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Full Length Research Paper

Simplified extraction of good quality genomic DNA from a variety of plant materials

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Depending on the nature and complexity of plant material, proper method needs to be employed for extraction of genomic DNA, along with its performance evaluation by different molecular techniques. Here, we optimized and employed a simple genomic DNA isolation protocol suitable for a variety of plant materials covering *in vitro* grown tender plantlets to relatively complex plant tissues such as field grown mature potato leaves and tubers. Unlike other methods, no detergent was included in the isolation steps. This protocol, based on Dellaporta's method as reported earlier, worked efficiently both at small and miniscale during handling large number of plant materials. DNA yield was found to be in the range of 70 to 120 µg per gram of the plant material; sufficient for most of the molecular techniques. Purity of DNA was checked by A_{260}/A_{280} ratio, and restriction analyses including the isoschizomers *Hpa*II and *Msp*I. The DNA preparations were successfully used in polymerase chain reactions using gene-specific primers for cloning of different genes. Prolonged storage did not affect the quality of the DNA samples. Taken together, this method could be a reliable substitute to frequently used chemical cetyl trimethylammonium bromide (CTAB) and commercial kits-based plant DNA isolation protocols.

Key words: Plant materials, genomic DNA isolation, restriction analyses, *Hpa*II and *Msp*I isoschizomers, gene-specific primers, polymerase chain reaction (PCR), molecular cloning, DNA storage

INTRODUCTION

Isolation of good quality genomic DNA from different plant materials is an important prerequisite for many molecular techniques related to both basic and applied research in the areas of plant molecular biology, crop improvement, biodiversity studies and conservation of genetic materials. Successful implementation of various crop improvement strategies through molecular breeding involves isolation of useful genes and gene regulatory sequences by exploring various plant genetic resources. For this purpose, the genomic DNA preparations should be suitable for restriction cleavage, construction of partial and complete gene library, polymerase chain reactions (PCR). Moreover, the genomic DNA samples are frequently used in Southern blot analyses, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), genome mapping and finger-printing, screening of transgenic lines and other relevant molecular techniques (Clark, 1997; Csaikl et al., 1998; Aljanabi et al., 1999; Sarwat et al., 2006). Apart from quality, overall yield is also important for various applications. However, isolation of good quality DNA for different molecular biological techniques is relatively difficult from plant tissues containing high polysaccharides, various secondary metabolites including polyphenolic compounds, and nucleases. It is also known that the extent of secondary metabolites varies in the same plant tissues with age and the prevailing growth

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Abbreviations: PCR, Polymerase chain reaction; **RFLP,** restriction fragment length polymorphism; **RAPD,** random amplified polymorphic DNA; **CTAB,** cetyl trimethyl ammonium bromide; **PVP,** polyvinylpyrolidone; **GWD,** α-glucan water dikinase.

conditions. Some macromolecules are often complexed with nucleic acids which become unsuitable for further molecular biology techniques (Guillemaut and Maréchal-Drouard, 1992). DNA isolation from somatic storage tissues such as potato tuber is difficult because the genomic DNA usually gets contaminated with poly-saccharides. They co-precipitate with DNA during alcohol precipitation to form highly viscous solution making DNA unsuitable for digestion by restriction enzymes and Southern blot hybridization (Do and Adams, 1991; Wulff et al., 2002). During homogenization of plant tissues rich in polyphenols, the phenolic compounds can become oxidized and irreversibly bind to the proteins and nucleic acids. This irreversible binding produces a gelatinous material, which is hard to separate from organelles and the DNA becomes unsuitable for PCR amplification and restriction analyses (Aljanabi et al., 1999; Porebski et al., 1997).

