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Comparative Analysis and Optimization of Different DNA Extraction Protocols in *Satureja khuzistanica*

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Abstract

Genetic studies of plants are base on high efficiency of purified DNA samples. In this study, we optimized DNA extraction and PCR conditions of *Satureja khuzistanica* from Iran. The aerial organs of this plant contain high levels of essential oil which makes it difficult to DNA extraction with high quality and thereby intervened with subsequent PCR expansion. Four published DNA extraction protocols include Dellaporta (1983), Doyle and Doyle (1990), Murry and Thompson (1980), Kang and yang (2004) were compared for their capability to produce suitable quality DNA from *Satureja khuzistanica*. The protocol that provided the foremost DNA quality in the *Satureja khuzistanica* is selected. In Dellaporta method, no acceptable results were found because SDS buffer extraction was not attached to proteins well. In Doyle and Doyle, the obtained DNA was negligible. In kang and yang method, the quality of extracted DNA was not satisfying. Finally, in modified Murray and Thompson method, the extracted DNA has proper quality. In this method, these factors had the considerable effect on the quality of extracted DNA include: change of incubation time, high NaCl concentration, temperature changes in centrifugation stages, use of proteinase K and TE with high amount of salt and use of plant leaves before flowering stage.

Keywords: DNA extraction method, quality of DNA, *Satureja khuzistanica*, PCR

1. Introduction

Different plant species often may not allow optimal DNA production from one extraction protocol. For instance, some related species of the same genus require different extraction protocols. Thus, for each plant species, an efficient protocol for extraction of DNA as well as the optimization of the PCR conditions is required. Different protocols for DNA extraction have been applied to many plant species which were modified to provide DNA extraction protocol, suitable for several kinds of genetic studies in plants (Doyle & Doyle, 1987; Wang & Taylor, 1993). *Satureja khuzistanica* of the family Lamiaceae is an aborigine plant of Iran that is widely distributed in the West and South west Provinces of Iran (Jamzad, 1994). It is famous for its medical uses as painkiller and antiseptic in traditional medicine (Amanlou et al., 2004; Abdollahi et al., 2003; Hajhshemi et al., 2002). The unique chemical composition of essential oil of *Satureja khuzistanica* and phytochemical analysis of extract of this plant has been reported recently (Farzam et al., 2004). Medicinal and aromatic plants utilization and protection has attracted global attention. This plant contains secondary metabolites such as essential oils, flavonoids, alkaloids, phenols and terpenes which would interfere with the DNA extraction procedures (Abdollahi et al., 2003; Padmalatha & Prasad, 2006). The problems encountered in the extraction and purification of DNA specially from these plants include inhibitor compounds like polyphenols, depreciation of DNA due to endonucleases, co-isolation of highly viscous polysaccharides and other substances known for binding tightly to nucleic acids during DNA isolation and interfering with subsequent processes and reactions (Pirttila et al., 2001). Many protocols for DNA extraction from plant cells have been reported (Doyle & Doyle, 1990; Li et al., 2001; Pirttilä et al., 2001; Drabkova et al., 2002; Shepherd et al., 2002; Mogg & Bond, 2003). However, due to medicinal and aromatic plants contain high amounts of many different secondary metabolites; it

is unseemly that just one DNA extraction protocol to be appropriate for this plants (Loomis, 1974). In present study, four DNA extraction protocols in plants (Dellaporta, 1983; Doyle & Doyle, 1990; Murry & Thompson, 1980; Kang & Yang, 2004) were tested and compared for their capability to produce high quality DNA from *Satureja khuzistanica*. The extracted DNA by selected protocol would be used in the subsequent experiments such as PCR, RAPD analysis, enzyme digestion and other genetic studies.

