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Original Article

# Comparative Analysis of the Genomic DNA Isolation Methods on *Inula* sp. (Asteraceae)

Emre SEVINDIK[1]*, Fatih COSKUN[2], Zehra Tugba MURATHAN[3], M. Yavuz PAKSOY[4], Veysel UZUN[2]

[1*]*Department of Agricultural Biotechnology, Faculty of Agriculture, Adnan Menderes University, Aydın, Turkey; ph.d-emre@hotmail.com (*correspomding author)*

[2]*Balikesir University, Department of Biology, Cagis Campus, Balıkesir, Turkey; fcoskun@balikesir.edu.tr*

[3]*Ardahan University, Faculty of Engineering, Department of Food Engineering, Ardahan, Turkey; ztugbaabaci@hotmail.com*

[4]*Tunceli University, Faculty of Engineering, Department of Environmental Engineering, Tunceli, Turkey; mypaksoy@gmail.com*

**Abstract**

Simple, fast, low-cost and high throughput protocols are required for DNA isolation of plant species. In this study, phenol chloroform isoamyl alcohol and commercial (Sigma) DNA isolation kit methods were applied on some *Inula* species that belong to Asteraceae family. Genomic DNA amounts, $A_{260}$, $A_{280}$, $A_{260}/A_{230}$ and purity degrees ($A_{260}/A_{280}$) that were obtained through both methods were measured through electrophoresis and spectrophotometer. Additionally, PCR amplification was realized by primer pairs specific to nrDNA ITS, cpDNA *ndh*F (972F-1603R) and *trn*L-F regions. Results showed that maximum genomic DNA in nanograms obtained by phenol chloroform isoamyl alcohol method. The study also revealed that *I. macrocephala* had the maximum DNA and *I. heterolepis* had the minimum DNA amount. $A_{260}/A_{280}$ purity degrees showed that the highest and lowest purity in gDNAs obtained through phenol-chloroform isoamyl alcohol method were in *I.aucheriana* and *I. salicina*, respectively. The highest and lowest purity degrees of gDNAs obtained through commercial kit was observed in *I. fragilis* and *I. macrocephala* samples, respectively. PCR amplification results showed that while band profiles of each three regions (ITS, *trn*L-F and *ndh*F) did not yield positive results in PCR amplifications using phenol-chloroform isoamyl alcohol method; PCR band profiles obtained through commercial kit yielded positive results. As a result, it is fair to say that the relation of genomic DNA with PCR was found to be more efficient although the maximum amount of genomic DNA was obtained through phenol chloroform isoamyl alcohol method.

***Keywords:*** amplification, commercial kit, genomic DNA isolation, Inula, PCR

## Introduction

Polymerase chain reactions have brought about radical changes in molecular biology and genetic studies for plants, animal and many microorganisms. Usage of molecular markers such as RAPD, ISSR, RFLP, SSR and QTL in plant breeding, PCR based DNA marker analysis, some crop plants, populations and species for genetic diversity and mapping studies with the development of PCR based markers within the last years has paved the way for many different studies (Lee, 1995; Sant *et al.*, 1999; Francia *et al.*, 2005; Liu *et al.,* 2005; Dwivedi *et al.*, 2007; Mackill, 2007; Akhtar *et al.,* 2010; Vural and Akçın, 2010). Characteristically, a considerable number of plaint species contain a significant level of polysaccharides, terpenes, polyphenols and other secondary metabolites. It was revealed that these substances bind to nucleic acids firmly during DNA isolation and inhibit Taq- polymerase enzyme and block PCR amplification (Fang *et al.*, 1992; Pirttila *et al.*, 2001; Kermekchıev *et al.*, 2009). For this reason, it is particularly essential to obtain DNA material to be used in the studies within a short time and in a pure form. A simple, rapid, low-cost, high yield protocol with minimum contamination and high safety is needed for DNA isolation of any plant species. The process of obtaining genomic DNA includes steps such as eliminating the cell membrane, disintegrating and removing the proteins and purifying the DNA after precipitation. Various chemicals must be used in different amounts in order to apply these methods (Bailes *et al.*, 2007).