Depending on the nature and complexity of the plant materials, different methods have been developed and modified by many laboratories for genomic DNA isolation (Murray and Thompson, 1980; Taylor and Powell, 1982; Dellaporta et al., 1983; Doyle and Doyle, 1987; Doyle and Doyle, 1990; Csaikl et al., 1998; Wulff et al., 2002; Sharma et al., 2008). Literature survey for the last two decades clearly reveals that the cationic detergent, cetyl trimethyl ammonium bromide (CTAB)-based DNA isolation protocols were more frequently used for different plant materials as compared to other protocols. Apart from cationic and/or anionic detergents, different chemicals and biochemicals, such as sodium perchlorate, lysozyme, polyvinylpyrolidone (PVP), pectinases, proteinase K and others were included at different steps for improving efficiency of the DNA isolation protocols. Moreover, a number of commercial kits and column materials have also been introduced for isolation and purification of genomic DNA from different plant materials (Csaikl et al., 1998; HwangBo et al., 2010). In Dellaporta's method as reported in 1983, the detergent sodium dodecyl sulfate (SDS) was used immediately after the cell lysis step, followed by addition of potassium acetate to the crude genomic DNA preparation in order to remove carbohydrates and other bulk impurities. This method was used for the leaf tissues from *Nicotiana, Lycopersicum, Amaranthus, Glycine max and Petunia* species. Later on, this method was adopted and modified for DNA isolation from other plant materials such as spruce needles, mature tree leaves, needles of Silver fir and oak, rice leaves, seaweeds (Guillemaut and Maréchal-Drouard, 1992; Ziegenhagen et al., 1993; Csaikl et al., 1998; Sadasivam and Manickam, 1996; Wattier et al., 2000).

At present, there is growing importance of potato, oilseed crop *Brassica*, and biodiesel crop *Jatropha* in many countries both in terms of basic and applied research. Keeping this in view, we have modified the Dellaporta's method for isolation of total genomic DNA both at small and miniscale. We used a variety of plant materials such as micropropagated potato (*Solanum tuberosum* L.) plantlets, germinating seedlings of oilseed *Brassica* species, the embryo culture raised *Jatropha* plantlets along with field-grown potato leaves and tubers. In other words, from simple and tender plant materials to complex plant tissues were included in this study.

MATERIALS AND METHODS

Plant materials

The micropropagated plantlets of some high yielding Indian potato (S. *tuberosum* L.) cultivars, namely Kufri Chipsona-1 and Kufri Chandarmukhi, were routinely maintained in our laboratory using Murashige and Skoog (MS) basal media. Surface sterile seeds of the *Brassica* cultivars namely Raya sarson, *B. juncea, B. napus* and Chinese sarson were allowed to germinate aseptically on MS basal medium. Seeds of elite varieties of *Jatropha curcas* L. corresponding to our different accessions were procured from the different locations in the state of Punjab, India. The seeds were used for generation of plantlets under aseptic conditions through embryo culture using MS basal medium supplemented with 100 mg/L polyvinyl pyrrolidone (PVP). Four to five weeks old *in vitro* grown potato plantlets, one to two weeks old germinating seedlings of *Brassica* and embryo culture raised *Jatropha* plantlets were used for both small and miniscale genomic DNA isolation. The potato plantlets after proper hardening and acclimatization were grown in the field for 70 to 80 days for collection of mature leaves and tubers, for further use in genomic DNA isolation. Moreover, large number of transgenic potato lines, generated *in vitro,* were used in miniscale DNA isolation.

Bacterial strains and plasmid vectors

For routine molecular cloning experiments pUC19 was used as a vector, and *E.coli* DH5α was used as host. During triparental mating, *E. coli* pRK2013 and *Agrobacterium tumefaciens* LBA4404 strains were used as helper and recipient, respectively. The Patatin promoter-GUS genetic construct was made in the binary vector pBI121 (GenBank Acc No AF485783), and used for transformation of the potato cv. Kufri Chipsona-1.

Genomic DNA isolation procedure

For small scale genomic DNA isolation, 2.0 to 3.0 g of plant material (thoroughly washed using running tap water followed by sterile distilled water in case of the field-grown plant materials) were pulverized to fine powder using mortar and pestle in the presence of liquid nitrogen, then transferred quickly to a conical flask containing 15 ml extraction buffer [50 mM Tris-HCl pH 8.0, 50 mM ethylene diaminetetraacetic acid (EDTA) pH 8.0, 250 mM NaCl, 15% (w/v) sucrose] maintained at 65°C, mixed well and incubated at 65°C for 20 min with intermittent gentle shaking. 5 ml of 5.0 M potassium acetate solution (pH 5.5) was added, mixed vigorously and incubated further on ice for 20 min and then centrifuged at 4000 *g* and 4°C for 25 to 30 min. The supernatant was filtered through two layers of fine cloth and 0.70 volume of isopropanol was added, mixed gently and incubated at -20°C for 2 to 3 h followed by centrifugation at 10000 *g* and 4°C for 15 min. The crude DNA pellet was washed with ice cold 70% ethanol, then air dried and suspended in 500 µl of TE buffer (10 mM Tris-HCl pH 8.0 and 1.0 mM EDTA pH 8.0). For further purification of DNA, DNase-free

Table 1. Spectrophotometric analysis of different plant genomic DNA preparations.

