INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–6–882–884
http://www.ijab.org

Comparing Study Between Four Different Methods of Genomic DNA Extraction from *Cyclamen persicum* Mill.

MITRA ALAEY[1], ROUHANGIZ NADERI, ALI VEZVAEI, AHMAD KHALIGHI AND ALIREZA SALAMI
Department of Horticultural Sciences, Faculty of Agriculture, University of Tehran, Karaj-Iran
[1]Corresponding author's e-mail: mitra_alaey@yahoo.com

ABSTRACT

The most essential principle in the modern molecular biology is extraction of DNA with a desirable quantity and quality, and this achievement and complete DNA sequencing of genome could be the main necessity for every genetic study till 2010. DNA extraction is obviously difficult because of negative effects obtained from carbohydrates, tannins, polyphenols and proteins. Therefore, the ideal method is the one, which highly reduces the presence of these materials. In the present study, four special methods are chosen to be compared: (1) Murray and Thompson (1980), (2) Dellaporta *et al*. (1983), (3) Doyle and Doyle (1990), and (4) Lodhi *et al*. (1994). Both young and mature leaves of Iranian cyclamen are used in the procedure. The quality and quantity of extracted genomic DNA gained from these methods are deliberated by means of spectrophotometry, electrophoresis in 1% agarose gel and PCR. In this regard, application of Murray and Thompson's method (1980) is chosen for young and mature leaves. The most value of qualified DNA, is extracted from young leaf tissue by 395 ng/µLit and by the same method it shows a significant difference with the elderly leaves by 355 ng/µL. According to the data, Murray and Thompson's Method (1980) is recommended for DNA extraction from young leaves of Iranian cyclamen.

Key Words: DNA extraction; Iranian cyclamen; Quality and quantity of DNA; PCR

INTRODUCTION

Now-a-days, molecular aspects of biological studies are highly valuated and the first approach to such fields is extraction of nucleic acids. Lots of limitations in genetic materials extractions are solved by some changes in compound and pH of functional buffers, so that extracted DNA is much more quantified and also better qualified (Lodhi *et al*., 1998). It's also incredibly important to use a method, not only needn't so many modern lab-accessories, but also can be done acceptably and economically too. Presence of some inhibitors in extracted DNA solution causes a reduction in taq-polymerase activities in PCR and hinders the function of cutter enzymes as well (Bushra *et al*., 1999). In this point of view, lots of researches on different plants have been operated (Lodhi *et al*., 1998; Bushra *et al*., 1999).

Cyclamen persicum taxonomically belong to *Primulaceae* family. As the native source and because of the suitable weather conditions, Iran is one of the best centers for plantation of Iranian Cyclamen and it may be the center of variation of *Cyclamen persicum*. It seems that the country is exceedingly rich of its germplasm (Naderi *et al*., 2000). So it takes for serious to collect the varieties of all its genotypes, to assay and classify them. For this movement extraction and preparation of proper DNA samples is certainly of importance. Classifying genetic resources, molecular methods play significant roles to manage and hoard such precious priceless collections.

Various methods are used for DNA extraction from plant tissues. So far Doyle and Doyle method is applied in horticulture and fruit trees (Doyle & Doyle, 1990; Jenderek *et al*., 1997). The extraction technique of Lodhi *et al.* (1998) has been also utilized for grape, apple, apricot peach, cherry and snapdragon. For rice, wheat, tobacco, cabbage, olive, potato and rose Murray-Thompson's process has been done Csaikl *et al*. (1998) introduced Dellaporta's an effective method to s extract genomic DNA from leaf samples of spruce, white poplar, pine, oak and corn. This process is also applied successfully for anthurium by Buldewo *et al.* (2002). Different species of marshmallow are treated by the method of Doyle and Doyle (1980). Although there are lots of ways used to extract DNA from plant tissues, there are only few studies related to cyclamen (Zhang *et al*., 1997; Laura *et al*., 2000).

