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An efficient DNA extraction protocol for medicinal plants

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Abstract

This paper communicates a simple and cost effective protocol for isolation of genomic DNA from dry parts of Berberis and Mentha. This protocol was applied to 5 species of Berberis and 4 species of Mentha collected from different locality of Kunhar Valley. In this protocol 5 M NaCl, 2% CTAB, 1% PVP and 0.1 % ß-mercaptoethanol were used and incubated at 60ºC for 25 minutes. Pure DNA extracted by this method was found sufficient and suitable for PCR amplification and Southern blot hybridization analyses, which are the important steps in crop improvement programme through marker development and genetic engineering techniques.

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Introduction

The use of molecular markers in genome studies has greatly enhanced the speed of crop improvement in breeding programs. A prerequisite for taking advantage of this technology is the isolation of DNA for PCR amplification. For DNA isolation a rapid, simple and reliable method is generally needed. As the size, content, organization of genome and contents of metabolites of different plants vary to a great extent, a single DNA isolation protocol is not likely to be applicable for all the plant tissues (Loomis, 1974). Medicinal and aromatic plants contain secondary metabolites which affect the enzymatic reactions reducing yield and quality of the extracted DNA (Weising *et al.*, 1995; Loomis, 1974; Porebski *et al.,* 1997). Polysaccharides and polyphenolic compounds causes difficulty in DNA extraction and purification (Fang *et al.*, 1992 and Howland *et al.*, 1991). Polysaccharides often react with DNA and thus reduce the action of DNA modifying enzymes i.e. restriction enzymes, DNA polymerase and ligase during DNA isolation (Sharma *et. al.*, 2002). Several methods for extracting DNA from different parts of plant materials are available (Dellaporta *et al.*, 1983; Keim *et al.*, 1988; Doyle and Doyle *et al.,* 1990; Khanuja*et al.*, 1999; Kumar *et al.*, 2003). The methods employed for extracting DNA from fresh and dried parts of medicinal plants are however time consuming and generally yield DNA in lesser quantity. This communication introduces as a simple, rapid, inexpensive and efficient protocol for DNA extraction from *Berberis* and *Mentha* species.

Materials and methods

Plant materials

Nine species of two important genera of medicinal plants i.e. *Berberis* and *Mentha* were collected from different areas of Kunhar valley Mansehra, Khyber Pakhtunkhwa Pakistan. Five species of *Berberis i. e. Berberis kunwarensis* (Kapigali)*, B. lyceum* (Balakot)*, B. orthobotrys* (Shogra), *B. pachyacantha* (Saiful Maluk) *& B. parkeriana* (Kaghan) and four species of *Mentha* i.e. *Mentha arvensis* (Kaghan)*, M. longifolia* (Balakot), *M. royleana* (Naran) *& M. spicata* (Mahandri). The specimens were identified with the help of Flora of Pakistan (Jafri, 1975 and Hedge, 1990). The DNA was extracted by the protocols of Barzegari, *et al.*, 2010; Khan *et al.*, 2007; Kumar *et al.,* 2003; Khanuja *et al.*, 1999; Lodhi *et al.*, 1994 and Doyle and Doyle, 1990 is in lesser quantity. Finally some modified procedure was used and best result was obtained.

Reagents

Sodium EDTA, Tris-HCl, NaCl, Cyltrimethylammonium bromide (CTAB), Polyvinylpolypyrrolidone (PVP), ß-mercaptoethanol, Chloroform, Isoamylalcohol, Ethanol, RNAase A

CTAB buffer with pH 8.0

Take 7.44g Sodium EDTA, 15.76g Tris-HCl, 81.82g NaCl, dissolve it in 1000ml of water and add 2.0% (w/v) i.e. 20g CTAB, CTAB will be dissolve by heating up to 60 ºC, adjust the pH to 8.0. Add 0.1 % of ß-mercaptoethanol and 01% PVP just before use.

Chloroform: Isoamyl alcohol 24:1 (v/v)

Take 24 ml of Chloroform and add with 1 ml of Isoamylalcohol.

5 M NaCl

Take 292.2 g of NaCl and dissolve in 1L of water that will form 5 M solution of NaCl.

TE buffer

Dissolve 1 M Tris, 0.5 M EDTA in 100 ml of water and adjust pH to 8.0.

Plant sample treatment

Fresh plant sample were collected and sun dried.