Plant material	Scale of isolation	A_{260}/A_{280} ratio	DNA yield (µg/g of plant material)
Potato cv. Kufri Chipsona-1 (micropropagated plantlets)	Small	1.79	120.0
Oilseed crop *Brassica* (germinating seedlings)	Small	1.95	107.0
Biodiesel crop *Jatropha* (plantlets from embryo culture)	Small	1.77	112.0
Potato cv. Kufri Chipsona-1 (field grown leaves)	Small	1.73	98.0
Potato cv. Kufri Chipsona-1 (field grown tubers)	Small	1.70	87.0
Transgenic potato plantlets	Mini	1.85	70.0

RNase treatment was carried out followed by solvent extraction twice using a mixture of phenol:chloroform:isoamyl alcohol (25:24:1). The DNA was then precipitated using 0.1 volume of 3.0 M sodium acetate (pH 5.5) and 2.0 volume of dehydrated ethanol, which was finally dissolved in 200 to 250 µl of TE buffer and stored at -20°C. For miniscale DNA isolation (0.1 to 0.2 g of plant material), we used small mortar and pestle during grinding with liquid nitrogen. Otherwise, the same protocol was followed by scaling down the necessary steps (usually, after grinding we used 1.0 to 1.5 ml of the extraction buffer). The DNA was finally dissolved in 50 to 60 µl of TE buffer. In the case of suspended macromolecules in DNA solution, small spin was required to remove the impurities; the clarified supernatant was then transferred to sterile microfuge tube and stored at -20°C for further use.

Checking of yield and quality of plant DNA samples

For checking the yield and the presence of dissolved impurities, the genomic DNA samples were used in spectrophotometric analysis for measuring absorbance at 260 nm and A_{260}/A_{280} ratio, respectively. The DNA samples were analyzed by agarose gel electrophoresis to see the possible degradation during isolation. For checking the presence of inhibitory substances, the DNA samples were digested with some restriction enzymes namely *Eco*RI, *Bam*H1, *Sau*3A1, *Hpa*II and *Msp*I (the restriction enzymes, *Taq* DNA polymerase and other enzymes for molecular cloning were procured from Bangalore Genei, India). *Hpa*II and *Msp*I isoschizomers were used to identify genomic DNA methylation.

PCR using different gene-specific primers

Polymerase chain reactions (PCR) were carried out using different cDNA and gene-specific oligonucleotide primers and the individual plant DNA sample as template. The details of oligonucleotide primers (synthesized by Bangalore Genei, India) used in this study are given: the forward primer K20-AI (5′-AGTACCATTCCAG-TTATGAC-3′), the reverse primer M20-AI (5′-CAATAGCATAGT-GATCTTGC-3′) and another reverse primer P20-AI (5′-ACTGGCGTTAGCTCAGATAG-3′) correspond to the potato soluble acid invertase cDNA (GenBank Acc No X70368); the forward primer D20-SPS (5′-CTAAGTTCT-CTCTCGC TGTC-3′) and the reverse primer E20-SPS (5′-ATGACAATTTCGGAAGCATC-3′) correspond to the potato sucorose-phosphate-synthase (SPS) cDNA sequence (GenBank Acc No X73477); the forward primer A20-R1 (5′-TTCATCGAATTTCTCGAAGC-3′) and the reverse primer B20-R1 (5′-TTAGCCTTGCTCGAATGTCC-3′) correspond to potato GWD (R1 protein) cDNA sequence (GenBank Acc No Y09533); the forward primer PT-F074 (5′-TAATTGACCGGAGACTATAC-3′) corresponds to the Class I patatin gene from potato (GenBank Acc No X87216); the reverse primer US-R6747 (5′-CAAGTCCG CATCTTC ATGAC-3′) was specific to GUS gene (GenBank Acc No AF485783). The forward primer FW-TUA4 (5′-AGCCTTCCAT GAGCAACTCT-3′) and the reverse primer RV-TUA4 (5′-CAGC ACCGACCTCTTCATAA-3′) were specific to the constitutive α-tubulin gene (GenBank Acc No NM_100360). PCR amplification was performed in thermal cycler (Applied Biosystems, USA). The thermal cycling parameters were, 94°C for 1 min, 55°C for 2 min and at 72°C for 3 min for 30 cycles with initial denaturation at 94°C for 1 min 30 s and final extension at 72°C for 5 min. Usually, 1.0 to 2.0 µg of plant genomic DNA was used as template in PCR. Amplified DNA products corresponding to different gene-specific primer pairs were analyzed by agarose gel electrophoresis. The different PCR products were polished with Klenow enzyme, purified, and cloned into the *Sma*I site of pUC19. The cloned DNA inserts were further characterized by PCR using internal primers, and partial sequencing as well.