With the assistance of morphological traits, some restricted genotype identification of cyclamen have been already done at Iran (Naderi *et al*., 2000), however because of impact of environmental factors on these traits, it's inevitable to proceed to molecular procedures to define dissimilar genotypes. Attaining to this goal, aforesaid genomic DNA is inspected in both young and mature leaves, in order to specify the best DNA extraction method for this plant.

MATERIALS AND METHODS

Four DNA extraction methods are examined on young and mature leaf samples of *Cyclamen persicum*. Factorial test in completely randomized design with three repetitions is set. In early January 2005, young and elderly leaf samples

of the plant were collected. In all extraction procedures 1 g leaf sample is used. Four different methods included Murray and Thompson (1980), Dellaporta *et al*. (1983), Doyle and Doyle (1990), Lodhi *et al*. (1994) are utilized to extract genomic DNA from leaf samples of cyclamen. After DNA extraction with foresaid methods and sedimentation, resulted plat are given a rinse with ethanol 75%, dried solved in 200 µL double distilled sterile water and overnighted in 4°C. This solution is stored in freezer to be available for next treatments. Spectrophotometer measured absorption of diluted extracted DNA in ratio of 1:50 (20 µL of DNA stock solution + 980 µL of double distilled sterile water) in a wavelength of 260 nm (as nucleic acids absorbing wavelength of light) and also in another wavelength of 280 µm (as the proteins absorbing wavelength of light) and obtained ratios of light absorptions of DNA solutions in 26 nm to 280 nm (DNA purity index: r = A 260/ a 280). Eventually Concentration of DNA stock is calculated.

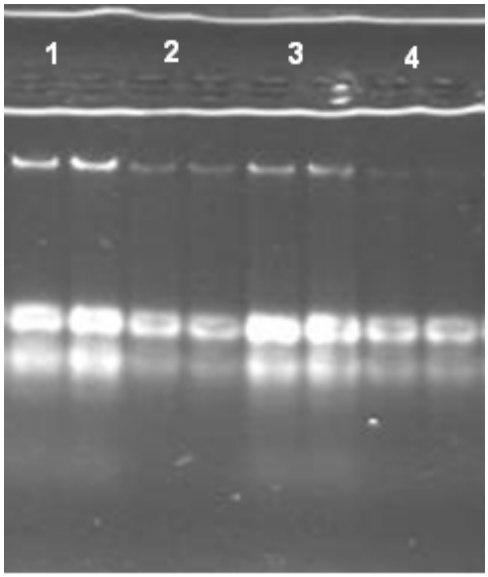
Taking advantage of DNA electrophoresis on 1% agarose gel, quality of DNA band is recognized for each sample; each one contained 5 µL of extracted DNA, 2 µL loading buffer and 5 µL double distilled sterile water, is put into the agarose gel sumps and loaded off in TBE buffer condition. Agarose gel was under a steady voltage of 100 for 1 h. After staining by ethidium bromide, the result is seen and photographed by Gelodoc© under UV lighting. Electrophoresis gel results on agarose certify the obtained from spectrophotometer.

The ability of amplification of extracted DNA strands is proved by a randomized 10-nucleotide-primer TIBMBD-09 by PCR. The final volume of 25 µl is tested in PCR reaction (2.5 µL PCR reaction buffer 10x, 0.875 µL $MgCl_2$ 50 mM, 0.5 µL dNTPs 10 mM, 1.0 µL primer 10 µM (5′ CCA CGG TCA G 3′), 0.2 µL taq-polymerase 5 Unit/µL, 2.0 µL template DNA (5 ng/µL). Thermocycling condition was scheduled as 1 cycle in 94°C for 4 min, 35 cycles including 1 min in 92°C, 1 min in 37°C and 2 min in 72°C, and at last 1 cycle in 72°C for 1 min. This all has been done by Bio-Rad® thermocycler, model "I-Cycler".

