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Full Length Research Paper

Efficient DNA isolation from *Emblica officinalis* for effective PCR

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Emblica officinalis **is a medicinal plant and distributed throughout the lower ranges of Himachal Pradesh. Its ph is low and has high amount of polysaccharides, polyphenols several pigments and other secondary metabolites. A slight modification of Warude et al. protocol for DNA isolation yielded good quality high molecular weight DNA free of contaminants from eight plants taken for analogy using RAPD as a potential marker. 120 mgPVP/g of tissue was used and the isolated DNA pellets were washed twice with 70% ethanol.**

Key words: Himachal Pradesh, medicinal plant, *Emblica officinalis*, polysaccharides, PCR, RAPD, PVP.

INTRODUCTION

Amla (*Emblica officinalis*) is a tropical, deciduous, small to medium sized tree with pale yellowish fleshy globose fruits. Leaves are narrow and pinnate and develop after the fruit sets. In Himachal Amla grows up to 1350 m in Nalagarh, Nahan, Mandi, Kunihar, Bilaspur, Hamirpur, Kangra, Solan and Rajgarh.

E. officinalis is famously known for its hepatoprotective and antioxidant activities (Jose and Kuttan, 2000; Bhattacharya et al., 1999) *E. officinalis* is rich source of Vitamin-C (Thorat et al., 1995). It is rich in quercetin, phyllambic compound, gallic acid, pectin and others (Chopra et al., 1956). The effective components are flavonoids, tannins, Vitamin-C and are found in maximum concentration and are anti-oxidant in action (Haque et al., 2001). Amla is a component of triphala and an important rasayan in Ayurvedic medicine (Kaur et al., 2002). Amla extract possess anticancer, antisclerotic, lipid lowering, hepatoprotective, anti HIV activities (Khan 2002; El-Mekkawy, 1995), and inhibits thioacetamide-induced oxidative stress and hyperproliferation in rat liver (Sultana et al., 2004). The alcoholic and aqueous extract of Amla has powerful retarding effect on ochratoxin induced haemolysis on RBC (Verma and Chakraborty, 2006).

Isolation of pure, intact and high quality DNA is so crucial for any genetic studies, especially because of high amounts of compounds present in plant tissues that may interfere with subsequent DNA manipulations. *E. officinalis* contain high level of polysaccharides, polyphenols, several pigments and other secondary metabolites (Wen and Deng, 2002), which makes DNA unusable for downstream work in molecular biology research (Levi et al., 1992; Michiels et al., 2003; Qiang et al., 2004). Although the new DNA based methods are highly specific, reproductive and sensitive and characterized by high discriminatory power, rapid processing time and with low costs, they are strongly limited by the presence of inhibitors in plant tissues. Polysaccharides and polyphenols are particularly abundant in *E. Officinalis* fruit samples and are not completely removed during classical extraction protocols; they remain as contaminants in the final DNA preparations. Polysaccharides make DNA viscous, glue like and non- amplifiable in the PCR reaction by inhibiting *Taq* enzyme activity and also interfere with accurate activity of restriction enzymes (Porebski et al., 1997). When cells are disrupted, these cytoplasm compounds can come into contact with nuclei and other organelles (Loomis et al., 1974). In their oxidized forms, polyphenols covalently bind to DNA giving it a brown color and reduce their maintenance time, making it useless for most research applications (Katter man and Shattuck, 1992). The presence of these compounds renders studies difficult due to long and tedious extraction procedures and often does not result in good standards in terms of yield and quality.

Although a number of methods for DNA isolation from

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plants containing high levels of secondary metabolites have been developed (Van Driesch et al., 1984; Porebski et al., 1997; Peterson et al., 1997; Cheng et al., 2003; Michiels et al., 2003; Quiang et al., 2004; Zeng and Yang, 2002). Recently, modified CTAB DNA extraction procedure (Gawal and Jarret, 1991) was used in the identifycation of DNA polymorphism in *E. officinalis* mutants using RAPD markers (Senthamizh et al., 2007). But, these DNA extraction methods are generally expen-sive and time consuming. The need for a rapid and efficient method of DNA isolation for *E. Officinalis* plant having high contents of polysaccharides is necessary when hundreds of samples need to be analyzed rapidly, such as in genome mapping and marker assisted selection (MAS) programs. As high purity DNA is required for PCR and restriction based techniques like RAPD, SSR, AFLP and RFLP.

