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Efficient DNA Isolation and Electrophoretic Methods for Molecular Analysis of Sweet Potato

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ABSTRACT

DNA isolation from sweet potato was a very difficult and laborious process due to the high content of polysaccharide and phenols which make it useless for molecular analysis like restriction digest, PCR amplification and hybridization. In recent years, PCR based DNA markers provide a powerful tool for genetic analysis, gene mapping and for breeding programme because of their simplicity and easy handling. DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. Hence, the present study highlight a simple, cost effective CTAB (Cetyl trimethyl ammonium bromide) based protocol for isolation of contaminant free DNA which preclude the use of proteinase K and toxic chemicals like phenol. Purity of the pelleted DNA was checked using spectrophotometric reading at A260:A280 as well as running on agarose gels and amplified using RAPD, ISSR and SSR markers. The proposed method makes use readily available reagents and thus provides an alternative to the use of commercial DNA isolation kits. Better resolution in more number of bands was observed in PAGE gels for SSR, RAPD and ISSR markers. This procedure is highly sensitive, avoids unspecific background staining without loss of contrast and detects up to nanogram quantities of DNA.

Key words: DNA extraction, ISSR, PAGE, RAPD, SSR markers, Sweet potato.

INTRODUCTION

Sweet potato (Ipomoea batatas (L) Lam), a member of the morning glory family, is the seventh most important crop in the world and a major source of food as well as a staple diet in developing. It was recommended as one of the best vegetable by World Health Organization and as best food by Centre for Science in Public Interest. This tropical American plant is considered to be a food security crop due to its long history of saving life during famines. In addition to utilization as food, it serves as a rich source of Vitamin A, fodder for animals and also processed into a variety of products viz., snacks, starch, liquor, flour.

The breeding of sweet potato is mostly through hybrid or polycross breeding and hence the screening for cross incompatibility is a requisite which is very time consuming. In recent years, PCR (Polymeraase Chain Reaction) based DNA markers provide a powerful tool for genetic analysis, gene mapping and for breeding programe because of their simplicity and easy handling. DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. A number of different molecular assays have been applied in sweet potato like Random Amplified Polymorphic DNA (Zhang et al., 1998), Inter Simple Sequence Repeat (Hu et al., 2003), Simple Sequence Repeat (Hu et al., 2004), Amplified Fragment Length Polymorphism (Zhang et al., 2004), Selective Amplification of Microsatellite Polymorphic Loci (Tseng et al., 2002), and DNA Amplification Fingerprinting (He et al., 1995). The success of any molecular assays relies on the availability of high quality PCR amplifiable DNA. Many plant molecular biology techniques are time consuming and labor intensive which is particularly true in case of DNA isolation. Sweet potato being rich in polysaccharides and polyphenols, isolation of pure DNA for PCR amplification often becomes difficult.

We have developed a new protocol based on Doyle and Doyle's (Doyle & Doyle, 1987) original procedure with few modifications for yielding clear DNA devoid of proteins, polysaccharides and RNA and compared it with modified Dellaporta method (Dellaporta et al., 1983).

MATERIALS AND METHODS

Plant materials:

Two clones - 'S-1' (white fleshed clone) and 'ST-14' (orange fleshed clone) were selected from the germplasm maintained at Central Tuber Crops Research Institute, Trivandrum, Kerala, India. Two separate DNA extractions were carried out for both clones. The extraction buffer consisted of ingredients as shown in Table 1. The extraction buffer was prewarmed at 65 0C before use and 15 mL was aliquoted equally into an oak ridge tube.

