Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 6, p. 222-225, 2015
http://www.innspub.net

INNSPUB
RESEARCH PAPER
OPEN ACCESS

A modified genomic DNA extraction method from leaves of sunflower for PCR based analyzes

Hossein Zeinalzadehtabrizi[1*], Arash Hosseinpour[2], Murat Aydin[2], Kamil Haliloglu[2]

[1]*Iranian Academic Center for Education, Culture and Research (ACECR), Hamedan, Iran*

[2]*Department of Field Crops, Faculty of Agriculture, Ataturk University, Turkey*

Article published on December 29, 2015

Key words: Agarose gel, CTAB, Electrophoresis, RNase, Spectrophotometry.

Abstract

Sunflower genomic DNA extraction for PCR (Polymerase Chain Reaction) analyses and similar molecular technics is a challenging issue because of high concentrations of secondary metabolites, i.e. polyphenolics, polysaccharides, tannins and other contaminants. Our modified (Cetyl Trimethyl Ammonium Bromide) CTAB protocol can be performed approximately in two hours and suitable for PCR analyses. It needed small amounts of plant leaves and good quality of sunflower genomic DNA can be isolated. Quantity and purity of extracted DNA were checked by spectrophotometry and agarose gel electrophoresis. This modified method takes less time, reliable and gives good quality of DNA even without using RNase can be used in various marker systems such as (Single Sequence Repeat) SSR and (Random Amplified Polymorphic DNA) RAPD.

***Corresponding Author:** Hossein Zeinalzadehtabrizi ✉ tabrizi@jdhamedan.ac.ir

Introduction

The polymerase chain reaction (PCR) method is extensively used for crop analyses, genetic diversity assessments, genotyping and other molecular techniques (Li *et al*., 2007; Amani, 2011).

Due to high concentrations of secondary metabolites, i.e. polyphenolics, polysaccharides, tannins and other contaminants in DNA extracts of sunflower leaves, a high efficient genomic DNA extraction protocol is essential for DNA-based molecular assays such as random amplified polymorphic DNA (RAPD) and single sequence repeat (SSR) (Horne *et al*., 2004; Li *et al*., 2007).

The CTAB based protocols are widely used for isolation of different plant genomic DNAs. Up to now, several CTAB-based sunflower genomic DNA protocols have been previously developed by various researchers (Encheva *et al*., 2005; Horne *et al*., 2004; Iqbal *et al*., 2008; Isaacs *et al*., 2003; Li *et al*., 2007; Zhizhong, 2008). However, their protocols have some advantages and disadvantages. For example, Li *et al*. (2007) described a high-quality DNA extraction method for mature sunflower leaves. Even though their method was accurate and reliable, it is very time-consuming (about 12-13 ha) and significant amounts of extracted DNA were lost during the extraction procedure. Furthermore, this method needs a long period for plant growth (mature leaves of sunflower are needed). On the other hand, the requirement to large amounts of plant tissue can be a disadvantage of protocols i.e Isaacs *et al*. (2003) and Encheva *et al*. (2005).

In the present study, a modified CTAB protocol that can be performed approximately in two hours and suitable for PCR analyses was evaluated. It needs small amounts of plant leaves and good quality of sunflower genomic DNA can be isolated.

Protocol for sunflower genomic DNA extraction

1. Sunflower young leaves (15-20 days old) were quickly ground in liquid nitrogen to fine powder using a mortar and pestle.
2. 200-300 mg of powder was transferred to a 2 ml Eppendorf tube and 1 ml preheated extraction buffer (2% CTAB (w/v); 1.42 M NaCl; 200 mM EDTA; 100 mM Tris-HCl, pH 8.0; 1% $Na_2S_2O_3$; 0.2% 2-mercaptoethanol) was added with uniform mixing to avoid clumping at the bottom.
3. Samples were placed in water bath and incubated at 65-70°C for 60 min with mixing after every 15 min.
4. Subsequently, samples were taken out from water bath and allowed them to cool down for 4-5 min at room temperature
5. Samples were centrifuged at 14000 rpm for 10 min at 24°C.
6. 750 µl of the aqueous phase was carefully transferred to a new tube, 750 µl Chloroform:Isoamyl alcohol (24:1 v/v) was added and gently mixed the solution for 1 min
7. Samples were centrifuged at 14000 rpm for 10 min at 4°C.
8. Approximately, 600 µl of the aqueous phase was carefully transferred to a new tube, followed by adding 600 µl Chloroform-Isoamyl alcohol (24:1 v/v) and gently mixed the solution for 1 min
9. Samples were centrifuged at 14000 rpm for 10 min at 4°C.
10. Carefully transfer approximately 500 µl of the upper phase to a new tube, add 100 µl sodium acetate 3 M (PH=5.5), 100 µl ammonium acetate 10 M and 500 µl pre-chilled isopropanol.
11. Mix the samples by gentle inversion for 1 min to precipitate the DNA (mucous-like, translucent cotton of extracted DNA can easily be seen in this step).
12. Samples were centrifuged at 14000 rpm for 10 min at 4°C to pellet the DNA.
13. The upper aqueous phase was poured off as much as possible and then inverted to dry the pellet DNA at room temperature.
14. Finally, the pellet was dissolved in 150 µl of TE buffer. Isolated DNA is stable in 4°C. For a long-period use, it can be stored at -20°C.