Genus *Inula* belongs to Asteraceae family and is distributed throughout the world. Many species of this genus are being used by the local people for different purposes. The species of this genus are known for anticancer, antibacterial, cytotoxic

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and anti-inflammatory properties. The investigation on *Inula* species has brought various significant bioactive components of this genus into view. These components are sesquiterpenes, lactones, flavonoids, glycosidases and phenolic compounds (Ulubelen *et al*., 1987; Vajs *et al*., 1989; Shao *et al*., 1996; Ahmed *et al*., 2003; Qi *et al*., 2008). It is difficult to obtain pure genomic DNA from these species, which are rich in secondary components. This is because their compounds pose barrier to obtain pure DNA and block successful PCR reaction. Various studies are being conducted and methods are being developed in order to prevent such disadvantages. The aim of this study was to obtain DNA from species of *Inula* spp. (Asteraceae) with different methods, to multiply different gene regions of DNA samples obtained via two different methods with different primers, and to determine the suitability of the methods and primers. To fulfill this goal, two different genomic DNA isolation methods, which are phenol chloroform and commercial kit (SIGMA) methods, were applied on plant samples. Genomic DNAs obtained with two different isolation methods were multiplied via primers specific to ITS (nrDNA), *trn*L-F and *ndh*F (972F-1603R) (cpDNA) regions, and it was determined which method was suitable for which primer. This study aimed to make contribution to the molecular biology, agricultural biotechnology and biochemical studies to be carried out in the years to come by optimizing DNA isolation method of some *Inula* species, which are economically valuable due to the secondary compounds that they contain.

## Materials and Methods

*Plant samples*

All plant materials used in this study were collected from different localities in Turkey during field works from Summer 2013. A total of 12 taxa (6 endemic taxa) *Inula* accessions were used in the current study. For each population collection of information related to the region and subgenera are shown in Table 1.

*DNA extractions*

*Isolation with modified phenol chloroform isoamyl alcohol protocol*

Dellaporta *et al.* (1983) modified and used DNA isolation method. In this method, almost 1 gr leaf tissue is crushed within liquid nitrogen. The samples crushed are put into eppendorf tubes, and 600 µl isolation buffer is added and dissolved. 500 µl phenol chloroform isoamyl alcohol were added onto the sample within the tube and centrifuge is performed. Thus, proteins precipitate and DNA rises to the top. The supernatant formed is decanted to the new tube and 3M NaAc, pH = 5.2, which equals to the 10% of supernatant volume, is added into it. 500 µl isopropanol is added. At this phase, DNA is seen with naked eye. DNA is precipitated through centrifuge, and pellet is formed on the bottom. The solution on the top is decanted into the waste bottle. 500 µl TE (10mM, pH=8) is added into the pellet formed (It is required to dissolve with pipetting). 5 µl RNaz A is added and the tube is turned upside down. Lipoid layer is made homogeneous after pipetting. It is incubated 37 °C for 30 minutes and RNA is migrated away. Later on, 50 µl NaAc (3M) is added and tube is turned upside down. Later on, 1 ml 90% ETOH is added and the tube is turned upside down. It is kept for 10 minutes at -80 °C and precipitation is achieved by performing centrifuge when the waiting time is over. Ethanol is decanted. Supernatant on the top is put into waste bin, and the pellet settles to the bottom. The remaining solution is washed with 70% ETOH (by performing pipetting) and centrifuge is performed. The ethanol on the top is removed after centrifuge and pellet settles on the bottom. The pellet formed is washed with 90% ETOH and centrifuge is performed. Following centrifuge, the ethanol on the top is removed. Once again, pellet is washed with 90% ETOH and centrifuge is performed. Later, ETOH is removed and pellet remains. The pellet settled on the bottom is put on the blotting paper and the ethanol is evaporated. Lastly, 50 µl TE is added on the pellet on the bottom and is made ready for use after dissolving properly.

*Isolations with Sigma DNA isolation kit*

Plant samples were isolated by using SIGMA G2N70 Plant Genomic DNA Miniprep Kit. After 1 gr plant sample is powdered with liquid nitrogen, 350 µl lysis solutions (Part A) is added on it. Next, 50 µl lysis solution (Part B) is added onto it and vortex is performed. 4 µl RNaz A is added onto this mixture. It is left to incubation at 65 °C for 10 minutes in water bath. 130 µl precipitation solution is added on the samples. They were put into ice for 5 minutes and centrifuge is performed consecutively. Liquid part is decanted to the blue filtered tube. Centrifuge is performed and column is removed and collection tube remains. 700 µl binding solution is added and almost a total of 1000 µl volume is formed. Later on, binding column is prepared. To this end, 500 µl column preparation solution is added to the red columned tubes and centrifuge is performed. Collection tubes are removed and red filtered colons are taken. Thus, binding colon becomes ready. 700 µl of the solution in collection tubes in the previous step is transferred to the new red columned tubes and centrifuge is performed and liquid is removed and collection tube remains. Red colons are put into collection tubes again. The remaining

