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Validation of a Simple and Rapid Method for Isolating Genomic DNA from Medicinal and Aromatic Plants for Subsequent Polymerase Chain Reaction

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A B S T R A C T

The robust technique of polymerase chain reaction (PCR) has revolutionized the field of plant molecular biology and has made the molecular characterization of crop plants easy, rapid and reproducible. Only a minute amount of input genomic DNA is required in PCR. But, the isolated DNA must be free from different contaminants, which can potentially act as PCR inhibitors. The efficiency of PCR might be reduced in case of the medicinal and aromatic plants, due to the presence of biomolecules acting as PCR inhibitors. Hence the present study reports the applicability of a simple and rapid method for isolating genomic DNA from medicinal and aromatic plants, which can be successfully applied for PCR-based genotyping of these plants. Using a modified form of the detergent (SDS)-potassium acetate method, genomic DNA from the mature leaf tissues of 7 different medicinal and aromatic plants, viz., slender dwarf morning-glory (*Evolvulus alsinoides* L.), horse mint (*Mentha longifolia* L.), centella (*Centella asiatica* L.), brahmi (*Bacopa monnieri* L.), ashwagandha (*Withania somnifera* L.), vasaka (*Adhatoda vasica* Nees.) and sarpgandha (*Rauwolfia serpentina* L.) was isolated and PCR was carried out with different inter short sequence repeat (ISSR) primers. Gel electrophoresis revealed the presence of distinguishable sharp amplicons, prompting us to advocate the utility of this method for genotyping of medicinal and aromatic plants.

Introduction

The application of DNA technology in plant molecular biology is attracting many researchers to do lots of work for increasing the production, conservation and genetic improvement of rare and endangered plant species. The medicinal and aromatic plants contain several important secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides, terpenes and quinines, which have huge applications as therapeutic, cosmetic, food additives, pesticides, and many more. The genetic improvement of these crops should be given priority, where modern techniques, like marker assisted selection (MAS) have to be adopted. With the invention of polymerase chain reaction (PCR), molecular characterization of plants has become easy, rapid and reproducible. In PCR, a minute but good quality genomic DNA is required as a template. But presence of different metabolites in medicinal and aromatic plants has remained

as a bottleneck in the isolation of good quality of genomic DNA, PCR reaction, cloning and other downstream applications. Presence of polysaccharides in the isolated genomic DNA of medicinal and aromatic plant make DNA viscous and glue-like, that stick to the wells during gel electrophoresis and becomes non-amplifiable in the polymerase chain reaction (PCR) by inhibiting *Taq*DNA polymerase activity and also interfere the activities of several other biological enzymes like DNA ligases and restriction endonucleases (Porebski *et al.,* 1997; Barnell *et al.,* 1998; Diadema *et al.,* 2003; Varma *et al.,* 2007). The presence of polyphenols, released from vacuoles during cell lysis, undergo irreversible interaction with nucleic acid causing browning of the DNA (Varma *et al.,* 2007). Hence, attempts have been made to optimize several protocols to isolate pure and high quality of genomic DNA from medicinal and aromatic plant (Doyle and Doyle, 1987; Haymes, 1996; Scott and Playford, 1996; Khanuja *et al.,* 1999; Sharma *et al.,* 2000; Pirttilä *et al.,* 2001; Shepherd *et al.,* 2002; Mogg and Bond, 2003).

Here we validate a rapid and reproducible method (Kumar *et al.,* 2017) for isolating genomic DNA from 7 different medicinal and aromatic plants. The utility of the method was tested through subjecting the isolated genomic DNA to PCR using different inter short sequence repeat (ISSR) primers. The presence of distinguishable sharp amplicons, as revealed through gel electrophoresis advocates the applicability of this method for genotyping of medicinal and aromatic plants at molecular level.

Materials and Methods

Plant materials

Leaf tissues were collected from 7 different medicinal and aromatic plants viz., slender dwarf morning-glory (*Evolvulus alsinoides* L.), horse mint (*Mentha longifolia* L.), centella (*Centella asiatica* L.), brahmi (*Bacopa monnieri* L.), ashwagandha (*Withania somnifera* L.), vasaka (*Adhatoda vasica* Nees.) and sarpgandha (*Rauwolfia serpentina* L.) from Botanical garden of Bihar Agricultural University, Sabour, Bhagalpur.

DNA isolation procedure

For the preparation of good quality of genomic DNA, ~50mg fresh leaf samples were collected in 1.5 ml microcentrifuge tubes. The tissue was homogenized through crushing, using a micro-pestle in 400µl of buffer [100 mM Tris-Cl, 50 mM EDTA, 500 mM NaCl, 1 % (w/v) SDS and 0.1 % (v/v) β-Mercaptoethanol]. Next, 128 µl of 5M potassium acetate was added and the mixture was centrifuged at 10,000 rpm for 10 s. Then 300µl supernatant was collected in a fresh microcentrifuge tube and equal volume (300µl) of chilled isopropanol was added. Following mixing, the sample was centrifuged at 10,000 rpm for 10 sto precipitate the genomic DNA. The DNA pellet was then washed with 200 µlof 70% (v/v) ethanol, dried and dissolved in sterile water.