RAPD is a common molecular approach in DNA fingerprinting analysis for genotypic differentiation, molecular taxonomy and other applications (Lin et al., 1996). Our aim was to derive an analogy between various plants taken from different regions based on the amplification of pure high molecular weight DNA from these plants as the plant contains high levels of polysaccharides and polyphenols (Katterman and Shattuck, 1983; Peterson et al., 1997), and has high pH. Few basic DNA protocols (Dellaporta et al., 1983; Doyle and Doyle, 1987) have been modified to a number of other isolation protocols. Unlike in non plant DNA isolation protocols, methods need to be adjusted to each species depending on the range of primary and secondary metabolites (Sangwan et al., 1998). As the pH of the plant is less and the quantity of secondary metabolites is more, the above protocols were modified to yield good quality DNA (Warude et al., 2003). A slight modification of Warude et al protocol in amount of PVP per gram of tissue yielded good quality high amount DNA. A putative marker has been identified by Warude et al. (2006) for *E. officinalis* through RAPD.

MATERIALS AND METHODS

Plant material reagents and chemicals

Leaves of *E. officinalis* were taken from eight locations (Nahan, Arki, Kasauli, Kunihar, Johadji, Darlaghat, Jawalaji, and Ghanhati) of Himachal Pradesh and coded as (Acc1, Acc2, Acc3, Acc4, Acc5, Acc6, Acc7 and Acc8) for genomic DNA isolation, respectively. The leaves were brought in aluminum foils in an ice box and stored at -20°C.

Reagents and chemicals

Solutions prepared

CTAB (10%) was prepared by dissolving CTAB in 100 ml of distilled water and autoclaved. 1 M Tris HCl (pH 8), was prepared in 100 ml of distilled water and autoclaved. 5 M NaCl was prepared in 100 ml of water and autoclaved, 3 M sodium acetate, 70% ethanol, chloroform-IAA (24:1[v/v]), polyvinylpyrrolidone (~ 40000 mol wt), beta –mercaptoethanol, absolute alcohol, 0.5 M EDTA (pH 8) was prepared in 100 ml of water and autoclaved.

Extraction Buffer (100 ml): was prepared by mixing 4 ml of 0.5 EDTA, 10 ml of 1 M Tris-Cl, 20 ml of 10% CTAB, 28 ml of 5 M NaCl and 38 ml of distilled water.

TE Buffer (100 ml): was prepared by mixing 1 ml of 1 M Tris-Cl, 2 ml of 5 M EDTA and 98.8 ml of distilled water.

Other: Taq DNA polymerase enzyme, dNTP mixture (A, T, G and C).

DNA isolation protocol

3 g of frozen leaf tissue of each of the samples was powdered in liquid nitrogen with 120 mg PVP/g of leaf tissue. Added freshly prepared extraction buffer with 20 µl of β-mercaptoethanol. Incubated at 65°C for 1 h in hot water bath. Allowed the solution to cool for 5 min at RT. Added equal volume of chloroform-IAA (24:1). Centrifuged at 8000 rpm for 15 min at 16°C and collected the supernatant. Precipitated DNA from aqueous layer by adding $1/10^{th}$ volume of 3 M Sodium Acetate and double chilled absolute alcohol incubated at -20°C overnight. Centrifuged at 7000 rpm for 10 min at RT. Decanted and collected the pellets. Added 5 ml of 70% ethanol to wash the pellets. Centrifuged at 7000 rpm at RT for 10 min. Decanted and collected the pellets. Dried the pellets at RT. Again washed with 70% ethanol. Dissolved the dried pellets in 200 µl TE buffer and kept at 4°C overnight. Isolated DNA was stored at -20°C for further analysis.

Quantification and PCR amplification

PCR for DNA amplification of DNA preparations was carried out in a 25 microlitre vol. of reaction mixture after isolated DNA was loaded on 0.8% agarose gel prepared in 1X TBE buffer containing 0.5 microgram/ml oh Ethidium Bromide along with sample control (Lambda DNA). The quality and concentration of each sample was estimated by comparing it with the control DNA. A reaction tube contained 25 ng of DNA approx., 0.6 U of Taq DNA Polymerase enzyme, 100 mM of each d NTP, 10X buffer and decanucleotide primers. Amplifications were carried out by using DNA Thermal cycler with following parameters: 94°C for 5 min; 45 cycles at 94°C for 1 min, 36°C for 1 min, and 72°C for 2 min; and a final extension at 72°C for 5 min. PCR products were subjected to agarose gel (1.2% [w/v]) electrophoresis in 0.5 X TBE buffer, along with DNA marker (Banglore Genie, India) as a size marker. DNA was stained with Ethidium Bromide and photographed under UV light. Two primers OPA-9(5'-GGGTAACGCC-3') and OPA-14(5'-TCTGTGCTGG-3') were run.