For our modified protocol which was based on Doyle & Doyle's original procedure, 1 g

of young leaves devoid of vein portions were finely powdered using liquid nitrogen in a mortar and pestle. This was quickly transferred into the prewarmed buffer and shaken well. The homogenate was then incubated at 65 0C for 60 min in a shaking water bath. This was followed by an addition of equal volume of chloroform and isoamyl alcohol (24:1) and centrifugation for 10 minutes at 15,000 rpm at 4 0C. The supernatant was transferred to fresh centrifuge tube and genomic DNA precipitated with 0.5 volume of ice cold isopropanol. The precipitated DNA was dissolved in 400 µL sterile double distilled water and transferred to a 1.5 mL Eppendorf tube. To this, 5 µL RNAse (10 mg/mL) was added and incubated for 1 hour at 37 0C followed by a mixture of Poly Ethylene Glycol (PEG) (2%): NaCl (1.5mM) solution (Kamal et al., 2008). The above mixture was subjected to two extractions with equal volume of chloroform and isoamyl alcohol (24:1) for two times. The DNA was precipitated using double the volume of ice cold ethanol after centrifugation at 12,000 rpm for 10 min. The pellets were washed in 70 % ethanol to remove the traces of PEG, NaCl and sheared pieces of DNA. Finally the pellets were air dried at 37 0C in an oven for two hr. The air dried pellets were dissolved in 100 µL of sterile double distilled water.

For the second protocol (Dellaporta et al., 1983) 1 g of fresh leaf sample was homogenised using liquid nitrogen in a pre-chilled mortar. The fine powder was dissolved in 15 mL of extraction buffer containing 30 µL of β- mercaptoethanol, PVP (Polyvinylpyrollidine) and 1 mL of 20 % SDS (Sodium dodecyl sulphate). The tubes were thoroughly mixed and incubated at 65 0C for 60 min in a water bath. 5 mL of 5 M potassium acetate (pH 5.5) was added to each tube and kept for 20 min at 4 0C in ice. The mixture was centrifuged at 15,000 rpm for 15 min and 10 mL of ice cold isopropanol was added to the supernatant and kept for incubation at 4 0C for 30 min. The solution was centrifuged at 15,000 rpm for 15 min and the pellets were dissolved in sterile double distilled water. The DNA solution was transferred to 2 mL eppendorf tube and treated with RNAse (10mg/mL) for 1 hr at 37 0C and 1 mL of chloroform:isoamyl alcohol (24:1) was added and centrifuged at 12,000 rpm for 15 min. The aqueous phase was transferred to another eppendorf tube with out disturbing the inter phase and ice cold ethanol was added to precipitate the DNA. The precipitate was centrifuged at 12,000 rpm for 10 min and the supernatant discarded. The pellet was washed in 70 % ethanol, air dried and finally dissolved 100 µL of sterile double distilled water.

PCR amplification:

The genomic DNA obtained was separated on an agarose gel (0.8 %) and quantified using a spectrophotometer at A260:A280. The DNA was subjected to RAPD, ISSR and SSR marker analysis and the amplified products were separated on agarose and denaturing polyacrylamide gels.

The PCR amplification was performed in a 20 µL reaction mixture containing 20 ng genomic DNA, appropriate quantities of forward and reverse primers, dNTPs, 1X buffer (10mM Tris HCl (pH 8.3), 1.5mM MgCl2 and Taq DNA polymerase. The PCR amplification was performed on an Eppendorf Thermal Cycler with a PCR profile for all

the markers (Table 2). Table 3 shows the detailed list and sequences of RAPD, ISSR and SSR marker used in the study. A volume of 8µl loading buffer (98 % formamide, 10mM EDTA, 0.005% each of xylene cyanol and bromophenol blue as tracking dye) was added to each of the amplified products and denatured at 95 0C for 5 minutes, snap cooled using ice.

DNA detection:

The amplified PCR products were separated and detected on a 3 % agarose gel incorporated with Ethidium bromide (0.1 %) and run on a Biorad (Biorad Laboraratories Inc., CA, USA) submarine system. The corresponding images were captured using Alpha imager 1100-genetic analyzer. The same samples were also resolved on a urea (7M) based denaturing polyacrylamide gel (5 %) which was finally silver stained and the expression of the bands scanned manually using a scanner.

RESULTS

Several methods of DNA isolation are available and are variants of a few principal protocols such as Dellaporta et al. (1985), Doyle and Doyle (1987) etc. The DNA extracted based on our modified protocol yielded high quality (Table.4) and quantity of high-molecular-weight DNA devoid of contaminants and could be amplified by means of PCR. Figure 1 and 2 shows the nucleic acids obtained from the different extraction protocols on 0.8 % agarose gel. Browning of the end product was not at all present in both the protocols. This might be due to the addition of increased concentration of PVP (4 %).

