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Comparison of some DNA isolation methods in Chamomile (*Matricaria chamomilla L.*)

Amin Azadi[1*], Maryam zandipour[2], Arghavan Ghamsari[3], Saeede Khani[2]

[1]*Department of Plant Breeding, College of Agriculture, Yadegar-e- Imam Khomeini (RAH) Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agronomy & Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[3]*Department of Plant Breeding, Ferdowsi University of Mashhad, Mashhad, Iran*

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Abstract

There are different methods for DNA extraction in different plants. Quality and quantity of extracted DNA are important parameters. The presence of inhibitory compounds such as polyphenols and secondary metabolites can affect direct or indirect on DNA extraction process and cause reduction of DNA concentration and impurities of extracted DNA. In order to compare available method for DNA extraction in chamomile, a factorial experiment was conducted with a completely random design in three replicates. Factors were different DNA extraction methods (five common methods) and leaf tissue (young/mature). Quality and quantity of extracted DNA was evaluated with the ratio of absorbance at 260 to 280 nm and agarose gel electrophoresis. Method Doyle and Doyle (1987) had the highest DNA concentration among others with 141ng/µl. Quality and quantity of extracted DNA was higher in young leaves than mature.

*** Corresponding Author:** Amin Azadi ✉ azadi.amin@gmail.com

Introduction

Chamomile with scientific name of *Matricaria chamomilla L.* is an important herbal plant. This plant has different attributes likes: anti-inflamatory (Pourohit and Vyas, 2004), antiseptic and therapeutic (Franke and Schilcher, 2007), antimicrobial (Letchamo and Marquard, 1993) and antispasmodic (Manifesto *et al.,* 2001). Capitol is the part of plant which is collected in the spring and summer and used. Although this plant is anthropophilous, but due to the extensive use in the word, it is cultivated in large area. The origin of this plant was different part of Mediterranean region.

There are three problems in the isolation of high molecular weight DNA from plant species: (1) partial or total DNA degradation due to the presence of endogenous nucleases, (2) co-isolation of highly viscus polysaccharides witch render the handling of sample difficult and (3) co-isolation of polyphenols and other secondary plant compounds which cause damage to DNA and/or inhibit restriction enzymes and *Taq* polymerase (Weishing *et al.*, 1995).

The first step for DNA isolation from plant tissues is breaking the cell walls. It has been done with liquid nitrogen, pestle and mortar. The next step is to disrupt the cell membranes by using detergents. There are two main detergents: sodium dodecyl solphate (SDS) and cetyl trimethyl ammonium bromide (CTAB). For protected DNA from endogenous nucleases EDTA disodium salt should be used.

Michiels *et al.*, (2003) optimized CTAB for isolation of genomic DNA from latex-containing plants. Key steps in the modified protocol were the use of etiolated leaf tissue for extraction and an overnight 25 ˚C isopropanol precipitation step.

It has been reported that wild marigold (*Tagetes minuta* L.) contains high concentrations of essential oils, flavonoids, polyphenols, and polysaccharides that interfere with DNA (Shahzadi *et al.*, 2010). They used sun-dried, shade-dried and fresh-leaf tissues, as well as seeds for DNA analysis. The DNA obtained from seeds and fresh-leaf tissues with a modified cetyltrimethylammonium bromide buffer protocol was of good quality, with no colored pigments and contaminants. DNA extraction from grape has been difficult due to the presence of contaminants such as polyphenols and polysaccharides. The presence of these contaminants in DNA preparations often makes the samples viscous and renders DNA unrestrictable in endonuclease digestion and unamplifiable in PCR (Lodhi *et al.*, 1994).

Due to the different types and amount of chemical compounds in each medicinal plant, there are different methods of DNA extraction for some of them and many researchers don't know which method have the best result. Also usually no one has compared available DNA extraction methods for many plants such as chamomile.

Here we compare five common methods to identify best DNA extraction procedure for chamomile.

Materials and methods

Plant materials

Leaf tissues (young/mature) of *Matricaria chamomilla L.* were applied in this experiment.

Experimental Design

a factorial experiment was conducted with a completely random design in three replicates. Factors were different DNA extraction methods (Dellaporta *et al.*, (1983), Murry & Thampson, (1984), Pirtila *et al.*, (20010, Saghai–Maroof *et al.*, (1984) and Doyle and Doyle, (1987) for,) and leaf tissue (young/mature).

Data Analysis

Quality and quantity of extracted DNA was evaluated with the ratio of absorbance at 260 to 280 nm and agarose gel electrophoresis. ANOVA was conducted with the GLM procedure of SAS 8.2 and the significant differences between treatments were determined using least significant difference (LSD) test at probability level of 0.01.

Results

As shown in Table 1, there were significant differences

at $P<0.01$ level among DNA extraction methods for all traits. The Doyle and Doyle, (1987) method was superior among all other methods (Table 2 and Fig. 1). After it, Murry & Thampson, (1984) had higher DNA concentration among others. The most appropriate absorbance ratios were belong to Doyle and Doyle, (1987) and Pirtila *et al.*, (2001), with 1.82 and 1.73 respectively (Table 2). Thus, the quality of extracted DNA was pure and suitable. Other DNA extraction methods didn't have acceptable results and concentrations of DNA were lower than 100 ng/µl (Table 1 and 2). There were significant differences at the level of 0.01were observed between types of leaf tissues for both traits. Leaf tissue used in present study was effective in variation of DNA concentration and absorbance ratios. Quality and amount of DNA was higher in young leaves. No significant interaction was observed between DNA extraction and leaf tissue methods.