Table1. Collection localities of the *Inula* taxa

| Taxa | Locations/Date | Taxa | Locations/Date |
|---|---|---|---|
| **Inula helenium* subsp. *orgyalis* (Boiss) Grierson | Kastamonu, Eflani, Turkey, 01.08.2013 | *Inula heterolepis* (Boiss) | Manisa, Sipil Mountain, Turkey, 26.07.2013 |
| **Inula macrocephala* (Boiss &: Kotschy ex Boiss) | Muş, Malazgirt, Turkey, 15.08.2013 | **Inula fragilis* (Boiss. & Hausskn) | Malatya, Beydağı, Turkey, 14.08.2013 |
| *Inula inuloides* (Fenzl) Grierson | Van, Çatak, Turkey, 16.08.2013 | **Inula anatolica* (Boiss) | Denizli, Pamukkale, Turkey, 27.07.2013 |
| *Inula salicina* (Linnaeus) | Konya, Taşkent, Turkey, 27.07.2013 | **Inula sechmenii* (Hartvig & Strid): | Antalya, Turkey, 28.07.2013 |
| *Inula mariae* (Bordz) | Ağrı, Doğubeyazıt, Turkey, 16.08.2013 | **Inula discoidea* (Boiss): | Muş, Malazgirt, Turkey, 15.08.2013 |
| *Inula britannica* (Linnaeus) | Çankırı, Atkaracalar, Turkey, 01.08.2013 | *Inula aucheriana* (DC) | Denizli, Pamukkale, Turkey, 27.07.2013 |

* Endemic

300 µl solution is passed from the colon and centrifuge is performed. Liquid and colon are removed. Colon is inserted into a new collection tube in order to be washed. 500 µl wash solution is added and centrifuge is performed. Liquid is removed and collection tube remains. Wash solution is added again and centrifuge is performed. Liquid is removed and collection tube remains. Wash solution is added again and is centrifuged. Liquid is decanted without contacting the colon. Colon is placed into new collection tubes. 100 µl elution solution is added onto it and is centrifuged. The colon is decanted without contacting the liquid and is placed into the collection tubes. 100 µl elution solution is added again and is centrifuged. Column is removed and DNAs are made ready to use.

*Determining purity and quantity of DNA samples*

The amount of the DNA samples isolated through two different methods was determined through gel electrophoresis. To this end, 0.8% gel electrophoresis was used. $A_{260}/A_{280}$ absorbance ratios were measured through spectrophotometer with the aim of determining genomic DNA purity, and purity and amount were calculated. $A_{260}/A_{280}$ nm absorbance ratio of 1,8 signifies a pure DNA sample. Lower values indicate contamination by proteins; the presence of RNA increases the ratio above 2,0 (Sambrook *et al*., 1989; Puchooa and Khoyratty, 2004). When the $A_{260}/A_{230}$ nm ratio is greater than 1,8 the DNA preparations are free from contaminations by polysaccharides (Peterson *et al*., 1997; Singh *et al*., 1999; Chen and Ronald, 1999; Ahmad *et al*., 2004; Aleskıc *et al*., 2012). Table 1 illustrates the spectrophotometric measurements and purity levels and amounts of the genomic DNAs of *Inula* taxa.