PCR analysis of DNA isolated by both the protocols using RAPD, ISSR and SSR markers were separated on agarose and PAGE gels. The Dellaporta based protocol showed smearing on the lanes with less number of amplified products. On the other hand, the samples from Doyle & Doyle showed clear and crisp bands. Comparing the two techniques- agarose and PAGE, the resolution of the size markers as well as amplified fragments showed significant differences in their separation on the two gels for SSR markers. PAGE gel for microsatellite amplified products revealed that, of the 27 primers screened, 23 showed amplification of which 11 were polymorphic and 12 were monomorphic. All the amplified products ranged between 100 to 200 bps and the bands ranged from 2 to 12 numbers (Fig 3 & 4). Agarose gel profiles of the same product showed a maximum of 2-3 smudged bands between 100-200 bp where no clear distinction of bands was observed here. Generally, both agarose and polyacrylamide gels are used for DNA analysis. Similarly the RAPD and ISSR (Fig 5) generated fragments also varied in their number in both the gels.

DISCUSSION

Interference of polyphenols which reduce the maintenance time of DNA thereby rendering it useless for research applications have been demonstrated by Katterman and Shattuck (1983). Neutralization of polyphenols can be achieved by using high

levels of PVP and β- mercaptoethanol in the lysis buffer (Dehestani & Kezemi, 2007). One major problem encountered in both the protocols was the severe polysaccharide contamination in the end product which prevented the dissolving of nucleic acid and hence the DNA remained in the wells during electrophoresis. Electrophoretic analysis of the modified Dellaporta protocol products revealed severe lighting of the entire lane. The RNA concentration was found to be very high in this method. One reason for this could have been the undissolved polysaccharide mass which prevented the action of RNAse as well as the chloroform isoamyl alcohol mix thereby contaminating the nucleic acid with proteins and RNA. This was overcome in the modified method by the addition of 2 % PEG and 1.5M NaCl solution to the polysaccharide rich mass. The spectrophotometer readings at A260: A280 revealed the DNA to be of good quality with a ratio of 1.8 – 2.0. A lesser OD (optical density) value denotes contamination by proteins and polysaccharides (Table 4). Similar results were observed in a study conducted in various tuber crops species (Kamal et al., 2008) where the incubation of the above mixture at 4 0C for up to one hour increased the final yield. Use of NaCl or NaCl combined with detergents such as SDS, CTAB, Sarkosyl etc are usually recommended for the nucleic acid isolation of polysaccharide rich plant as well as fungal cultures (Murray & Thompson, 1989). Addition of NaCl at the isopropanol precipitation level has found to prevent co-precipitation of polysaccharides in Pinus radiata (Crowley et al., 2003). Co-precipitation of contaminants like polysaccharides, the secondary metabolites and phenolic compounds during ethanol precipitation adversely affect the purity and suitability of the isolated DNA for future molecular use has been reported by various authors (Dellaporta et al., 1983; Do & Adams, 1991). Acidic polysaccharides are found more problematic than neutral polysaccharide as they inhibit the activity of enzymes like polymerases, ligases and endonucleases (Do and Adams, 1991; Pandey et al., 1992; Fang et al., 1992; Weishing et al., 1995; Scott & Playford, 1996). Dilution of the DNA samples has been recommended as an effective way to overcome this problem though, excessive dilution also dilutes the DNA which makes it unsuitable for Southern analysis (Pandey et al., 1992).

The level of polymorphism detected after PCR analysis is found to vary with the detection method used (Reddy et al., 2002). PAGE in combination with radioactivity was shown to be most effective followed by silver staining of PAGE and then agarose-ethidium bromide system of detection. Sambrook & Russel (2001) have reported that DNA of same size can differ in mobility by up to 10 % in agarose. Moreover, the volume of PCR mixture used to load in agarose gel is comparably high (10 µL) with that of PAGE (3 µL). Again, agarose gels could not be stored for long and even if stored the ethidium bromide fades away rendering the bands invisible. Contrary to this, the PAGE gels can be sandwiched in blotting paper and stored indefinitely. Similar research by comparing the agarose and PAGE gels of RAPD fragments were reported by Stift et al (2003). The study put forward that advantages of PAGE gels such as production of more number of clearer bands, need of less DNA, better cost efficient as much as 60 % compared to agarose and avoidance of mutagenic agents like EtBr. The silver staining procedure is more sensitive, displaying bands which had not been detected by ethidium bromide. Essentiality of the DNA to be free of proteins for the silver staining

procedure has been demonstrated by Antonio et al (1990).