DNA isolation procedure

Grind 0.026g dry part of plant in pestle and mortar without liquid nitrogen. Transfer the powder material to 1.5 ml eppendorf tube and add 500 µl of Cetyltrimethylammoniumbromide (CTAB) buffer, 01(w/v)% Polyvinylpolypyrrolidone (PVP) and 0.1 % (0.5µl) ß-mercaptoethanol. Incubate the mixture for 35 minutes at 65 ºC. Cool to room temperature and add one volume of Chloroform Isoamylalcohol and

mix gently for 1-2 minute. Centrifuge at 6000 rpm for 15minutes. Transfer the supernatant to another tube, if impurities are present than again add one volume of Chloroform Isoamylalcohol and mix gently for 1-2 minute. Again centrifuge at 6000 rpm for 15minutes. Transfer the supernatant to another tube and add 0.5 volumes of 5M NaCl. Add one volumes of ice cold pure ethanol and kept at 6 – 7 °C for 15-20 minutes or even more for 12 hour. Centrifuge at 3000 rpm for 3 minutes and then Centrifuge at 8000 rpm for 5 minutes Discard the supernatant and wash the pellet with 70% ethanol. Dry the pellet and dissolve it in 15µl TE buffer. Keep at 6 – 7 °C for 1 hour or overnight to dissolve the DNA completely.

Gel Electrophoresis

Quality and quantity of the DNA was checked on 1% agarose / TBE gel. For gel preparation 0.5 gram of agarose powder was dissolved in 50 mL TBE. The mixture was boiled on hot plate at 100°C. After agarose was dissolved completely, 5 µl ethidium bromide was added and gel was casted in a gel tray with comb. After solidifying, gel was placed in gel tank containing 1X TBE. 5 µl DNA from each samples was taken, mixed with 3 µl loading dye and loaded in the wells. Gel was then run at constant voltage of 70 volts for approximately 30 minutes. The gel was observed under UV light using "Uvitech" gel documentation system. If RNA is present than add 1µl "RNAse A" and incubate at 35 °C for 15 minutes to remove RNA.

Results

In spite of several attempts we were unsuccessful to isolate a consistent amount of DNA with good quality and quantity suitable for amplification through PCR and restriction analyses from different species of *Berberis* and *Mentha*. The methods used include; Barzegari, *et al.*, (2010); Kumar, *et al., (*2003); Khan *et al.*, (2007); Khanuja *et al.*, (1999) Lodhi, *et al.*, (1994) and Doyle & Doyle (1990). The DNA of high quality and quantity was however extracted by a modified protocol. Details of modification made in the protocols are given in Table 1. The DNA isolated by this protocol from dried parts of plants **belaying** to both the genera is free of polysaccharides and secondary metabolites. The DNA isolated was checked by 1% agarose gel electrophoresis, the result showed that the DNA was neither contaminated nor degraded (Figure 1). The DNA of all specimens was run independently with a primer for PCR amplification, the result of PCR amplification is represented in figure 1.

Table 1. The following modifications have made in the protocols of Doyle and Doyle (1990) and Lodhi, *et al.* (1994).

S.No	Doyle and Doyle (1990) protocol	Lodhi, *et al.* (1994) protocol	Present protocol
1.	Fresh leaves were used	Young unexpanded leaves were used	All parts of the plant (mature or immature)
2.	Liquid nitrogen is used for collection & preservation of material	Liquid nitrogen is used for collection & preservation of material	Materials is collected without liquid nitrogen & dried in sunlight
3	10 mM ammonium acetate is used	Not used	Not used
4.	0.2 % ß-mercaptoethanol	0.2 % ß-mercaptoethanol	0.1 % ß-mercaptoethanol
5.	chloroform:Isoamylalcohol is used	chloroform:octanol is used	chloroform:Isoamylalcohol is used
6.	Incubated the sample for 30 minutes at 60°C	Incubated the sample for 25 minutes at 60°C	Incubated the sample for 25 minutes at 60°C
7.	Spin at 6000 rpm for 10	Spin at 3000 rpm for 3minutes and then increase speed to 5000 rpm for an additional 3 minutes	Spin at 3000 rpm for three minutes and then increase speed to 8000 rpm for an additional 5 minutes
8.	Wash pellet with 70% ethanol at room temperature.	Wash pellet with 76% cold ethanol	Wash pellet with 70% ethanol at room temperature.