RESULTS

Isolation of plant genomic DNA, and checking of the yield and purity

We adopted and optimized the protocol for isolation of total genomic DNA and tested it both at small and miniscale using a variety of plant materials such as micropropagated potato (*S. tuberosum* L.) plantlets including transgenic ones, germinating seedlings of oilseed *Brassica* species and the biodiesel crop *Jatropha* plantlets raised through embryo culture technique along with complex plant tissues such as field-grown potato leaves and tubers. The A_{260}/A_{280} ratio of the DNA samples appeared to be in the range of 1.70 to 1.90 indicating the absence of the impurities such as proteins and phenolic compounds. The DNA yield was found to be in the range of 70 to 120 µg per gram of plant material (Table 1) sufficient for different molecular biological techniques. The genomic DNA samples from different plant materials were analyzed in agarose gel. The DNA bands appeared to be compact with negligible smearing indicating little degradation during isolation (Figure 1A, B, C, D). In the case of miniscale genomic DNA pre-parations as shown in Figure 1D, some material appeared to be stuck in the wells indicating the formation of insoluble complex between a small fraction of DNA and other macromolecules.

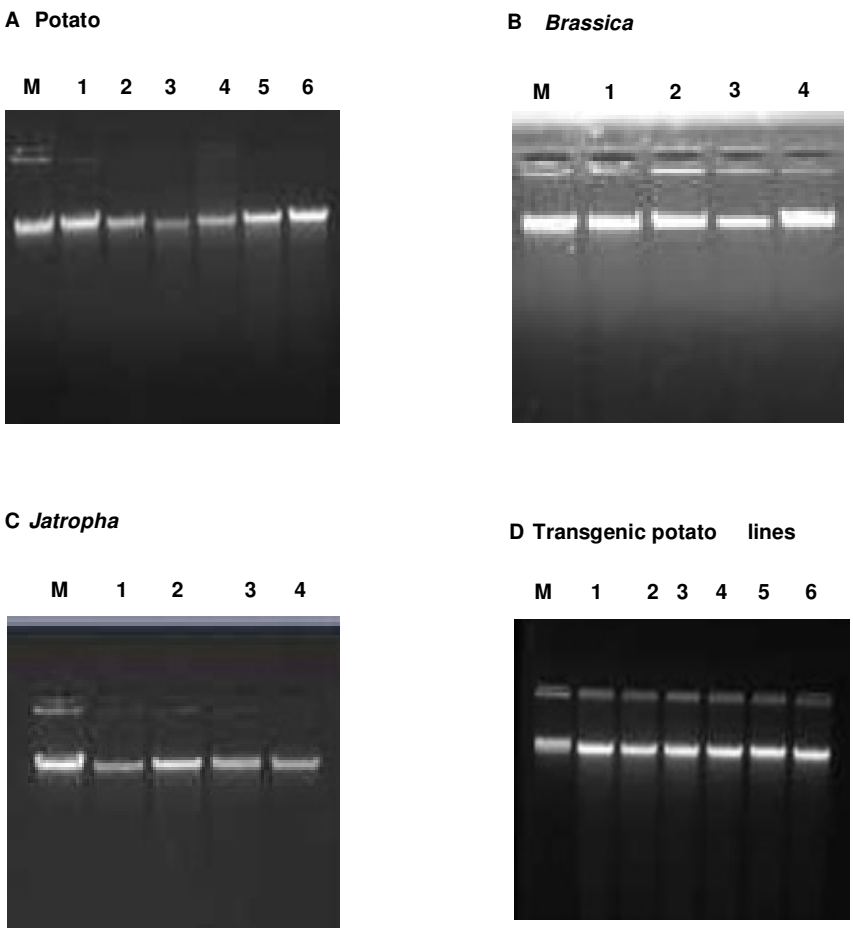

Figure 1. Agarose gel (0.7%) electrophoresis of the genomic DNA isolated from different plant materials. A to C refer to small scale, and D refers to miniscale genomic DNA isolation. M, bacteriophage λ DNA as marker for A to D: A) Lanes 1, 3 and 5, genomic DNA from micropropagated plantlets, field-grown tuber and leaves, respectively of the potato cv. Kufri