RESULTS AND DISCUSSION

Quality and quantity of extracted DNA is tested in three spectrophotometer methods, electrophoresis on agarose gel (Fig. 1) and PCR (data not shown). By spectrophotometer procedure, absorption of double-stranded DNA in wavelength of 260 nm is 50 µg μL^{-1}. In due to the fact, when the ratio of absorption amount resulted in 260 nm to the result of 280 nm is between 1.7 and 2, it shows the most absorption is done by nucleic acids and therefore extracted DNA is well-qualified and its purity is acceptable. So these results confirm that extracted DNA executed by Murray and Thompson Method from young and mature leaves possess better quality and quantity in compare with

Fig. 1. Electrophoresis on agarose gel for DNA extracted from four different protocols. *Left to right*: 1. Murray and Thompson (1980) 2. Lodhi *et al*. (1994) 3. Doyle and Doyle (1990) 4. Dellaporta *et al*. (1983)



the other methods. Using 1 g leaf material in this method gained the most DNA extraction 395 ng μL^{-1} of young leaves, and 355 ng μL^{-1} of elderly leaves. Though the quality of extracted DNA by Doyle and Doyle method was agreeable, its value was less than what has been extracted by the Murray and Thompson's: only 122.5 ng μL^{-1}. DNA extractions by Lodhi *et al*. (1998) and also Dellaporta *et al*. (1983) methods produced superfluous materials so that the ratio of absorption in 260 nm to 280 nm equaled a range between 1 and 1.2, which DNA electrophoresis confirmed as well. All scores, resulted from spectrophotometer are analyzed as a factorial test in completely randomized design. In comparison with the other methods, for both young and mature leaves, the amount of extracted DNA by the Murray and Thompson's was higher than Doyle and Doyle's. Its quality was much better than the result of other methods too (Table I).

Comparing four different genomic DNA extraction procedures (Murray & Thompson, Doyle & Doyle, Ziegenhagen & Lodhi), Talebi *et al*. (2003) have also introduce Murray and Thompson's method as the most appropriate one in aspect of quality of DNA extracted from young leaves of pomegranate.

Jenderek *et al*. (1997) has found the method of Doyle and Doyle as the best quality resulting method for DNA extraction form marshmallow, but its quantity was too low. PCR tests outcomes show that extracted DNA by Murray and Thompson (1980) from either young or elderly leaf samples brings an acceptable quality forth for PCR, and the candescence of amplified DNA bands apperceives this truth

Table I. Analysis of variance for efficiency of type of DNA extraction methods and leaf type (young - mature) on concentration of extracted DNA and the absorption ratio in leaf samples of cyclamen

S.O.V	Freedom Degree	Means of square	
		DNA Concentration (ng.µl^{-1})	Absorption Ratio (260nm/280nm)
Extraction Method	3	14876.37 **	2.12 **
Leaf type	1	3812.76 **	0.62 *
Extraction Method × Leaf type	3	215.53 *	0.1 *
Error	16		
C.V. %		5.91	10.42

* = Significant at P = 0.05, ** = Significant at P = 0.01

Table II. Comparison between quantity and qualities of extracted DNA from young and mature leaf samples of cyclamen

Extraction Methods	DNA Conc. Young leaves (ng.µl^{-1})	DNA Conc. mature leaves (ng.µl^{-1})	Absorption Ratio (260nm/280nm) Young leaves	Absorption Ratio (260nm/280nm) mature leaves
Murray and Thompson	395a	354.16 b	1.91 a	1.66 b
Doyle and Doyle	120.83 c	92.5 d	1.49 b	1.02 c
Dellaporta *et al.*	46.66 ef	31.66 f	0.7 d	0.38 e
Lodhi *et al.*	58.33 e	41.66 f	0.7 d	0.45 e

Mean separation within columns by Duncan's Multiple Range Test.

(Table II).

Chemicals such as PVP, NaCl, ß-mercaptoethanol etc. in the method of Murray and Thompson are utilized in proper concentrations, and this is in fact an effective issue to increase DNA extraction quality and quantity. PVP makes a complex, making hydrogen bandings with polyphenols and it simplifies their release from DNA strands (Kadkhodaee, 2002). Remnant polysaccharides are a group of chemicals which decrease DNA quality. To omit these materials, NaCl 1-2.5 M is recommended (Zhang *et al*., 2003). In this procedure, ß-mer captoethanol (5%) works as an antioxidant agent and forbids oxidation in polyphenols (Porebski *et al*., 1997; Bushra *et al*., 1999) and this can also elevate the extraction process during the method of Murray and Thompson.