*PCR amplifications*

ITS (nrDNA), *trn*L-F and *ndh*F (cpDNA) primers were used in multiplying gDNAs obtained with the aim of determining the suitability degree of DNA isolated from the samples for PCR reactions. Amplification of the whole ITS region (ITS1 + 5.8S+ ITS2) was performed with primers ITS5A 5' CCTTATCATTTAGAGGAAGGAG 3' and ITS4 5'TCCTCCGCTTATTGATATGC 3' (White *et al.,* 1990). The amplification process was performed in 25 µl of PCR reaction volume. Each PCR reaction contained 2.5 µl of Taq buffer, 1.5 µl of magnesium chloride ($MgCl_2$), 0.4 µl of dNTP, 2.5 µl for ITS4 and 2.5 µl for ITS5 primers, 0.3 µl of Taq DNA polymerase, 1.5 µl of total genomic DNA (sometimes, 1µl, 0,5µl, $10^{-1}$,$10^{-2}$ dilitued) and 11,8 µl of $ddH_2O$. PCR amplification was performed with an initial denaturation step of 94 °C for 5 min, followed by 35 cycles of strand denaturation at 94 °C for 1 min, annealing at 50 °C for 45 s, and primer extension at 72 °C for 1 min, and a final elongation at 72 °C for 5 min.*trn*L-F moleculer marker analyzed in this study belongs to the chloroplast genome (cpDNA). Polymerase Chain Reaction (PCR) amplifications of *trn*L-F cpDNA were performed using the primers designed by Taberlet *et al.* (1991) *trn*Le 5' GGTTCAAGTCCCTCTATCCC 3', *trn*Ff 5'ATTTGAACTGGTGACACGAG 3' for all taxa included in this work. The amplification process was performed in 25 µl of PCR reaction volume. Each PCR reaction contained 2.5 µl of Taq buffer, 1.5 µl of magnesium chloride ($MgCl_2$), 0.4 µl of dNTP, 2.5 µl for ITS4 and 2.5 µl for ITS5 primers, 0,3 µl of Taq DNA polymerase, 1,5 µl of total genomic DNA (sometimes, 1µl, 0,5µl, $10^{-1}$,$10^{-2}$ diluted), and 11,8 µl of $ddH_2O$. PCR amplification was performed with an initial denaturation step of 94 °C for 5 min, followed by 35 cycles of strand denaturation at 94 °C for 30 sec, annealing at 53 °C for 45 s, and primer extension at 72 °C for 90 sec, and a final elongation at 72 °C for 8 min *ndhF* moleculer marker analyzed in this study belongs to the chloroplast genome (cpDNA). Polymerase Chain Reaction (PCR) amplifications of *ndh*F cpDNA were performed using the primers designed by Olmstead and Sweere (1994) *ndh*F 972F5'GTCTCAATTGGGTTATATGA3',*ndh*F1603R5' GCATAGTATTTCCCGTTTCATGAGG 3'. The PCR amplification of *ndh*F DNA was carried out in total reaction volume of 25 µl containing 2.5 µl of Taq buffer, 1.5 µl of Magnesium chloride ($MgCl_2$), 0.4 µl of dNTP, 2.5 µl for *ndh*972F and 2.5 µl for *ndh*1603R primers, 0.3 µl of Taq DNA polymerase, 2.0 µl of total genomic DNA, and 10.8 µl of $ddH_2O$. The thermocycling profile consisted of an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 30 sec. 94°C, 45 sec. from 50 to 55°C, 1 min at 72 °C and final extension step of 10 min at 72 °C. The amplification products were resolved by electrophoresis in %0.8 agarose gels. The bands were visualized using ethidium bromide staining.

**Results and Discussion**

After two different DNA isolation methods were applied on the *Inula* genus taxa, both genomic DNAs and PCR products were processed within 0.8% agarose gel at 120V for 35 minutes, and photos of the gel were taken (Figure 1-8). Both phenol chloroform isoamyl alcohol method and commercial kit (Sigma) DNA isolation protocols were applied on 12 taxa of *Inula* genus in the study. It was found that the highest nanogram (ng) level of DNA is obtained through phenol chloroform isoamyl alcohol method in 1µl stock. The highest and the lowest DNA amounts were obtained from *I. macrocephala* (908 ng) and *I. heterolepis* (148 ng), respectively with this method (Table 2). It was found that the highest and lowest DNA amounts obtained using commercial kit (SIGMA) were from *I. fragilis* (204 ng) and *I. discoidea* (86 ng), respectively (Table 2). When the $A_{260}/A_{280}$ absorbance rates of the DNA are examined, it was seen that the highest and the lowest purity degrees in gDNAs obtained through commercial kit isolation were observed in *I.fragilis* (1.457) and *I.macrocephala* (1.046), respectively. The highest and lowest purity degrees in gDNAs obtained through phenol chloroform isoamyl alcohol were observed in *I.aucheriana* (1.337) and *I.salicina* (1.023) samples, respectively (Table 2). When the gel images obtained from DNA isolations were examined, it was seen that the DNA bands obtained through phenol chloroform isoamyl alcohol method were brighter compared to the ones, which were obtained through commercial kit (SIGMA) method. It was also found that the amount of DNA obtained through phenol chloroform isoamyl alcohol method is higher than the amount obtained through commercial kit (SIGMA) in all samples; however, PCR results were worse in the DNAs obtained through phenol chloroform isoamyl alcohol (Table 2).