PCR amplification

Polymerase chain reaction (PCR) amplification of isolated genomic DNA was carried out in a total 10µl volume. The PCR reaction mixture contained template DNA (1 µl), 1X PCR buffer with $MgCl_2$ (Xcelris), 0.2mM of dNTP mix (Xcelris), 0.1µM of primer and 1U of *Taq*DNA polymerse (Xcelris). PCR amplification was performed in an automated thermal cycler (Veriti 96 well thermal cycler, Applied Biosystems) using the thermal profile consisting of an initial denaturation at 94°C for 4min followed by 45 cycles of 30s at 94°C, 60s at 36°C annealing temperature, 120s at 72°C and ended with final extension at 72°C for 10min followed by

hold at 4°C. The sequence of the ISSR primers reported earlier (Lu *et al.*, 2009) and used in this study is presented in Table 1. The amplicons were subjected to 1.2% (w/v) agarose gel electrophoresis in presence of ethidium bromide and imaged in gel documentation system (Genei, Bangalore).

Results and Discussion

The rapid DNA isolation procedure adopted in this study allowed the isolation of genomic DNA from the leaf tissues in ~20 min time. All the isolated genomic DNA were found to give amplification with the 6 ISSR markers, tested in this study. The amplicon profiles, obtained in this study are summarized in Figure 1. All the primers gave distinguishable banding pattern. Furthermore, the banding pattern of the 7 medicinal and aromatic plant samples was found to be distinct from each other for all the 6 ISSR markers tested. This indicated the efficiency and quality of isolated genomic DNA from medicinal and aromatic plants. The scorable and distinct bands were then taken for analysis, where it was found that in case of the ISSR 4F primer, total 35 amplicons were obtained, where the amplicon size ranged from ~1000 bp (vasaka) to ~247 bp (centella). A total of 29 amplicons (ranging from ~1014 bp in sarpagandha to ~277 bp in brahmi) were generated in case of the ISSR 8F primer, whereas the total number of amplicons generated was 29 (ranging from ~943 bp in SD morning-glory to ~282 bp in brahmi) in case of the ISSR 22F primer. In a similar manner, the ISSR primer 43 F, 44 F and 51 F generated a total number of 24, 29 and 22 amplicons, with a range of ~1060 bp (in sarpagandha) to ~254 bp (in vasaka), ~786 bp (in sarpagandha) to ~275 bp (in vasaka) and ~700 bp (in SD morning glory) to ~261 bp (in brahmi and ashwagandha), respectively. Thus, the genomic DNA isolated in this rapid method was found to be suitable for downstream application in genotyping of medicinal and aromatic plants using PCR.

Fig.1 Amplicon profile of isolated genomic DNA from 7 medicinal and aromatic plants using 6 different ISSR primers. A. Amplification with ISSR 4 primer. B. Amplification with ISSR 8 primer. C. Amplification with ISSR 22 primer. D. Amplification with ISSR 43 primer. E. Amplification with ISSR 44 primer. F. Amplification with ISSR 51 primer. *L* = 100 bp DNA ladder (Genei, Bangalore)

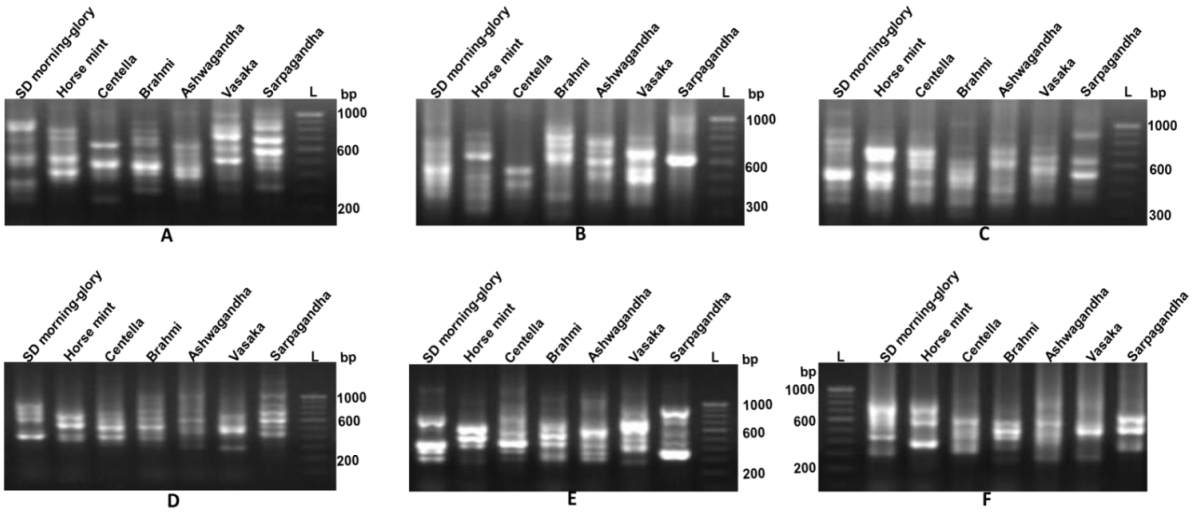

Table.1 Sequence of the ISSR primers used in this study

ISSR Primer	Sequence (5´-3´)
ISSR 4	ACACACACACACACACACAG
ISSR8	ATGATGATGATGATGATG
ISSR22	ACACACACACACACACAA
ISSR43	ACACACACACACACACCT
ISSR44	ACACACACACACACACGA
ISSR51	TGTGTGTGTGTGTGTGAT

A minute amount of isolated genomic DNA was found to be sufficient for PCR, enabling the researcher to use the isolated DNA for several hundred independent PCRs. In this method, the isolated genomic DNA was found to remain stable for several months, when stored at -20°C. Hence, we advocate the applicability of this simple, rapid and reproducible method of DNA isolation for molecular characterization of medicinal and aromatic plants through PCR.

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