Chipsona-1; lanes 2, 4 and 6, the same plant materials of the potato cv. Kufri Chandramukhi; B) lanes 1 to 4, genomic DNA from the *Brassica* cultivars namely Raya sarson, *B. juncea, B. napus* and Chinese sarson, respectively; C) lanes 1 to 4, genomic DNA from four high yielding *Jatropha* varieties, respectively; D) lanes 1 to 6, genomic DNA isolated from six transgenic potato plantlets.

Restriction analyses

The quality of the plant DNA preparations were further checked by restriction analyses using both hexacutter and tetracutter enzymes namely *Eco*R1*, Bam*H1 and *Sau*3A1. More extensive smear was obtained in the case of the tetracutter *Sau*3A1 as expected compared to the hexacutter ones (Figure 2A, B, C). *Hpa*II and *Msp*I isoschizomers were also used to show the pattern of genomic DNA digestion (Figure 2D, E).

Polymerase chain reaction (PCR) analyses

In this study, we successfully carried out PCR amplifications using a number of gene-specific primers and different plant genomic DNA as template. Depending on the primer design, a number of partial genes encoding α-glucan water dikinase (GWD; formerly known as starch-related R1 protein), soluble acid invertase, and sucrose-phosphate-synthase (SPS) were isolated using the potato genomic DNA. The sizes of the amplified products were approx. 3.7 kb, 3.2 kb and 4.0 kb, respectively (Figure 3A). The sizes of these DNA products were found to be larger than the respective cDNA sequences, clearly indicating the presence of one or more introns. For further confirmation, the amplified DNA products were cloned into pUC19 vector and characterized by PCR using the respective internal primers followed by partial sequencing (data not shown).