In PCR performed by using primers specific to nrDNA, ITS and cpDNA (*trn*L-F and *ndh*F) regions, DNA samples

Table 2. Spectrophotometric measurements, purity amounts, and DNA amounts of the DNAs belonging to the *Inula* taxa collected (C: Commercial Kit (Sigma), F: Phenol-cloroform isoamil)

| Taxa | Plant amount (g) | A260 | A280 | A230 | A260/280 | A260/230 | DNA amounts (ng) |
|---|---|---|---|---|---|---|---|
| *I. anatolica* | 0,96 | 0,056(C)<br>0,090(F) | 0,051(C)<br>0.084(F) | 0.096(C)<br>0.142(F) | 1.098(C)<br>1.071(F) | 0.583(C)<br>0.633(F) | 112(C)<br>180 (F) |
| *I. fragilis* | 0,95 | 0.102(C)<br>0.125(F) | 0.070(C)<br>0.114(F) | 0.177(C)<br>0.192(F) | 1.457(C)<br>1.096(F) | 0.576(C)<br>0.651(F) | 204(C)<br>250(F) |
| *I.sechmenii* | 0,82 | 0.051(C)<br>0.284(F) | 0.048(C)<br>0.252(F) | 0.083(C)<br>0.398(F) | 1.062(C)<br>1.126(F) | 0.614(C)<br>0.713(F) | 102(C)<br>568(F) |
| *I.mariae* | 0,89 | 0.051(C)<br>0.167(F) | 0.045(C)<br>0.145(F) | 0.113(C)<br>0.227(F) | 1.133(C)<br>1.151(F) | 0.451(C)<br>0.735(F) | 102(C)<br>334(F) |
| *I.heterolepis* | 0,81 | 0.054(C)<br>0.074(F) | 0.050(C)<br>0.068(F) | 0.098(C)<br>0.110(F) | 1.080(C)<br>1.088(F) | 0.551(C)<br>0.672(F) | 108(C)<br>148(F) |
| *I.macrocephala* | 0,90 | 0.068(C)<br>0.454(F) | 0.065(C)<br>0.399(F) | 0.174(C)<br>0.622(F) | 1.046(C)<br>1.137(F) | 0.390(C)<br>0.729(F) | 136(C)<br>908(F) |
| *I.britannica* | 0,96 | 0.058(C)<br>0.102(F) | 0.050(C)<br>0.091(F) | 0.089(C)<br>0.167(F) | 1.160(C)<br>1.120(F) | 0.651(C)<br>0.610(F) | 116(C)<br>204(F) |
| *I.salicina* | 0,93 | 0.069(C)<br>0.133(F) | 0.056(C)<br>0.130(F) | 0.257(C)<br>0.208(F) | 1.232(C)<br>1.023(F) | 0.268(C)<br>0.639(F) | 138(C)<br>266(F) |
| *I.inuloides* | 0,93 | 0.068(C)<br>0.157(F) | 0.057(C)<br>0.143(F) | 0.108(C)<br>0.260(F) | 1.192(C)<br>1.097(F) | 0.629(C)<br>0.603(F) | 136(C)<br>314(F) |
| *I.helenium subsp. orgyalis* | 0,91 | 0.060(C)<br>0.114(F) | 0.051(C)<br>0.102(F) | 0.094(C)<br>0.177(F) | 1.176(C)<br>1.117(F) | 0.638(C)<br>0.644(F) | 120(C)<br>228(F) |
| *I.aucheriana* | 0,89 | 0.053(C)<br>0.115(F) | 0.047(C)<br>0.086(F) | 0.097(C)<br>0.145(F) | 1.127(C)<br>1.337(F) | 0.546(C)<br>0.793(F) | 106(C)<br>230(F) |
| *I.discoidea* | 0,97 | 0.043(C)<br>0.277(F) | 0.033(C)<br>0.264(F) | 0.068(C)<br>0.471(F) | 1.303(C)<br>1.049(F) | 0.632(C)<br>0.588(F) | 86(C)<br>554(F) |

Table 3. PCR amplification results of ITS, *trn*L-F and *ndh*F primers and success rates

| | nrDNA (ITS) | | cpDNA (*ndh*F) | | cpDNA (*trn*L-F) | | Success rates | | |
|---|---|---|---|---|---|---|---|---|---|
| Isolation Protocol | + band | - band | + band | - band | + band | - band | ITS | *ndh*F | *trn*L-F |
| Phenol Chloroform Isoamyl Alcohol | 3 | 9 | 5 | 7 | 4 | 8 | %25 | %41 | %33 |
| Commercial Kit | 9 | 3 | 11 | 1 | 10 | 2 | %75 | %91 | %83 |