CONCLUSION

In the genetic improvement process, it is desirable to use molecular markers for screening of accessions, choosing of parents and selection of progeny. The present study was aimed at developing a suitable protocol for the isolation of sweet potato DNA free of polysaccharide and phenolic contaminants for molecular analysis. The presence of certain metabolites and polyphenols can hamper and reduce the yield and purity of extracted DNA (Loomis, 1974; Porebski et al., 1997). Our protocol involves the use of increased concentration of PVP and NaCl which are nontoxic and eliminates the use of phenol whose caustic properties pose health hazards. The isolated DNA has proved amenable to polymerase chain reaction (PCR) amplification. The proposed method makes use readily available reagents and thus provides an alternative to the use of commercial DNA isolation kits. Better resolution in more number of bands was observed in PAGE gels for SSR, RAPD and ISSR markers. This procedure is highly sensitive, avoids unspecific background staining without loss of contrast and detects up to nanogram quantities of DNA.

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References

Antonio MG, Nedia SN and Carlos MM (1990). An improved silver staining procedure for Schizodeme analysis in polyacrylamide gradient gels. Memorias do Instituto Oswaldo Cruz, Rio de Jeneiro. 85(1):101-106.

Crowley TM, Muralitharan MS and Stevenson TW (2003). Isolating conifer DNA: A superior polysaccharide elimination method. Plt. Mol. Biol. 21: 97a-97d.

Dehastani A and Tabar SKK (2007). A rapid efficient method for DNA isolation from plants with high levels of secondary metabolites. Asian J. Plt. Sci. 6(6): 977-981.

Dellaporta SL, Wood J and Hicks JB (1983). A plant DNA mini-preparation: version II. Plt. Mol. Biol. 1: 19-21.

Do N and Adams RP (1991). A simple technique of removing plant polysaccharides contaminants from DNA. Biotechniques. 10: 162-166.

Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure from small quantities of

fresh leaf tissue. Phytochem. Bull. 19: 11-5.

Fang G, Hammer S and Groumet R (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. Biofeedback. 13: 52-54.

Kamal S, Ajay KM and Raj SM (2008). A simple and efficient method for extraction of genomic DNA from tropical tuber crop. Afr. J. Biotech. 8: 1018-1022.

Katterman FRH and Shattuck VL (1983). An effective method of DNA isolation from the mature leaves of Gossypium species that contains large amounts of phenolics, terpenoids and tannins. Prep. Biochem. 13: 347-359.

Loomis MD (1974). Overcoming problems of the phenolics and the quinines in the isolation of plant enzymes and organelles. Meth. Enzym. 31: 528-544.

Murray MG and Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucl.Acid. Res. 8: 4321-4325.

Pandey RN, Adams RP and Flournoy LE (1996). Inhibitions of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. Plt. Mol. Bio. 14: 17-22.

Porebski S, Balley LG and Baum BR (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharides and polyphenols component. Plt. Mol. Bio. 15:8-15.

Reddy PM, Sarla N and Siddiq EA (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphy. 128:9-17.

Sambrook J and Russell DW (2001). Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA, p.6-11.

Scott KD and Playford J (1996). DNA extraction technique for PCR in rain forest plant species. Biotechniques. 20: 974-978.

Stift G, Pachner M and Lelley T (2003). Comparison of RAPD Fragment Separation in Agarose and Polyacrylamide Gel by Studying Cucurbita Species. Cucurbit. Genet. Cop. Rep. 26:62-65.

Weishing K, Nybom H, Wolff K and Meyer W (1995). DNA isolation and purification. In: DNA fingerprinting in plants and fungi. CRC Press, Boca Raton, Florida, p.44-59.