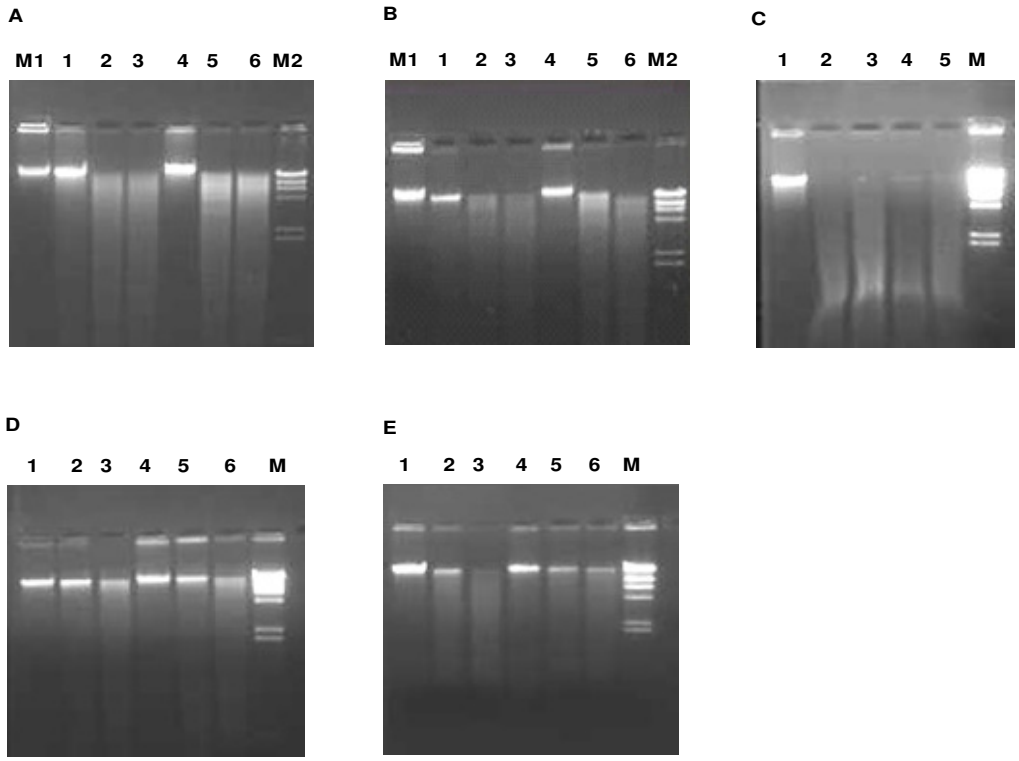

Figure 2

Figure 2. Restriction analysis of genomic DNA, isolated in small scale from different plant materials (0.7% agarose gel electrophoresis). A) M1, bacteriophage λ DNA; lane 1, DNA from the potato cv. K. Chipsona-1 plantlets; lanes 2 and 3, the same DNA digested with *Eco*R1 and *Bam*H1, respectively; likewise, lanes 4 to 6 correspond to the same order for the potato cv. K. Chandramukhi; M2, λ DNA digested with *Hind*III; B) M1, bacteriophage λ DNA; lane 1, *Jatropha* genomic DNA; lanes 2 and 3, the same DNA digested with *Eco*R1 and *Bam*H1, respectively; lane 4, *Brassica* genomic DNA; lanes 5 and 6, the same digested with *Eco*R1 and *Bam*H1, respectively; M2, λ DNA digested with *Hind*III; C) lane 1, potato genomic DNA as control; lanes 2 to 5, *Sau*3AI digested genomic DNA from potato plantlets, potato tuber, *Brassica* and *Jatropha,* respectively; M, λ DNA digested with *Hind*III; D) lane 1, genomic DNA from the potato cv. K. Chipsona-1 plantlets; lanes 2 and 3, the same DNA digested with *Hpa*II and *Msp*I, respectively; lane 4, genomic DNA from tuber of the same potato cultivar; lanes 5 and 6, the tuber DNA digested with *Hpa*II and *Msp*I, respectively; M, λ DNA digested with *Hind*III; E) lane 1, genomic DNA from *Brassica*; lanes 2 and 3, the same DNA digested with *Hpa*II and *Msp*I, respectively; lane 4, genomic DNA from *Jatropha*; lanes 5 and 6, the same DNA digested with *Hpa*II and *Msp*I, respectively; M, λ DNA digested with *Hind*III.

Likewise, partial α-tubulin gene was amplified from potato, *Brassica* and *Jatropha* as shown in Figure 3B. Apart from the expected amplicon (~0.5 kb) specific to α-tubulin gene, a few more bands appeared in the case of potato genomic DNA as templates (lanes 1 and 2, Figure 3B) suggesting some non-specific amplifications. The miniscale DNA preparations from a number of transgenic potato lines were found suitable for amplification of approx 3.0 kb corresponding to the Class I patatin-GUS fusion gene (Figure 3C). We also assessed the quality of the miniscale DNA preparations after prolonged storage, and found it to be stable as evident from agarose gel electrophoresis, restriction analysis and PCR using gene-specific primers (Figure 4A, B, C, D). Likewise, prolonged storage did not affect the quality of the plant genomic DNA isolated in small scale (data not shown).

DISCUSSION

We have optimized and employed a simple, efficient, rapid and cost-effective method for plant genomic DNA isolation. This protocol worked efficiently both in small and miniscale from a variety of plant materials as used in