RESULTS AND DISCUSSION

Significant PCR amplification occurred using the modified Warude et al protocol which in itself was a modification of previous protocols. The protocol differed in the amount of PVP added /g of leaf tissue which was 100 mg PVP/g of leaf tissue in case of Warude et al protocol and 120 mgPVP/g of leaf tissue in the above. The DNA pellets were washed twice with 70% alcohol instead of once in the Warude et al protocol. Two primers efficiently ran

Table 1. Jacquard's coefficient matrix.

Acc	1	2	3	4	5	6	7	8
1	1.000							
2	0.600	1.000						
3	0.570	0.440	1.000					
4	0.440	0.360	0.420	1.000				
5	00.25	0.200	0.400	0.500	1.000			
6	0.420	0.330	0.750	0.500	0.500	1.000		
7	0.360	0.210	0.300	0.550	0.370	0.350	1.000	
8	0.370	0.440	0.600	0.420	0.400	0.750	0.330	1.000

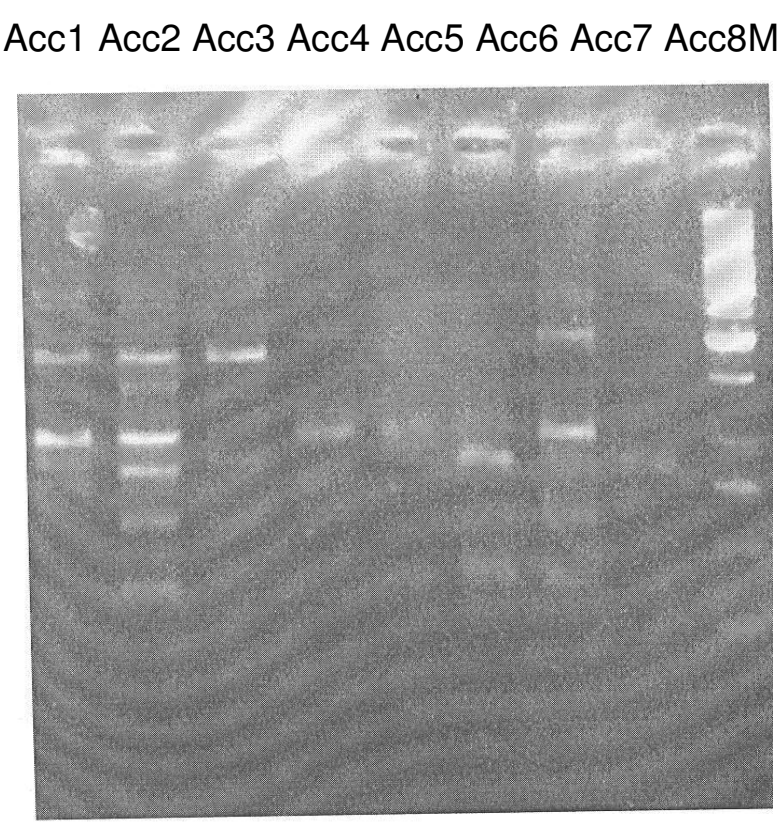

Figure 1. RAPD profile of *Emblica Officinalis* using OPA-9 Primer.

resulting in scorable bands.OPA-9 yielded nine bands all of which were polymorphic (Figure 1). OPA-14 yielded a total of five scorable bands, three were polymorphic and two were monomorphic (Figure 2). The protocol can be used for efficient DNA isolation and further studies on Genetic variation and polymorphism can be carried out in *E. officinalis* using the protocol (Table 1).The data matrices were analyzed by the SIMQUAL programme of NTSYS-PC (version 2.2j) and similarity between accessions was estimated using Jaccard coefficient. Eight primers were used, but only two primers (OPA-9[5'-GGGTAACGCC-3'], OPA-14 [5'-TCTGTGCTGG]) could bind generating a unique set of amplification products. The primers in this analysis yielded a total of 14 scorable bands. RAPD analysis with primer OPA-9 yielded nine bands, all of which were polymorphic. With primer OPA-14, a total of five scorable bands were produced. Three bands were polymorphic and two were monomorphic. Each primer generated a unique sequence of amplification products ranging in size from 300 - 2500 bp (OPA-9) and 650 - 2000 bp (OPA-14) (Figure 3).