He G, Prakash CS and Jarret RL (1995). Analysis of genetic diversity in a sweetpotato (Ipomoea batatas) germplasm collection using DNA amplification fingerprinting. Genome. 38: 938-945.

Hu J, Nakatani M, Lalusin AG, Kuranouchi T and Fujimura T (2003). Genetic analysis of sweetpotato and wild relatives using Inter-Simple Sequence Repeats (ISSRs). Breeding

Science. 53: 297-304.

Hu J, Nakatani M, Mizuno K and Fujimura T (2004). Development and characterization of microsatellite markers in sweetpotato. Breeding Science. 54: 177-188.

Tseng YT, Lo HF and Hwang SY (2002). Genotyping and assessment of genetic relationships in elite polycross breeding cultivars of sweet potato in Taiwan based on SAMPL polymorphisms. Botanical Bulletin of Academia Sinica. 43: 99-105.

Zhang D, Ghislain M, Huaman, Golmirzaie A and Hijmans R (1998) RAPD variation in sweetpotato [Ipomoea batatas (L.) Lam.] cultivars from South America and Papua New Guinea. Genetic Resources Crop Evolution. 45: 271-277.

Zhang D, Rossel LG, Kriegner A and Hijmans R (2004) AFLP assessment of diversity in sweetpotato from Latin America and the Pacific region: its implications on the dispersal of the crop. Genetic Resources Crop Evolution. 51: 115-120.

TABLES

Table 1: Constituents of extraction buffer followed for the different protocols

Constituents (100mL stock)	Dellaporta (100mL working solution)	Modified protocol based on Doyle & Doyle
Tris HCl 10mM (pH 8.0)	5 mL	10mL
EDTA 0.5Mm (pH 8.0)	5 mL	4 mL
NaCl 5M	5 mL	28 mL
PVP	1 %	4 %
CTAB	-	2 %
SDS (20 %)	1 mL	-
β- mercaptoethanol	30µL	10µL

Table 2. PCR cycles for RAPD, ISSR and SSR markers.

MARKER	REACTION MIXTURE	PCR CYCLE
RAPD	2µL of 5µM primer 0.5 µL of 200µM dNTPs 2 µL of 1X buffer 1 µL of 1.5mM $MgCl_2$	94 ^{0}C for 5 min 35 cycles of 94 ^{0}C for 1 min 35 ^{0}C for 1 min 72 ^{0}C for 2 min

	0.3µL of 3U Taq enzyme 12.2µL of sterile water	72 ^{0}C for 5 min
ISSR	5µL of 5µM primer 0.5 µLof 200µM dNTPs 2 µL of 1X buffer 1 µL of 1.5mM $MgCl_2$ 0.3µL of 3U Taq enzyme 10.2 µL of sterile water	94 ^{0}C for 5 min 40 cycles of 94 ^{0}C for 1 min 45 ^{0}C for 1 min 72 ^{0}C for 2 min 72 ^{0}C for 5 min
SSR	2µL of 5µM primer 0.1 µL of 200µM dNTPs 2 µL of 1X buffer 1 µL of 1.5mM $MgCl_2$ 0.1µL of 3U Taq enzyme 13.8µl of sterile water	94 ^{0}C for 5 min 30 cycles of 94 ^{0}C for 1 min 58 ^{0}C for 2 min 72 ^{0}C for 2 min 72 ^{0}C for 5 min

Table 3. Nucleotide sequences of the primers used in this study

Primer	Sequence(5'→3')
RAPD	
S-21	CAGGCCCTTC
S-22	TGCCGAGCTG
S-24	AATCGGGCTG
S-31	CAATCGCCGT
S-36	AGCCAGCGAA
ISSR	
813	CTCTCTCTCTCTCTCTT
818	CACACACACACACACAG
824	TCTCTCTCTCTCTCTCG
825	ACACACACACACACACT
836	AGAGAGAGAGAGAGAGYA
SSR	
IB 242 F R	GCGGAACGGACGAGAAAA ATGGCAGAGTGAAAATGGAAC
IB 3-31 F R	TTCCCTTTCCTTTCCTTCCC ACCCCAAATCCCAACTCCA
IB 324 F R	TTTGGCATGGGCCTGTATT GTTCTTCTGCACTGCCTGGATTC
IB R12 F R	GATCGAGGAGAAGCTCCACA GCCGGCAAATTAAGTCCATC

