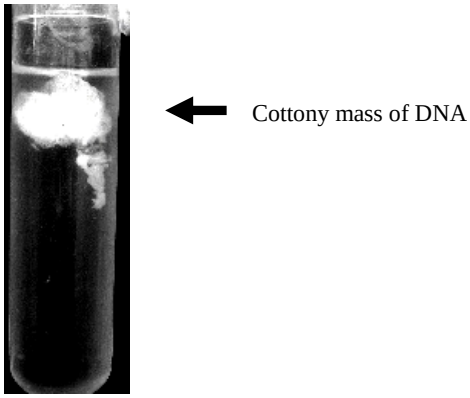

Figure 1. White DNA pellet of fresh mature leaf of *Mangifera indica* formed in isopropanol after polysaccharides were removed.

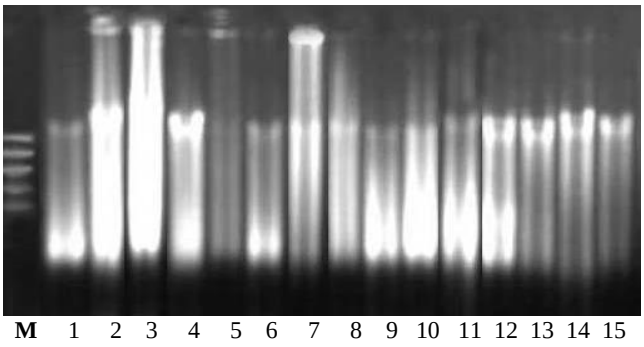

Figure 2. Quality test of DNA samples of 15 *Mangifera* spp. on 1% agarose gel. Lane M – λ DNA; Lane 1-15: Fresh mature leaf samples (DNA extracted by using CTAB Method). Samples serious smear and DNA concentrated on the well and appeared bright glow when placed under the UV light.

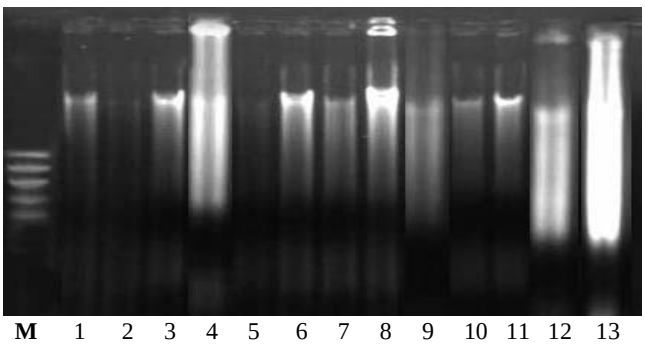

Figure 3. Quality test of DNA samples of 13 *Mangifera indica* on 1% agarose gel. Lane M – λ DNA; Lane 1-13: Fresh mature leaf samples (DNA extracted by using SDS Method). Samples serious smear and DNA concentrated on the well and appeared bright glow when placed under the UV light.

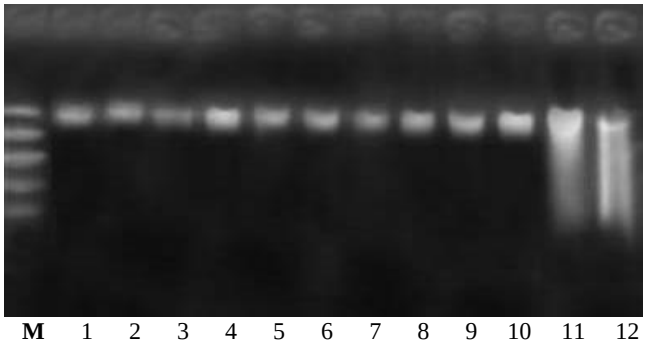

Figure 4. Quality test of DNA samples of 12 *Mangifera indica* on 1% agarose gel. Lane M – λ DNA; Lane 1-10: Fresh mature leaf samples; Lane 11: Withered old leaf; Lane 12: Frosted old leaf (DNA extracted by using Water-Saturated Ether with NaCl method).