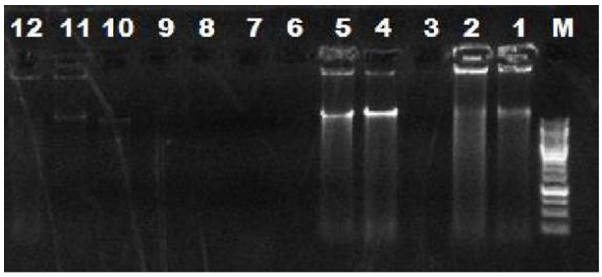


Fig. 1. Gel image of *Inula* gDNA's obtained by Phenol Chloroform Isoamyl Alcohol Protocol 1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina*, 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

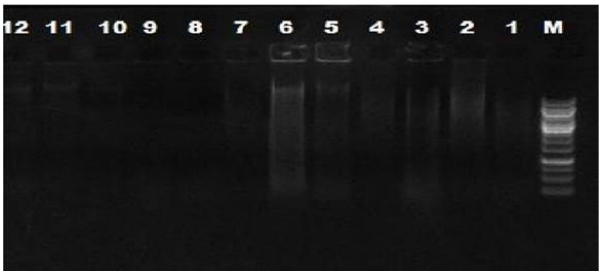


Fig. 2. Gel image of Inula gDNA's obtained by Comercial Kit. 1. *I. anatolica,* 2. *I. fragilis,* 3. *I. sechmenii,* 4. *I. mariae,* 5. *I. heterolepsis,* 6. *I. macrocephala,* 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides,* 10 . *I. helenium subsp. orgyalis,* 11. *I. aucheriana,* 12. *I. discoidea*

obtained with commercial kit (SIGMA) gave better results. ITS PCR results of the DNAs obtained through phenol isoamyl alcohol method gave positive results in only 3 out of 12 samples. A test was performed using different amounts of gDNA (0.5ul, 1ul, 2ul, 2.5ul) while isolation mixture was being prepared. However, the result remained the same. PCR success rate in phenol chloroform isoamyl alcohol method based on ITS primers was 25% (Table 3). PCR procedure of the gDNAs obtained through commercial kit (SIGMA) was very successful and while 9 out of 12 samples gave positive results, no result was obtained in 3 samples. Success rate of PCR rose to 75% with this method (Table 3). When PCR photos obtained using primers specific to cpDNA *ndh*F regions of gDNAs obtained through phenol chloroform isoamyl alcohol method were examined, it was seen that only 5 samples gave positive results and no result was obtained from 7 samples. In this case, PCR success rate became 41% (Table 3). However, when PCR results of gDNAs obtained through commercial kit (SIGMA)

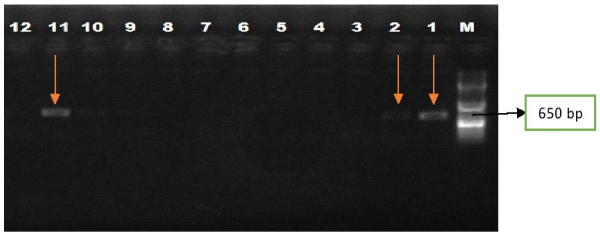


Fig. 3. Gel image of ITS primer amplified *Inula* gDNA's by Phenol Choroform Isoamyl Alcohol Protocol1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

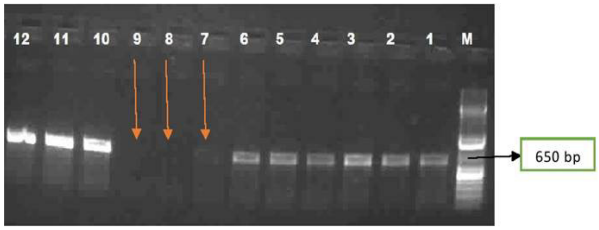


Fig. 4. Gel image of ITS primer amplified *Inula* gDNA's by Comercial Kit.1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