IB S18 F	CTGAACCCGACAGCACAAG
R	GGGAAGTGACCGGACAAGA
IB 3-28 F	TCGCCTTTCTCTTTGCACC
R	CCCCTCTCTTCTACAACCCTTC
IB R13 F	GTACCGAGCCAGACAGGATG
R	CCTTTGGGATTGGAACACAC
IB 2-30 F	ACGCATAAGGGTATTGGTGAA
R	ACGGAGGATGGTTCAGGTG
IB 316 F	CAAACGCACAACGCTGTC
R	CGCGTCCCGCTTATTTAAC
IB 2-55 F	CGTCCATGCTAAAGGTGTCAA
R	ATAGGGGATTGTGCGTAATTT
IB 3-01 F	CCTTCATCACCTTCCATTCCT
R	CTCCCAGTTAACCAAAACCTG
IB S 11 F	CCCTGCGAAATCGAAATCT
R	GGACTTCCTCTGCCTTGTTG
IB R20 F	CTTCACTCTGCTCGCCATTA
R	GTACTTGGACGGGAGGATGA
IB 2-45A F	TAACCTACGGCTGCATTA
R	TAATGCAGCCGTGGGTTAT
IB 2-66 F	CACACCACCACCATCACC
R	TTGATTTTCCCTTAGGTTTGTG
IB S09 F	GCTGCTCAATCCCTCTCTT
R	GGAACTCGATACAGCGTGGT
IB 297 F	GCAATTTCACACAAACACG
R	CCCTTCTTCCACCACTTTCA
IB S 07 F	GCTTGCTTGTGGTTCGAT
R	CAAGTGAAGTGATGGCGTTT
IB255 F	TGGGCATTCTCATATTTTGCT
R	AAGGACCACCGTAAATCCAA
IB S 10 F	CTACGATCTCTCGGTGACG
R	CAGCTTCTCCACTCCCTAC
IB R03 F	GTAGAGGTGAAGAGCGAGCA
R	CCATAGACCCATTGATGAAG
IB R16 F	GACTTCCTTGGTGTAGTTGC
R	AGGGTTAAGCGGGAGACT
IB2-45B F	TTGCAATACCATGATAACCC
R	TGCGAACTTATTGATCCATAC
IB 3-21 F	AATCCAAATGAGTCATACACC
R	CGAAAAATCTCTGGTTACGTT
IBR 08 F	GGCGACACCTTAGAGTAT
R	CACCCCCTATTCACAA
IB R14 F	CCTATGGCAATTCGGTCACT

R	GGAACATTGCCTACATCTG
IB 2-38 F	CCAGATTATTGCCCACTC
R	CATTATTGTTACCATGCACACT

Table 4. DNA purity ratio of sweetpotato sampled for the two methodologies A (modified) and B (Dellaporta et al., 1983) tested.

SL NO	CLONES	OD value			
		260	280	Ratio A_{260}: A_{280}	(ng/µL)
A					
1	S 1 (white fleshed)	0.064	0.044	1.882	220
2	ST-14 (orange fleshed)	0.065	0.044	1.912	220
B					
1	S 1 (white fleshed)	0.013	0.010	1.3	50
2	ST-14 (orange fleshed)	0.007	0.009	1	45