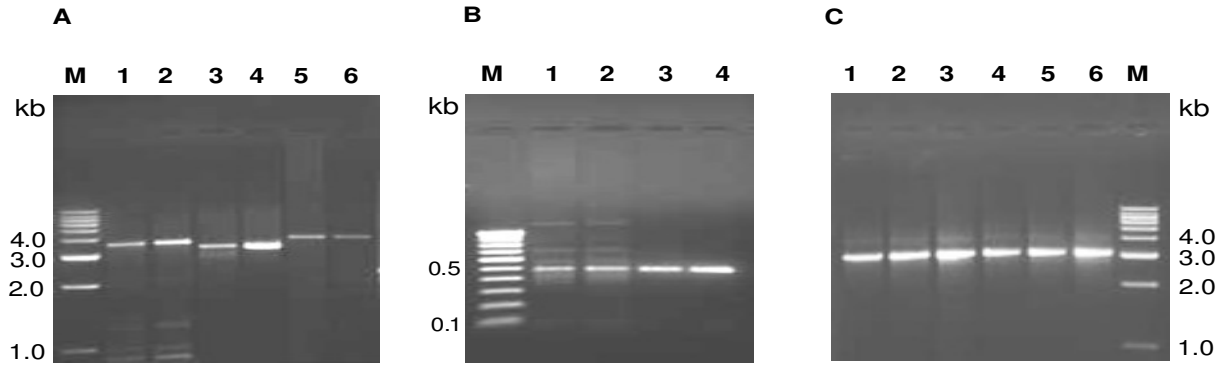

Figure 3

Figure 3. PCR amplification products using different gene-specific primers and different plant genomic DNA as template (A and B correspond to small scale, and C corresponds to mini scale isolation). 0.7% agarose gel electrophoresis for A and C, and 1.0% agarose gel electrophoresis for B. (A) M, 1.0 kb DNA ladder; lanes 1, 3 and 5, PCR amplified products correspond to the potato genes encoding GWD (starch-related R1 protein), acid invertase, and sucrose-phosphate-synthase (SPS), respectively using genomic DNA from micropropagated plantlets of the potato cv. Kufri Chipsona-1; lanes 2, 4 and 6, PCR amplified products correspond to the same genes, respectively using tuber DNA (see the text for approximate size of the amplicons); (B) M, 100 bp DNA ladder; lanes 1 to 4, PCR amplified products correspond to the conserved α-tubulin gene and template DNA from potato plantlets, potato tuber, *Brassica* and *Jatropha,* respectively (The size of the amplicon was nearly 0.5 kb in each case); C) lanes 1 to 6, PCR amplified products, corresponding to the approx 3.0 kb Class I patatin-GUS fusion gene, using genomic DNA from six independent transgenic potato lines; M, 1.0 kb DNA ladder.

this study. The composition of the extraction buffer was simple, and the method did not involve expensive/ specialty chemicals and/or any commercially available kits/column materials unlike many other protocols as reported in the literature. This method, although based on Dellaporta's original DNA isolation protocol, included no detergent in any step during genomic DNA isolation. Moreover, the nature of some plant materials as used in this study was different as compared to the earlier reports. The plant genomic DNA extraction buffer contained high concentration of EDTA. The ability of EDTA to sequester metal ions such as Mg^{2+} and Ca^{2+} from cellular membranes in combination with incubation of the pulverized plant materials at higher temperature could be considered as a substitute for detergent action. Moreover, this step helped in preventing the activities of endogenous nucleases. All the DNA preparations were found to be considerably pure. Restriction digestion patterns indicated that the genomic DNA preparations were essentially devoid of inhibitory substances. Moreover, digestion with *Hpa*II and *Msp*I clearly showed the varying smearing patterns indicating genomic DNA methylation which is common in plants. DNA yield, both in small and miniscale, was found to be sufficient for most of the molecular biological techniques. In the area of modern plant molecular biology and agricultural biotechnology, one of the focus areas includes isolation and characterization of desired genes, and their regulatory sequences by conventional and/or PCR approaches. For this purpose, isolation of suitable plant genomic DNA is an important prerequisite. In this study, we were able to carry out PCR amplifications of a number of genes from potato. The quality of genomic DNA did not get affected even after prolonged storage. Most of the plant materials were collected from *in vitro* grown plantlets under aseptic conditions which were easy to procure year around, and also less time consuming compared to growing them in the field. This helps in getting pure plant genetic materials essentially free from the genomes of microbial contaminants. This DNA isolation method was found to be equally effective for relatively complex plant tissues such as field grown potato leaves and tubers having more secondary metabolites and storage polysaccharides. Particularly, miniscale method could be employed for genomic DNA isolation from large number of plant materials which is very useful for screening of the large number of transgenic lines as well as for genetic diversity studies. In the Dellaporta's method, leaves from a number of plant species were used for genomic DNA isolation; the yield was 50 to 100 µg per gram of tissue and found to be suitable for different molecular biological techniques (Dellaporta et al., 1983). Csaikl et al. (1998) carried out a comparative analysis of different DNA extraction protocols including commercial kits from the leaves or needles of several plant species. The overall yield of plant genomic DNA as reported in this study is comparable to the earlier mentioned protocols, and proved to be useful for different molecular techniques. In conclusion, this method could be a reliable substitute to the frequently used CTAB and commercial kits-based