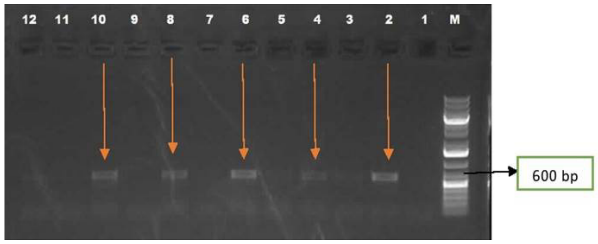


Fig. 5. Gel image of ndhF primer amplified *Inula* gDNA's by Phenol Choroform Isoamyl Alcohol Protocol 1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

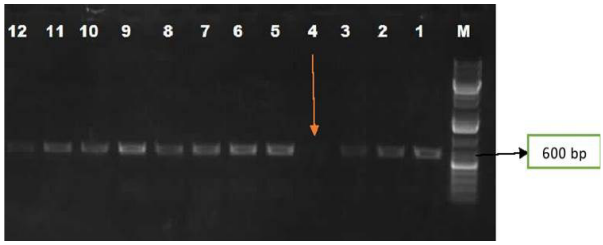


Fig. 6. Gel image of ndhF primer amplified *Inula* gDNA's by Commercial Kit. 1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

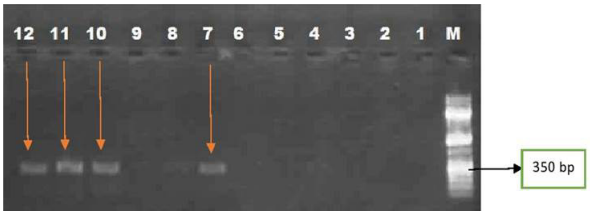


Fig. 7. Gel image of trnL-F primer amplified *Inula* gDNA's by Phenol Choroform Isoamyl Alcohol Protocol 1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

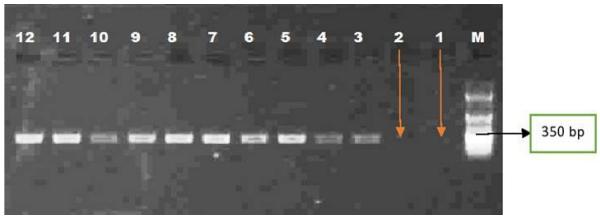


Fig. 8. Gel image of trnL-F primer amplified *Inula* gDNA's by Commercial Kit. 1. *I. anatolica*, 2. *I. fragilis*, 3. *I. sechmenii*, 4. *I. mariae*, 5. *I. heterolepsis*, 6. *I. macrocephala*, 7. *I. britannica*, 8. *I. salicina,* 9. *I. inuloides*, 10 . *I. helenium subsp. orgyalis*, 11. *I. aucheriana*, 12. *I. discoidea*

method were examined, it was seen that no result was obtained only in 1 sample and highly positive results were obtained in 11 samples. In this case, PCR success rate became almost 91%. According to the PCR results obtained using primers specific to cpDNA *trn*L-F regions of gDNAs obtained through phenol chloroform isoamyl alcohol method, positive results were obtained from 4 samples and PCR success rate was 33% (Table 3). When the PCR results of gDNAs obtained through commercial kit (SIGMA) method were examined, no positive results were obtained only from 2 samples, whereas 10 samples gave very positive results. In this case, PCR success rate rose to 83% (Table 3).

Previous studies performed in the past applied different genomic DNA isolation methods on plants, likens and algae and new techniques were developed. Cingilli and Akçin (2005) applied mini-prep CTAB and micro-prep CTAB methods on 49 chick peas (*Cicer* ssp.). At the end of the study, higher amount and higher quality genomic DNAs were obtained in comparison with the genomic DNAs obtained through mini prep method. The method was found to be highly economic with respect to labor force, cost and chemical material usage. It was also proven with band profiles through which suitable pure DNA was obtained by amplifying chick pea gDNAs obtained through mini prep CTAB protocol by using UBC181 RAPD primers. Aras and Cansaran (2006) applied a different genomic DNA isolation method for a series of analysis in herbarium samples of some lichens. In the study, as a result of the comparison of the protocol based on hexadecyltrimethylammonium bromide (CTAB) and the protocol based on SDS it was found that gDNAs obtained