FIGURES

Fig 1: Purified DNA from modified Doyle and Doyles method

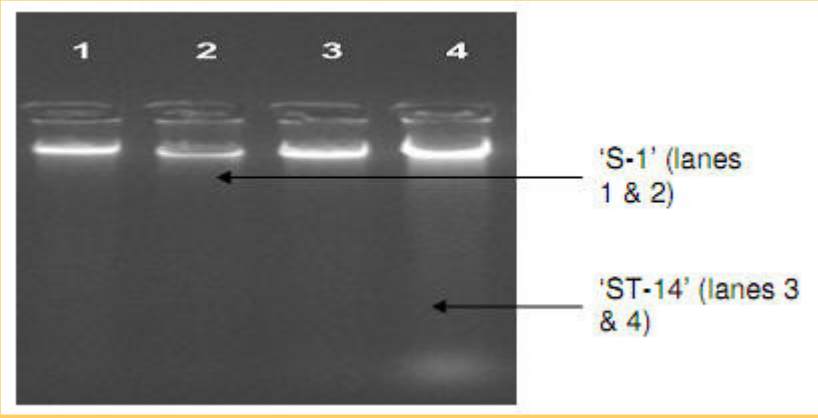


Fig 2: Low quality DNA with impurities obtained from the Dellaporta method. Lanes 1 & 2 shows DNA from 'S-1' while lanes 3 and 4contains 'ST-14'

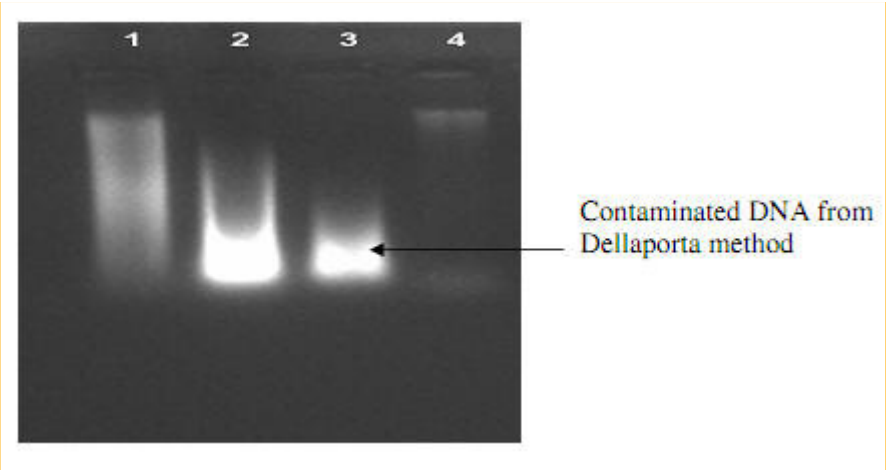
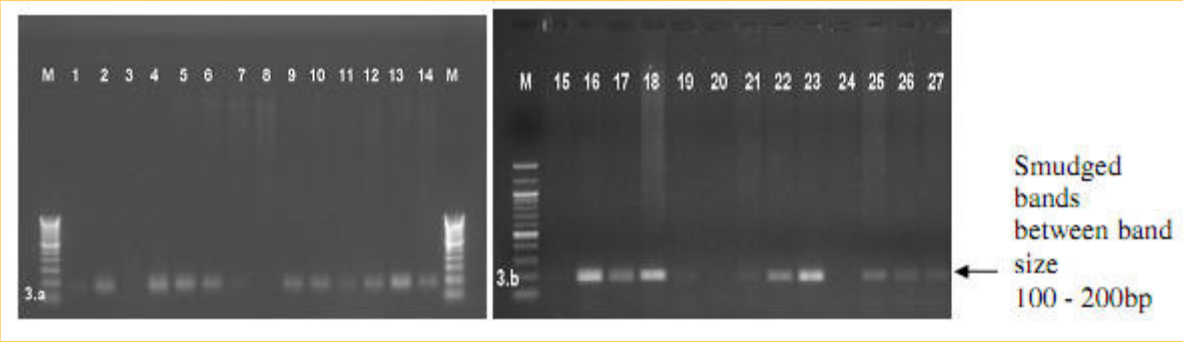


Fig 3 a & b: Agarose gel profile of 27 SSR primer amplified products for the clone 'S-1'.



*Gel 1, Lane 1 to 14 shows amplification products of primer 1 to primer 14 and gel 2, lane 15 to 27 shows amplified products from primer 15 to primer 27

Fig 4: Comparision of 27 SSR amplified products on denaturing polyacrylamide gel (PAGE) for 'S-1' and 'ST-14'.

*Lane 1 to 27 shows amplification products of primer 1 to primer 27 for 'S-1' and 'ST-14' loaded alternately