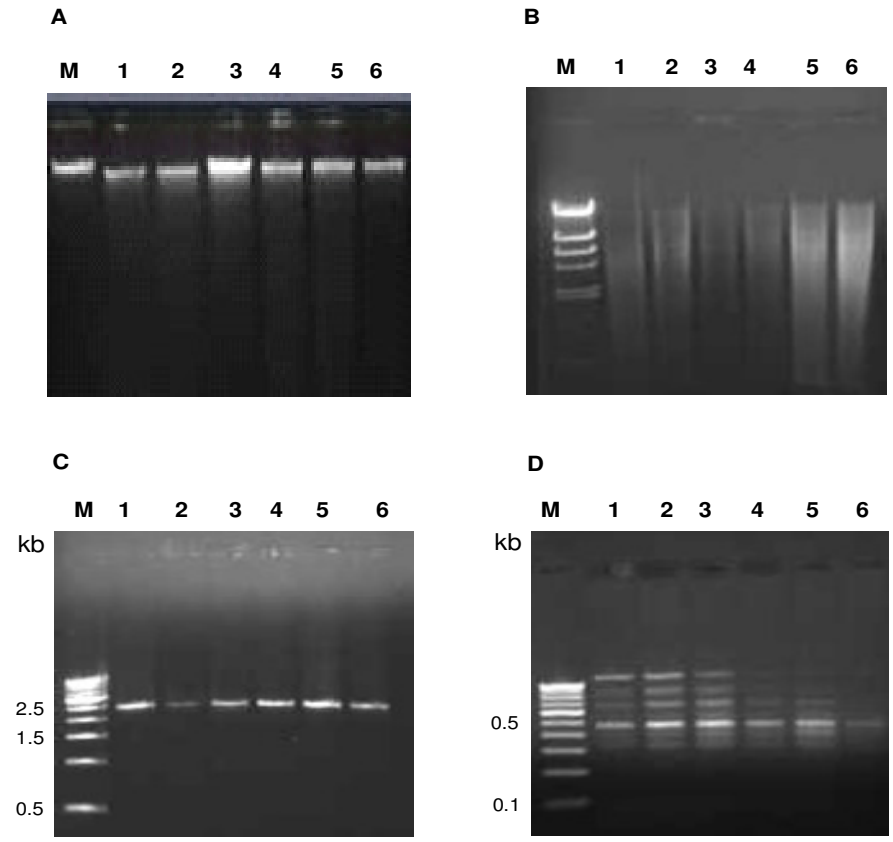

Figure 4

Figure 4. Quality checking of the miniscale plant DNA preparations after prolonged storage (two to three years) at -20°C (0.7% agarose gel electrophoresis for A to C, and 1% agarose gel electrophoresis for D). A) M, bacteriophage λ DNA; lanes 1, 3 and 5 correspond to the genomic DNA from *in vitro* grown plantlets, field-grown tubers and leaves of the potato cv. K. Chipsona-1; lanes 2, 4 and 6 correspond to the similar DNA samples of the cv. K. Chandramukhi; Lane-wise the same plant DNA samples were used in the following restriction analysis and PCR amplifications: (B) M, λ DNA digested with *Hind*III; lanes 1 to 6, the DNA samples digested with *Eco*RI; C) M, 500 bp DNA ladder; lanes 1 to 6, PCR amplification products using acid invertase cDNA-specific primer pair, K20-AI and M20-AI (the size of the amplicon is ~2.5 kb in each case); D) M, 100 bp DNA ladder; lanes 1 to 6, PCR amplification products using α-tubulin gene-specific primers (the size of the amplicon is nearly 0.5 kb in each case).

DNA isolation protocols as reported in the literature.

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