Table 1. Analysis of variance for DNA concentration and ratio of absorbance at 260 to 280 nm as affected by DNA extraction methods and leaf tissue in Chamomile.

Source of Variations	Degree of freedom	Mean square	
		ratio of absorbance 260/280	DNA concentration
DNA extraction methods	4	0.17^{**}	31730.48^{**}
leaf tissue	1	0.67^{**}	2235.33^{**}
DNA extraction× leaf tissue methods	4	0.002^{ns}	9.20^{ns}
Error	20	0.001	8.88
CV (%)		1.86	3.41

ns, *, **: No significant difference and significant difference on 1 and 5 % levels of probability, respectively.

Table 2. comparisons of mean for main effect.

↓ treatment traits →	ratio of absorbance 260/280	DNA concentration(ng/µl)
DNA extraction methods		
)Dellaporta *et al.*, 1983(	1.70b	42.1d
)Murry & Thampson, 1984(	1.59c	99.4b
)Pirtila *et al.*, 2001(	1.73b	75.0c
)Saghai–Maroof *et al.*, 1984 (	1.68b	79.3c
)Doyle and Doyle, 1987(	1.82a	141.0a
leaf tissues		
young	1.85a	95.5a
mature	1.55b	79.24b

Means with the same letter at each column are not significantly different at $P<0.01$ level.

Discussion

With access to a single protocol for DNA extraction in each plant, many problems due to consumption of chemicals and time-consuming will reduced and may provide a cost-effective method for DNA extraction. Concentration of DNA in Doyle and Doyle, (1987) method was above 100 ng/µl which is suitable for most of molecular markers (Table 2). This method was used in fruit tress (Degani *et al.*, 2003). Absorbance ratios based on user manual of NanoDrop, used to assess the purity of extracted DNA and RNA according to Table 2. Because of using restriction enzyme in AFLP and RFLP markers, purity and concentration of DNA are very important. It seems that the application of an appropriate concentration of 2 - mercaptoethanol and PVP on extraction buffer of this method, eliminated polyphenols in the leaf samples and therefore DNA

quality has increased. PVP creates complex via hydrogen bonds with polyphenolic substances and makes it possible to separate them from the DNA molecule. High levels of phenolic compounds, especially flavonoids have been proven in this plant (Kato *et al.*, 2008; Mortazaei *et al.*, 2012). Certain concentration of NaCl can be used for removing polysaccharides (which reduce the quality of the DNA) (Cheng *et al.*, 2003). Average concentration of extracted DNA in Doyle and Doyle (Doyle and Doyle, 1987) method was 141 ng/ µl that was higher than other methods. DNA extracted by other methods did not have favorable results in terms of the concentration. Changes in the amount and concentration of the materials used to prepare the extraction buffer may be the most likely reason. CTAB is used to cause lysis of the cell membrane. The CTAB as a detergent and PVP remove polyphenols and polysaccharides, while the ascorbic acid, DIECA and 2- mercaptoethanol reduce oxidation (De la Cruz *et al.*, 1997).

Table 3. Ratio of sample absorbance at 260 and 280 nm according to Nanodrop manual.

ratio of absorbance at 260/280 nm	Result
~1.8	DNA pure
~2.0	RNA pure
appreciably lower in either case	presence of protein, phenol or other contaminants

Absorption ratio of less than one represents the inability of methods for the removal of phenolic compounds, proteins and other compounds that may be extracted along with the DNA, because these compounds have a higher absorption at 280 nm.

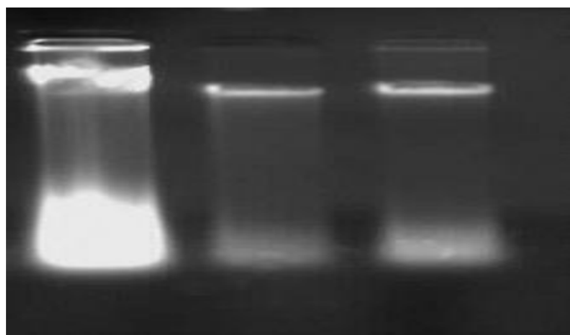

Fig. 1. Agarose gel electrophoresis for Doyle and Doyle (1987) Method.

Secondary metabolites which are abundant in fruit trees, medicinal herbs and more desert plants cannot be fully removed in the classic methods of DNA extraction. The presence of these compounds, cause to lower quality of results. Jenderek *et al.*, (1997) used Doyle and Doyle, (1987) method for extracting DNA in hibiscus plant.

In general, young leaves have less secondary compounds and polysaccharide than other parts of the plant. It led to use young leaves for DNA extraction in many cases. This leaves could be easily powder by liquid nitrogen and then extraction buffer easily reach to all parts.

Conclusion

In each of DNA extraction methods, attention to phenolic compounds, polysaccharides and other contaminants, may increase the quality of extracted DNA is the classical methods.

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