2. Materials and Methods

2.1 Plant Material

Plant samples were collected at Research Farm of Khorraman Company, located at Khoramabad, Iran 35 km west of Khoramabad, Latitude 33°20', longitude 48°20', altitude 1190m , during growing season of 2011. The fresh and young leaves of *Satureja khuzistanica* were collected during the three ontogenesis stages (include: before flowering, flowering and after flowering) in July, August and September 2011 from Khorraman Company in Khorramabad (Lorestan province) and stored frozen in liquid nitrogen until DNA extraction. In laboratory samples after rapid freezing in liquid nitrogen were powdered. DNA extraction tests were done in 2011 in genomics laboratory Islamic Azad University, Khorramabad branch.

2.2 Testing DNA Extraction Protocols

The first protocol examined in this research was the Dellaporta DNA extraction protocol. Extraction buffer in this protocol was 10% SDS, 100 mM NaCl, 50 mM Tris/HCl, 10 mM EDTA, pH 8.0. 20 µL/mL mercaptoethanol and 0.01g polyvinylpyrrolidone (PVP) immediately added to extraction tubes as well as 5M potassium acetate, 3M sodium acetate, Isopropanol, 70% ethanol, TE-RNase solution and TE buffer.

The second method used in this study was Doyle and Doyle, as a "classical" protocol. Modified Acetyl Trimethyl Ammonium Bromide (CTAB) extraction protocol was used, which was applied in many plant species. Extraction buffer in this protocol was included: 2% CTAB, 100 mM Tris/HCl, 2% PVP, 1.4 M NaCl, 20 mM Ethylene Diamine Tetra Acetic acid (EDTA), pH 8.0. Add 20 µL/Ml mercaptoethanol, Chloroform: Isoamyl Alcohol 24:1 (CIA), Isopropanol, 70% ethanol, TE-RNase solution: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, 10 mg/mL RNase, immediately prior to use.

In third protocol we tested the method of Kang and Yang genomic DNA extraction procedure (Kang & Yang, 2004). A piece of plant leaves (about 40 mm^2) were put in a 2-ml microfuge tube. The young and small leaves tissue was homogenized in 50 µl DNA extraction buffer (500 mM NaCl, 100 mM Tris-HCl and 50 mM EDTA, pH 7.5). After an initial homogenization with homogenizer and then was added 150µl of buffer and homogenized with the same homogenizer for 20 seconds. Then, 20µl of 20% SDS were added to microfuge tube and vortexes for 30 seconds. The samples were incubated at 65°C for 10 minutes. An equal volume of isoamyl alcohol/chloroform/phenol (1:24:25) was added to the microtubes, vortexed for 30 seconds, and then centrifuged at 10,000g for 3 minute at 4°C. The supernatant was diluted 5 folds, and supernatant was used as the DNA template. About 1.5 g of powdered plant tissue was put in a 2-ml microfuge tube.

Extraction buffer solutions for modified DNA extraction Murray and Thompson were prepared according to Table 1.

Table 1. Preparation of 50 ml DNA extraction buffer for final concentration of storage volume interpretation solutions

solutions Reserved	Removable volume	Final concentration
%2 CTAB	1g	% 2
NaCl	ml 14	1.4M
8 = pH 1M, Tris HCl	ml 5	100 Mm
8 = pH 0.5 M, EDTA	2ml	20 Mm
ddH_2O	28	----

Modified DNA extraction method Murray and Thompson was as follow: About 1.5 g of powdered plant tissue and extraction buffer which was became already hot on the tissue powder and then dumped after one hour 65°C water bath placed and every five minutes gently shaking the samples. Proteinase K added to the samples at 37°C

was incubated for half an hour. Then Isoamylalcohol/Chloroform (1:24) was added to the samples and placed in centrifuge. After this stage, the supernatant removed and added high concentration of NaCl and then samples were centrifuges. Temperature used in the centrifuge process was changed to 27°C, and then added absolute ethanol to the supernatant and the samples were re-centrifuged. After about 15-20 minutes samples were maintained at 20°C. For rinsed the sample, 70% ethanol was added three times and each time the samples were centrifuged (at 4°C). After drying, Tris-EDTA (TE) buffer were added to the samples and stored in -20°C. Visualize DNA on 0.8% agarose gel and also spectrophotometer according to the wavelength of 260 nm absorbed by DNA and the ratio of 260/280 to determine the quantity and quality of DNA. Samples extracted are desired according to quantitative and qualitative tests of about 500 ng DNA which had 260/280 absorbance ratio in the 1.8-1.9.