Compared with two protocols (SDS and CATB), the WSE method removed polysaccharides efficiently before DNA precipitation. White DNA pellets formed (Figure 1) and were quickly soluble in TE buffer. The $A_{260/280}$ ratios ranged from 1.7 to1.9, and the $A_{260/230}$ ratio was greater than 2. The absence of a peak at 270 nm indicated that residual phenols were removed. DNA samples can be stored at 4^0C for 1.5 years. Results of the agarose gel test and PCR or RAPD analysis indicated that polysaccharides had been efficiently removed and the DNA quantity had been enhanced (Figures 2-6)**.**

Two classic DNA extraction methods: CTAB (Saghai-Maroof et al. 1984) and SDS (Doyle and Dolye 1990) were inefficient in removing polysaccharides (Figures 2 and 3)**.** Several modified DNA protocols that remove poly-saccharide have recently been reported (Fang et al. 1992; Moller et al. 1992; Luro et al. 1995; Cruz et al. 1997; Porebski et al. 1997). All were unsuccessful in removing polysaccharides from crops of *Mangifera indica*, *Citrus* spp. and other fruit crops. Isolating high-quality DNA for RFLP analysis from some materials, such as withered and old frosted *Citrus* spp. leaves, was difficult. DNA samples were hyaloplasm gel-like (almost insoluble in TE buffer) (Cheng et al. 2003); $A_{260/280}$ ratios were always less than 1.5; and a peak of 270 nm corresponding to the peak of a combination of phenol and polysaccharides, was usually scanned (Tesniere and Vayda 1991). When tested on 1% agarose gel, it was observed that DNA samples with severe smear and the DNA concentrated on the well, appeared brightly glow when placed under the UV light (Figures 2 and 3). Conducting PCR analysis or enzyme digestion was difficult because of polysaccharides inhibited enzyme activity. DNA samples showed minimum number of polymorphic bands with maximum smearing and failed to create amplification with primer (Figure 5). Fang et al. (1992), Porebski et al. (1997), Schlink and Reski (2002) and Sharma et al. (2002) reported similar results from the materials of fruit crops. Therefore, the modified protocol should be used for fruit crops plant like *Mangifera* spp. and other tropical and subtropical woody fruit crops to avoid the contamination of DNA samples from high concentration polysaccharides level.

The data on the DNA contents of sixty mango genotypes (Table 1) showed that on an average, there had been 70-90% increase in the DNA contents of the studied materials over the average (1.012 µg/µL) of SDS method. Compared with the three studied DNA extraction protocols of mango, it was found that WSE method with NaCl had the highest value of average percentage (85.44%) in DNA contents of the mango genotypes. In this method, MI09 had the highest amount of DNA content (11.283 µg/µL), which was closely preceded by MI28 (10.450 µg/µL) and MI27 (10.217µg/µL), while the least amount of DNA content was recorded in MI03 (5.05 µg/µL). In the case of CTAB method, the average percentage of DNA content of mango was 71.79%. MI04 had the highest amount of DNA content (5.850 µg/µL) followed by MI94 (5.516µg/µL) and MI95 (5.05 µg/µL).

The minimum amount of the DNA contents were recorded in MI88 (2.017µg/µL). On the contrary, SDS showed the average DNA content (1.012 µg/µL) in the studied mango genotypes. In this method, MI61 had the highest DNA content (1.767µg/µL) but MI82 had the lowest DNA content (0.30µg/µL).

The WSE with NaCl method removed polysaccharides efficiently before DNA precipitation. White DNA pellets formed (Figure 1) and were quickly soluble in TE buffer. The DNA samples could be stored at 4^0C for 1.5 years. Results of the agarose gel test and PCR or RAPD (Figures 4 and 6) analyses indicated that polysaccharides had been efficiently removed and DNA quality had been enhanced (Figure 4).

Classic CTAB (Saghai-Maroof et al. 1984) and SDS (Doyle and Dolye 1990) protocols, when combined with the NaCl and water-saturated ether treatment, produced satisfactory results. In addition, the concentration of the DNA samples were too low for RAPD analysis. Those problems were resolved in the molecular analyses of *Mangifera indica* and other fruit crops using the studied modified DNA extraction protocol.

Molecular marker is powerful tool over conventional fruit breeding. Breeders occasionally find interesting mutants under extreme environmental conditions or on some genetically abnormal phenotypes (Cheng et al. 2003). Nonetheless, vigorous tissue and chilling equipment were unavailable, which limited the extraction of DNA according to CTAB and SDS method.