Discussion

The secondary metabolites produced by medicinal plants possess important medicinal properties and are used in food, pharmaceutical, cosmetics and pesticide industries (Khanuja *et al.*, 1999). Secondary metabolites and polysaccharides cause great problems in DNA isolation (Puchooa and Venkatasamy 2005) and isolated DNA that contains secondary metabolites is not suitable for PCR amplification and restriction digestion Lodhi *et al.* (1994). For DNA extraction from *Berberis* and *Mentha* species different protocols were used. The results of these protocols were; Kumar *et al.* (2003) protocol is relatively simple and cheaper but not applicable in case of *Berberis* and *Mentha* species because the DNA extracted by this protocol was in brown color most probably of secondary metabolite polysaccharides, as suggested by Lodhi *et al.* (1994), which are considered to be a PCR blocker. Hence the extracted DNA was not suitable for PCR amplification. The DNA pellet obtained through using Khanuja *et al.* (1999) protocol was not single mass rather powdery in nature, which was not suitable for PCR amplification. The degraded nature of genomic DNA has also been reported by Puchooa and Venkatasamy (2005). The protocol adopted by Barzegari *et al.* (2010) was good enough for DNA extraction but was very lengthy and time consuming. It had at least 16 steps for DNA extraction and mostly the steps involved centrifugation and centrifugation damage the intact DNA. The DNA isolated with the protocol of Khan *et al.* (2007) was highly viscous, sticky and was difficult to be resolved on the agarose gel. Similar problems with using this protocol were also reported by Thakare *et al.* (2007).

The protocol of Doyle and Doyle (1990) was very simple and failed for DNA from *Berberis* and *Mentha* species. Lodhi *et al.* (1994), protocol was found best when partially expanded leaves were used for DNA isolation. In old/ mature leaves the yield was low and the DNA was not completely digestible. Later in the season DNA extraction was difficult and the DNA obtained was unstable for long term storage. The extracted DNA by such protocol was partially soluble in TE buffer and therefore the quality and quantity of the DNA was also not satisfactory. Hence it was imperative to develop a new protocol for DNA extraction from *Berberis* and *Mentha* species. Thus a modified protocol came up with some basic changes in the protocols of Lodhi, *et al.* (1994) and Doyle & Doyle (1990). Details of modification are represented in table 1. This protocol is useful for DNA isolation from dried parts of *Berberis* and *Mentha* species.

Berberis and *Mentha* species contain high polysaccharides which can easily remove by adding more than 0.5 M of NaCl with CTAB (Paterson et al., 1993). In this protocol high concentration (5 M) of NaCl is used to remove polysaccharides. The concentration range of NaCl used in DNA extraction protocol varies from 0.7 M (Clark, 1997) to 6 M (Aljanabi *et al.*, 1999) depending upon plant species.

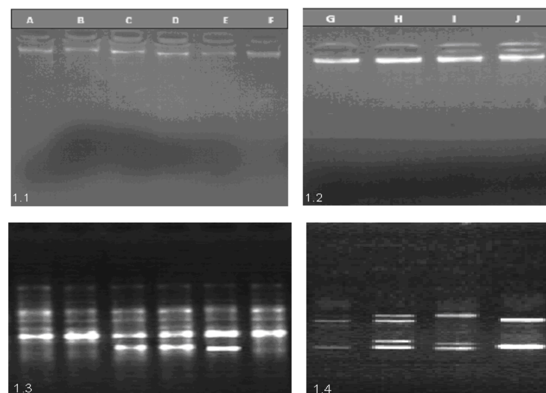

Fig. 1. DNA extracted from root (AB), Stem (CD) and Leaves (EF) of *Berberis* (1.1). DNA extracted from Stem (GH) and Leaves (IJ) of *Mentha* (1.2). Plates 1.3 and 1.4 show the PCR amplification of Berberis and Mentha DNA respectively obtained through modified protocol.

This protocol is also easy as it avoids the use of liquid Nitrogen. The problem with liquid nitrogen is not only it's availability but its storage for long time in mountains during collection is nearly impossible. Secondly fresh leaves might not be available in every season so alternative to that one may use other parts of the plant i.e. shoots, roots etc. The modifications suggested here in this paper will not only facilitate researcher in extraction of DNA from genera like *Beberis* and *Mentha* but will also reduce the expenses of the research.

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