3. Results and Discussion

Among of the four methods used in this study, modified Murray and Thompson methods showed the best results in Saturija khuzistanica. The quantity and quality of DNA extracted in this procedure was higher than other methods (Figures 1 & 2).

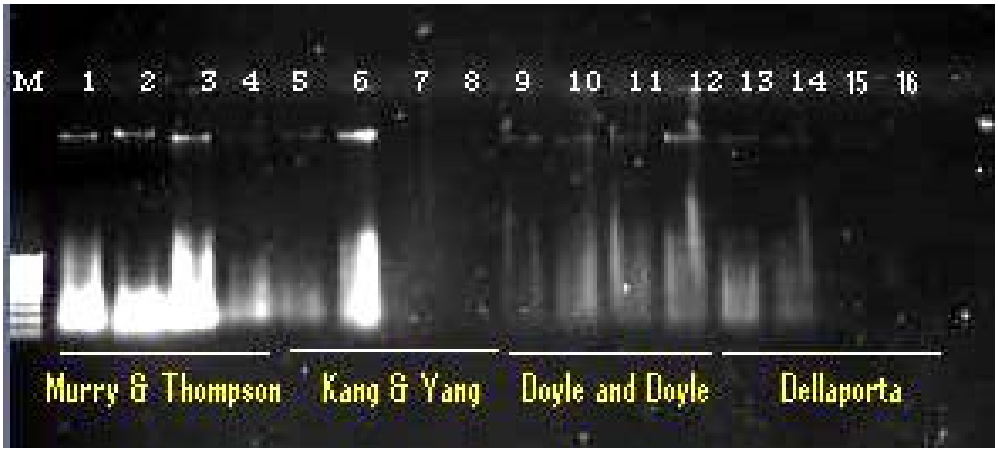

Figure 1. Comparison of DNA extracted in four methods before using the RNase. (5 µL DNA added to each one well)

Lane 1-4 DNA extracted in Murry and Thompson method. Lane 5-8 DNA extracted in Kang and Yang method. Lane 9-12 DNA extracted in Doyle and Doyle method and Lane 12-16 DNA extracted in Dellaporta method. M= 100bp size marker.

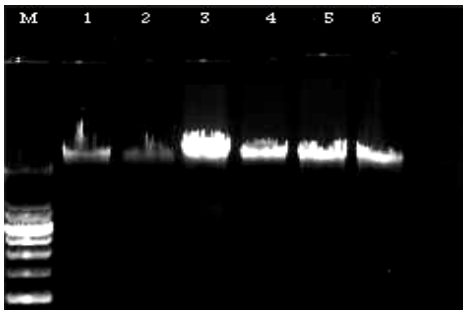

Figure 2. DNA extraction with Murray and Thompson method

Lane 1 and 2 = DNA extracted after flowering and lane 3, 4, 5 and 6 = extracted DNA before flowering. (5 µL DNA added to each well). M= 100bp size marker.