Table 1. Variation of the DNA contents in 60 mango genotypes in three different extraction methods (SDS, CTAB, WSE method)

Genotypes	DNA concentration (µg/µL)			Genotypes	DNA concentration (µg/µL)			Genotypes	DNA concentration (µg/µL)		
	SDS	CTAB	WSE		SDS	CTAB	WSE		SDS	CTAB	WSE
1. MI01	0.933	4.167	8.783	21. MI38	0.717	4.633	5.167	41. MI74	0.783	3.667	5.633
2. MI02	0.733	4.000	5.983	22. MI39	0.850	2.900	5.667	42. MI75	1.433	4.000	6.200
3. MI03	0.667	3.467	5.050	23. MI40	0.450	2.383	5.950	43. MI77	1.300	3.083	6.333
4. MI04	1.40	5.850	8.817	24. MI41	0.750	2.500	5.150	44. MI80	0.917	2.700	5.650
5. MI08	1.00	4.650	5.750	25. MI43	1.067	2.717	5.333	45. MI81	0.567	3.283	6.433
6. MI09	1.133	4.667	11.283	26. MI44	0.833	2.867	5.483	46. MI82	0.300	2.533	5.267
7. MI12	1.533	3.567	5.300	27. MI45	0.550	2.93	5.800	47. MI83	0.817	4.150	8.600
8. MI16	0.817	3.433	8.500	28. MI46	0.583	4.650	5.667	48. MI84	1.417	3.167	7.217
9. MI19	0.717	2.900	7.450	29. MI47	0.583	4.600	7.133	49. MI85	1.033	2.817	6.500
10. MI20	0.850	2.417	5.750	30. MI48	0.717	2.333	6.033	50. MI86	0.833	3.400	6.633
11. MI21	1.300	2.417	5.750	31. MI49	1.033	3.367	5.383	51. MI88	1.300	2.017	4.900
12. MI22	0.450	4.300	6.567	32. MI50	1.267	4.517	6.767	52. MI90	0.467	3.050	5.183
13. MI23	0.550	4.133	9.617	33. MI51	1.067	3.583	6.450	53. MI91	1.050	2.816	6.416
14. MI24	1.300	4.200	10.116	34. MI52	0.800	3.583	5.867	54. MI92	0.883	3.050	6.100
15. MI25	1.517	3.633	9.467	35. MI54	1.200	3.400	8.583	55. MI93	1.400	2.150	5.083
16. MI26	1.583	4.533	9.350	36. MI58	1.567	2.883	6.300	56. MI94	1.517	5.516	9.867
17. MI27	1.717	4.700	10.217	37. MI60	1.367	3.200	6.167	57. MI95	1.350	5.050	9.433
18. MI28	1.417	4.483	10.450	38. MI61	1.767	3.567	8.617	58. MI96	0.417	3.567	4.867
19. MI29	0.933	4.367	8.367	39. MI64	1.150	4.350	7.883	59. MI97	1.183	3.917	9.633
20. MI33	1.200	2.300	5.417	40. MI70	0.917	4.150	8.833	60. MI98	0.750	3.950	4.817
								Range	0.30-1.767	2.017-5.850	5.050-11.283
								Mean	1.012	3.587 (71.79%)	6.951 (85.44%)

Note: Data in the parentheses indicate increase percentage of DNA concentration over the average of SDS method