through CTAB method were more efficient for lichens. Şimşek *et al.* (2008) obtained gDNA by applying MiniPrep DNA isolation method and the modified versions of this method on hazelnut, avocado, persimmon, tangerine. In the methods tested, isolation buffers were prepared in a way in which CTAB is single, CTAB-PVP are together, CTAB- SDS are together and SDS is single. Concentration and quality of these DNAs obtained after this isolation method were compared and interpreted. Ogunkanmi *et al*. (2008) obtained genomic DNA from different pepper tissues by revising DNA isolation protocol modified by Dellaporte *et al.* (1983). During isolation, no liquid nitrogen or phenol chloroform extraction was required. It was determined that $A_{260}/A_{280}$ absorbance ratio of gDNAs obtained from tissues was between 1.7 and 1.9. It was revealed that DNA amount obtained from fleshy mesocarp tissue was higher than the DNA amount obtained from the seeds of the same weight. PCR amplification was performed by using RAPD primers. As a consequence, it was revealed that PCR and RAPD analyses performed with less efficient DNAs were more efficient. Tuney *et al.* (2010) used modified CTAB method on the brown marine algae and obtained genomic DNA. PCR was performed on gDNA using 4 different RAPD primers. The results indicated that CTAB method applied on the marine algae were suitable for molecular analysis. Roychowdhury *et al.* (2012) isolated genomic DNA from *Oryza sativa* (rice) tissues (seedling, leaf, root, grain, kernel, straw and embryo genic callus). $A_{260}/A_{280}$ absorbance ratios of the gDNAs obtained were measured. No toxic chemicals such as liquid nitrogen or phenol were used in the DNA isolation method that they developed and they obtained a DNA efficient for PCR from a low amount of plant tissue. At the end of the analysis, it was revealed that the best purity degree was obtained from callus tissues of gDNA (1.77), maximum DNA concentration was 9.52 ug/ml and was obtained from the seed tissues. The gDNAs isolated were multiplied via rice microsatellite (RM). Microsatellite based DNA fingerprint revealed that DNAs obtained from different tissues provided repeatable bands. Aleskic *et al.* (2012) developed a simple and efficient DNA isolation method for *Salvia officinalis* L. Of the gDNAs that they obtained, the mean efficiency was found to be 330.6 ugDNA/g, $A_{260}/A_{280}$ ratio was found to be 1,909 and $A_{260}/A_{230}$ ratio was found to be 1.894. Later, gDNAs were subjected to PCR amplification using primers specific to nuclear 26S rDNA and chloroplast *rps*16-*trn*K regions. As a result, they stated that the DNA isolation protocol that they performed gave positive bands and could be used in the DNA based molecular studies to be carried out in the future. Poyraz (2014) obtained gDNA by using CTAB method modified by Doyle and Doyle (1987) from seeds of *Nigella sativa* L. (Black cumin) plant. About 20 ng genomic DNA was obtained from each 150 mg dry seed. No ultracentrifuge or column purification was used during isolation. The gDNAs obtained were subjected to PCR amplification by using RAPD and ISSR markers. As a result, it was found that gDNAs obtained were efficient and suitable for PCR amplification.

## Conclusions

Consequently, in this study, two different genomic DNA isolation methods were applied on 12 taxa of *Inula* genus that is distributed throughout Turkey, and PCR amplification was performed using primers specific to ITS, *trn*L-F and *ndh*F regions. Various DNA isolation methods are available for different species and genera. It is harder to apply phenol choloform isoamyl alcohol method in comparison with commercial kit (SIGMA), however, it is cost-efficient. On the other hand, it is easy to apply commercial kit method and it is advantageous in terms the fact that it is time saving and the chemicals used are pure. In our study, it was revealed that the amount of the genomic DNAs obtained through phenol method was higher than the genomic DNA amount obtained through commercial kit. However, PCR results obtained by using primers specific to ITS, *trn*L-F and *ndh*F (972F-1603R) regions indicated that the highest efficiency was achieved from genomic DNAs by using commercial kit with respect to band profiles. *Inula* samples, which are the materials of the study, contain secondary metabolites rich in allochtones and terpenes. For this reason, phenolic compounds within gDNAs could not be purified through phenol chloroform isoamyl alcohol method and it is considered that it affects PCR amplification. However, that kit method purifies these compounds in an efficient manner rendered PCR amplification more successful. It is thought that washing with Wash solution twice at gDNA isolation step with kit was effective in terms of clearing the pure DNA of the secondary metabolites. At the last step of phenol isoamyl alcohol method in the study, washing with 90% ETOH was performed twice. However, the desirable result could not be obtained. For this reason, PCR amplification of gDNAs obtained through kit method was found to be more successful than PCR amplification of gDNAs obtained through phenol chloroform method. This study provides information concerning which method is more suitable in DNA based studies to be conducted on *Inula* genus or different plant genera.

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