In Dellaporta method, potassium acetate was used for protein deposition. This protocol uses SDS as detergent and the addition of potassium acetate resulted in the removal of some polysaccharides and proteins as a complication with the potassium-SDS precipitate (Ribeiro & Lovato, 2007). DNA extracted with this method

had not good quantity and quality. This extraction method for *Satureja khuzistanica* did not show acceptable results because SDS buffer that used in this method attached to the secondary metabolit and prevented extraction DNA with high quality. Furthermore these results are in agreement with the findings of Ziegenhagen & Scholz (1993). The protocol of Doyle and Doyle is based on lyses and purification with CTAB that selectively precipitates DNA while maintaining the solubility of many polysaccharides (Ribeiro & Lovato, 2007). Padmalatha and Prasad (2006) proposes the addition of PVP along with CTAB which may bind to the polyphenolic compounds by forming a complex with hydrogen bonds and may help in removal of impurities to some extent. But this method in *Satureja khuzistanica* also had much success. And the extracted DNA obtained from this protocol had not good quality and quantity. DNA extracted with this method was surrounded by Ammonium acetate which causes formed gelatinous deposit. The protocol of Kang and Yang (2006) is suitable for extracting DNA from fresh calluses and plant leaves that is applicable to a variety of organisms regardless of the complexity of their genomes. Applying this method to extract DNA from *Satureja khuzistanica* due to the use of phenol, proteins and phenolic compounds bind to genomic DNA were separated well and DNA had good quality but quantity was very low.

Extraction DNA in *Satureja khuzistanica* before flowering stage had better result than after the flowering stage. The results showed that the use of fresh tissue in young plants have not considerable effect in the quality of DNA extracted from this plant. As shown in Figure 2, lane 1 and 2 are DNA extracted from after flowering stages and lane 3, 4, 5 and 6 are extracted DNA before flowering. As is observed DNA in lane 3, 4, 5 and 6 (extracted before flowering) had a better quality. According to spectrophotometer results, amount of DNA obtained before the flowering stage are 600-500 ng/µl and after flowering stage are 80-100 ng/µl, respectively. These results confirm the results of of Majd et al. (2009) investigation. Majd et al. (2009) showed that the highest amount of essential oil was observed before flowering stage and lowest amount was observed after flowering stage. The essential oils from *Satureja khuzistanica* contained carvacrol as the major constituents in all oil samples at three ontogenical stages. Lowest variability was observed in before flowering stage (9 compounds for dry and 11 compounds for the fresh material) and highest variability was observed in after flowering stage (18 compounds for dry and 17 compounds for the fresh material). Therefore, low variation in essential oil components before flowering can be the major reason for better quality of extracted DNA in this ontogenical stage.

Polymerase chain reaction (PCR) Using random RAPD primer OPA10 (sequence GTGATCGCAG) was carried out to compared DNA extracted quality (Figure 3). As the observed DNA extracted from the selected method (modified Murray and Thompson method), had good amplified and also had the good banding pattern (Figure 3).

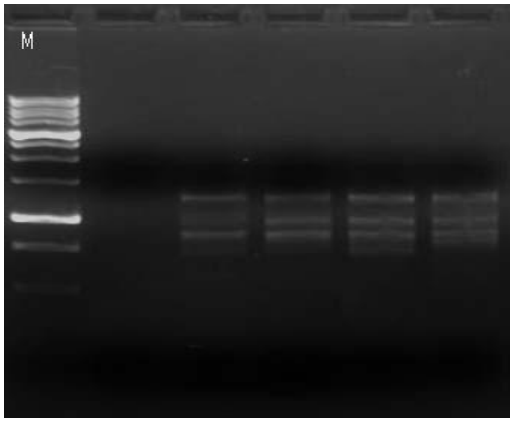

Figure 3. Polymerase chain reaction using OPA10 primer on DNA from leaf samples using modified Murray Thompson. M= 100bp size marker

4. Conclusion

The results showed that among of the four methods that were used for extraction DNA in *Satureja khuzistanica*, modified Murray and Thompson, in comparison with other methods had the best result which can subsequently be used for PCR extension. The DNA extracted with this method is suitable for other purposes include marker analysis. The method described here is simple and efficient for the isolation of DNA from medicinal plants that possess a wide range of secondary metabolite such as terpenoid compounds that can interfere with DNA extraction.

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