Foresaid factors cause an apposite quality and quantity of DNA extraction using Murray and Thompson's method for cyclamen leaves.

According to lower density of useless materials such as polysaccharides and secondary materials in younger leaves, these are more preferred in lots of DNA extraction procedures to be employed (Doyle & Doyle, 1990; Bushra *et al*., 1999). Eventually, because of proper concentrations of chemicals and shorter length of time to extract DNA and its higher quality and quantity of extraction as well, the method set up by Murray and Thompson is the best extraction method for cyclamen young and mature leaf samples.

Acknowledgements. This project was supported by Agriculture Faculty, Department of Horticulture of Tehran University. Tehran, Iran. The authors wish to express their thanks to Mr. Yashar Javan for the English translation of the manuscript.

REFERENCES

Buldewo, S. and Y.F. Jaufeerally-Fakhim, 2002. Isolation of clean and PCR-Amplifiable DNA from *Anthurium andreanu*. *Plant Mol. Biol. Rep.,* 20: 71a–71g

Bushra, C., Y. Afshan., H. Tayyab and S. Riazuddin, 1999. Mini-scale genomic DNA extraction from cotton. *Plant Mol. Biol. Rep.,* **17**: 1–7

Cheng, Y.J., W.W. Guo, H.L. Yi., X.M. Pang and X. Deng, 2003. An efficient protocol for genomic DNA extraction from Citrus species. *Plant Mol. Biol. Rep.,* 21: 177a–177g

Csaikl, U.M., H. Bastian, R. Brettschneider, S. Gauch, A. Meir, M. Schauerte, F. Scholz, C. Sperisen, B. Vornam and B. Ziegenhagen, 1998.Comparative analysis of different DNA extraction protocols: A fast, universal maxi-preparation of high quality plant DNA for genetic evaluation and phylogenetic studies. *Plant Mol. Biol. Rep.,* 16: 69–86

Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep.,* 1: 19–21

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. *Focus,* Vol. 12.pp. 11–5

Jenderek, M.M., K.A. Shierenbeck and A.J. Olney, 1997. Development of random amplified polymorphic DNA markers characteristic of *Hibiscus rosa-sinensis* and *H. syriacus.* Center For Irrigation Technology. California State University, USA

Kadkhodaee, S., 2003. A simple method for nucleic acid extraction from plants containing high polysaccharide and polyphenol components: *Optimizing DNA extraction method in almonds,* III. Congress of Biotechnology, Iran

Laura, M., L. De Benedetti, S. Bruna, G. Burchi, T. Berio, A. Giovannini and B. Ruffoni, 2003. *Cyclamen persicum* mill: Somatic embryogenesis and RAPD analysis of embryogenic callus. *Acta Hort.,* (ISHS) 625: 137–44

Lodhi, M.A., G.N. Ye, N.F. Weeden and B.I. Reisch, 1994. A simple and efficient method DNA extraction from grapevine caltivars and vitis species. *Plant Mol. Biol. Rep.,* 12: 6–13

Murray, M. and W.F. Thopson, 1980. Rapid isolation of molecular weight plant DNA. *Nucleic Acid Res.,* 8: 4321–5

Naderi, R., A. Khalighi, K. Arzani and M. Babalar, 2000. Effects of storage temperature, chemical treatment, harvesting stage and genotype on vase life of cut flowers of cyclamen (*Cyclamen persicum* Mill.). *Seed and Pl. J.,* (SPII), Vol 16: 240–51

Porebski, S., L. Grant Bailey and B.R. Boum, 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Mol. Biol. Rep.,* 15: 8–15

Talebee, M., 2003. *Genetic diversity in pomegranate cultivars in Iran by RAPD marker,* III Congress of Biotechnology, Iran

Zhang, J., M.B. McDonald and P.M. Sweeney, 1997. Testing for genetic purity *Petunia* and *Cyclamen* seed using random amplified polymorphic DNA markers. *J. Amer. Soc. Hort. Sci*., 32: 246–7

(Received 20 July 2005; Accepted 12 August 2005)