RESEARCH PAPER

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 % β-mercaptoethanol were used and incubated at 60°C 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; Lodhi et al., 1994 and Doyle and Doyle, 1990 is in lesser quantity. Finally some modified procedure was used and best result was obtained.

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 kunwarenensis (Kapigali), B. lyceum (Balakot), B. orthobotrys (Shogra), B. pachycanthera (Saiful Maluk) & B. parkeriiana (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, Cylytrimethylammonium bromide (CTAB), Polyvinylpolypyrrolidone (PVP), β-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 °C, adjust the pH to 8.0. Add 0.1 % of β-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) β-mercaptoethanol. Incubate the mixture for 35 minutes at 65 °C. Cool to room temperature and add one volume of Chloroform Isoamylalcohol and
mix gently for 1-2 minute. Centrifuge at 6000 rpm for 15 minutes. 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 15 minutes. 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 casting 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).

<table>
<thead>
<tr>
<th>S.No</th>
<th>Doyle and Doyle (1990) protocol</th>
<th>Lodhi, et al. (1994) protocol</th>
<th>Present protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fresh leaves were used</td>
<td>Young unexpanded leaves were</td>
<td>All parts of the plant (mature or immature)</td>
</tr>
<tr>
<td></td>
<td>used</td>
<td>used</td>
<td></td>
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<tr>
<td>2.</td>
<td>Liquid nitrogen is used for</td>
<td>Liquid nitrogen is used for</td>
<td></td>
</tr>
<tr>
<td></td>
<td>collection &amp; preservation of</td>
<td>collection &amp; preservation of</td>
<td></td>
</tr>
<tr>
<td></td>
<td>material</td>
<td>material</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 mM ammonium acetate is used</td>
<td>Not used</td>
<td>Not used</td>
</tr>
<tr>
<td></td>
<td></td>
<td>chloroform:octanol is used</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.2 % ß-mercaptoethanol</td>
<td>0.2 % ß-mercaptoethanol</td>
<td>0.1 % ß-mercaptoethanol</td>
</tr>
<tr>
<td>5</td>
<td>chloroform:isoamylalcohol is</td>
<td>chloroform:octanol is used</td>
<td>chloroform:isoamylalcohol is used</td>
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<tr>
<td></td>
<td>used</td>
<td>used</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Incubated the sample for 30</td>
<td>Incubated the sample for 25</td>
<td>Incubated the sample for 25</td>
</tr>
<tr>
<td></td>
<td>minutes at 60°C</td>
<td>minutes at 60°C</td>
<td>minutes at 60°C</td>
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<tr>
<td>7</td>
<td>Spin at 6000 rpm for 10</td>
<td>Spin at 3000 rpm for 3 minutes</td>
<td>Spin at 3000 rpm for three minutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and then increase speed to 5000</td>
<td>and then increase speed to 8000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rpm for an additional 3 minutes</td>
<td>rpm for an additional 5 minutes</td>
</tr>
<tr>
<td>8</td>
<td>Wash pellet with 70% ethanol at</td>
<td>Wash pellet with 76% cold</td>
<td>Wash pellet with 70% ethanol at</td>
</tr>
<tr>
<td></td>
<td>room temperature.</td>
<td>ethanol</td>
<td>room temperature.</td>
</tr>
</tbody>
</table>
**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.](image)

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.
References


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