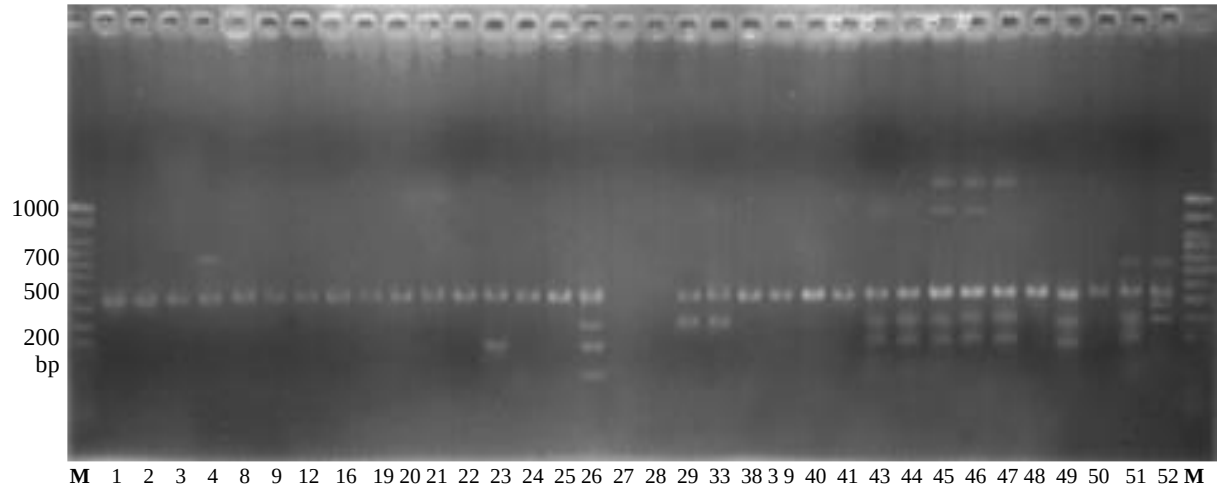

Figure 5. Analysis of 34 samples of *Mangifera indica*, by using RAPDs with OPC-12 primer. M, 20 bp ladder. DNA bands separation were not good (where DNA samples were used, extracted by using CTAB Method).

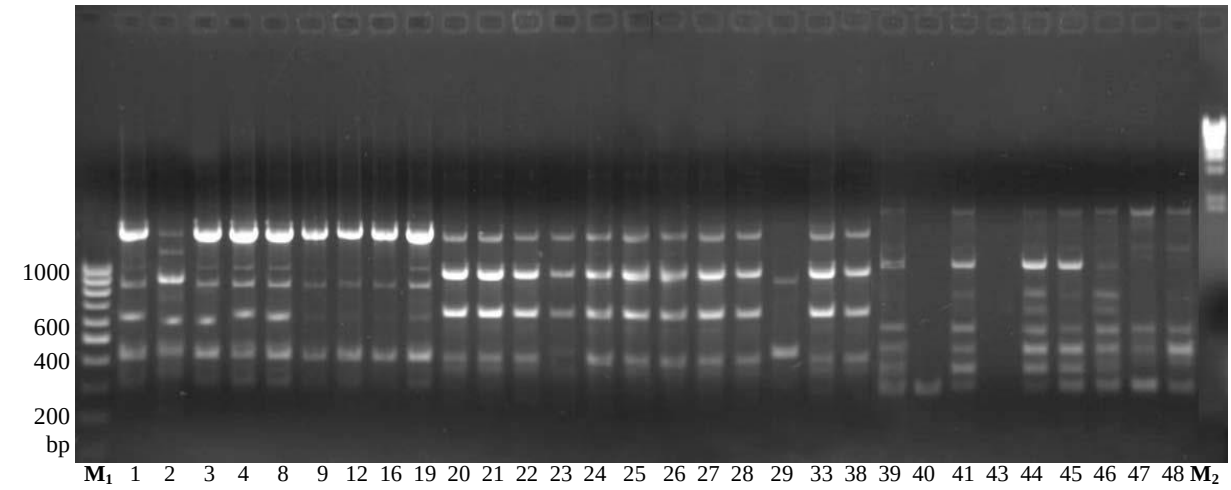

Figure 6. Analysis of 30 samples of *Mangifera indica*, by using RAPDs with OPC-12 primer. M_1 100 bp ladder & M_2 λ - DNA. DNA bands were properly separated (where DNA samples were used, extracted by using Water-Saturated Ether with NaCl method).

CONCLUSION

Using the modified protocol water-saturated ether with NaCl, the DNA was isolated from several tissues including matured, withered and frosted leaves, but the quality of DNA isolated from recently mature leaves was high enough to perform DNA marker analyses (Figure 7). The protocol has been performed in our laboratory since 2007. In the past 3 years, more than 600 DNA samples have been extracted from different developmental stages of mango leaves. Recently, good quality DNA samples were obtained from old leaves of other tropical and sub-tropical fruit crops. Results also proved the reproducibility, reliability, and practicality of this customized protocol.

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