This indicated a fair range of variability in the similarity coefficient values suggesting a wide genetic base of eight accessions taken. The highest value of 0.75 was found between accessions from Ghanhati and Darlaghat; Darlaghat and Kasauli. Though two primers are not

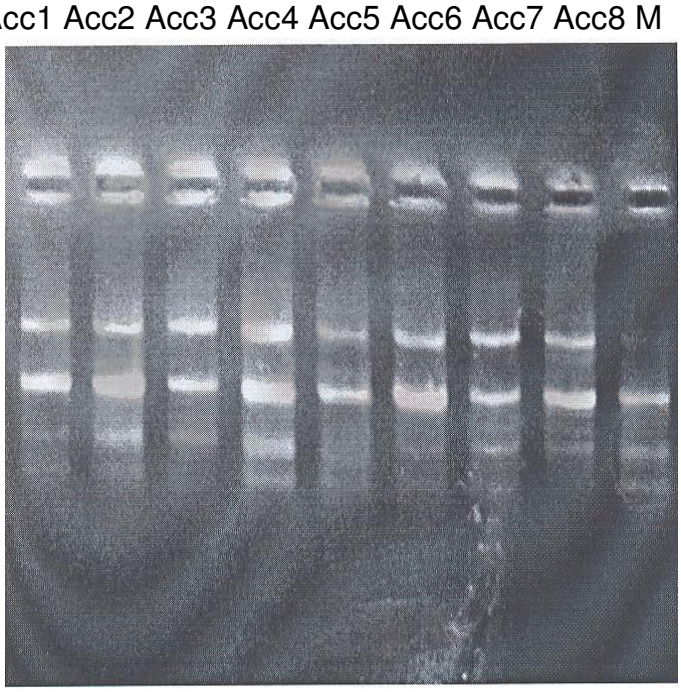

Figure 2. RAPD profile of *Emblica officinalis* using OPA-14 Primer.

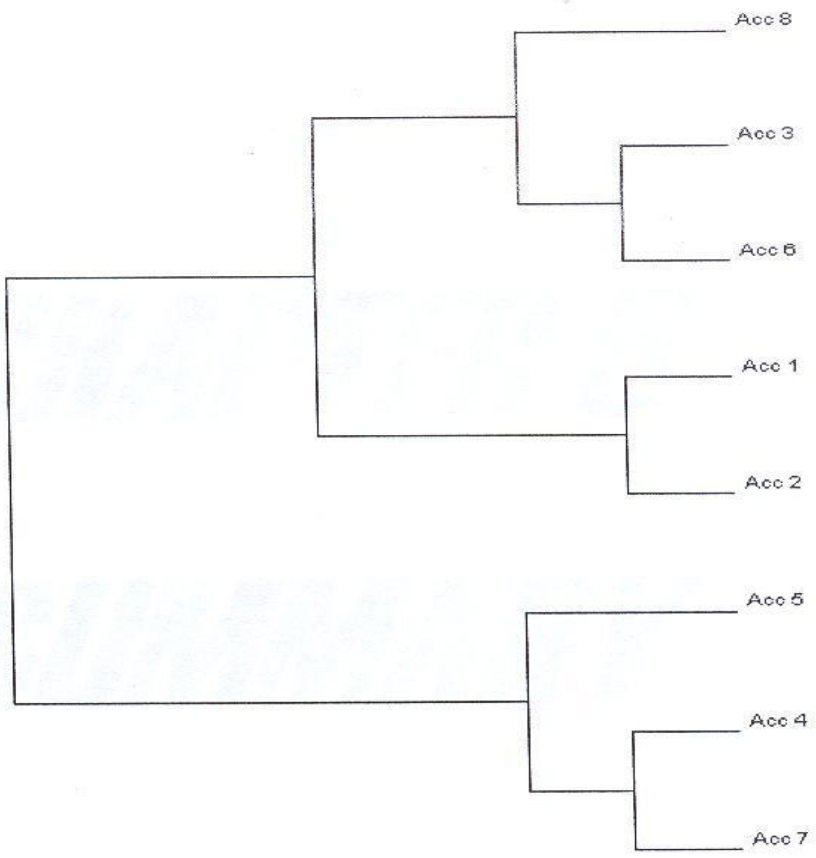

Figure 3. Dendogram of 8 genotypes of *Emblica Officinalis*, based on RAPD marker.

enough to draw any kind of conclusion about the similarity between the plants but definitely highlight differences between the plants.

Conclusion

The protocol can be used for efficient DNA isolation and further studies on genetic variation and polymorphism in *E. officinalis*. In case of plant DNA isolation; protocols can be modified and varied accordingly, so, the protocol can be used as a base for the isolation of DNA from plants having high amount of polysaccharides and polyphenols.

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