Important notes

- 2-mercaptoethanol should be added to CTAB buffer just before use.
- The temperature of centrifugation should be about 24°C. Too low temperatures might cause CTAB precipitation and lose DNA.
- To get good results and capture more amounts of DNA, protocol steps should be prepared on ice (after step 8).
- It is not necessary to use RNase in this protocol since it does not interfere with PCR.
- This protocol is partially based on the method described by Encheva *et al.* (2005).
-

Quantity and purity of extracted DNA

The concentration of extracted DNAs was measured at A260/A280 for protein contaminations and A260/A230 for polyphenol and polysaccharide compounds in a ND-3300 spectrophotometer (NanoDrop Technologies, USA).

The quality of DNA was checked by running 5 µL DNA on 0.8% agarose gel electrophoresis prepared in 1X SB buffer (Fig. 1).

In this modified protocol, concentration of DNA was ranged 500-800 ng/µl, A260/A280 ratio was between 1.7-2.1 and A260/A230 ratio was measured as 1.3-1.9 (Tab. 1).

Table 1. Comparison between previously reported sunflower DNA extraction protocols and our modified protocol.

	Our method	Isaacs *et al.* (2003)	Encheva *et al.* (2005)	Li *et al.* (2007)	Iqbal *et al.* (2008)
Time requirement	about 2 ha	Over night	2-2:30 ha	12-13 ha	about 2 ha
Amount of Leaf tissue	200-300 mg	Leaf pieces	2.5 g	200-300 mg	200-300 mg
Leaf tissue age	15-20 days old	N/A	N/A	Mature leaves	6 days old
Final concentration extracted DNA	of 500-800 ng/µl	N/A	N/A	N/A	N/A
260/280 ratio	1.7-2.1	N/A	N/A	1.8-1.89	N/A
260/230 ratio	1.3-1.9	N/A	N/A	>2	N/A
RNase usage	optional	mandatory	mandatory	mandatory	optional

DNA Amplification and SSR Analysis

A reaction mixture containing 1X reaction buffer, 25 mM $MgCl_2$, 0.2 mM dNTPs, 10 µM SSR primers, 2.5 unit Taq DNA polymerase and approximately 50 ng sample DNA was prepared and optimized in a total volume of 20 µL.

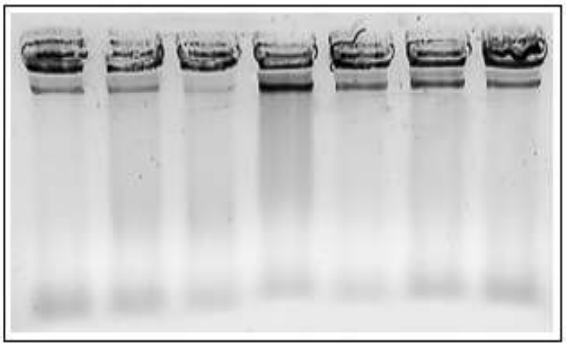

Fig. 1. Electrophoresis of sunflower genomic DNA extracted by this protocol on 0.8% agarose gel in SB 1X buffer.

For SSR analysis (using ORS 166 primers), DNA amplification reactions were performed in MultiGene Gradient Thermal Cycler (TC9600-G-230V, Labnet International, Inc.). The PCR profile was as follows: one cycle of 96°C for 2 min, 30 cycles of 94°C for 30 s, 55°C for 40 s, 72°C for 1 min, and a final extension for 10 min at 72°C.

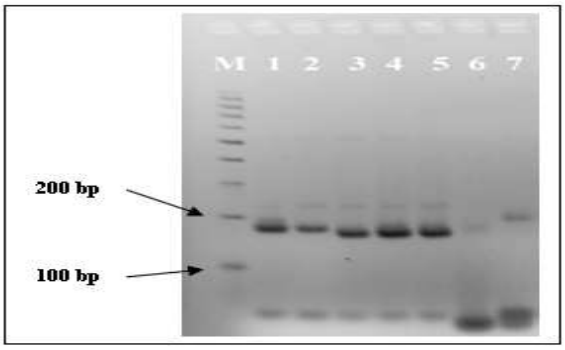

Fig. 2. Electrophoresis of PCR products from genomic DNA on 2% agarose gel. Lane 1: DNA molecular weight marker (DirectLoad™ PCR 100 bp Low Ladder, Sigma-Aldrich, Germany); lane 2-6: sunflower genotypes.

PCR products were separated on 2% agarose gel, stained with ethidium bromide and then was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, France) (Fig. 2).

In this study, a fast and efficient genomic DNA extraction from sunflower leaves, which can be used in PCR based studies was developed. This method takes less time (around two hours), reliable and gives good quality of DNA even without using RNase that can be used in various marker systems such as SSR and RAPD.

Acknowledgements

The Authors would like to thank Ms. Pinar Uysal and Ms. Atefeh Varmazyari for their kind helps.

References

Amani J, Kazemi R, Abbasi AR, Salmanian AH. 2011. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. Iran J Biotechnol. **9,** 69-71.

Encheva J, Köhler H, Christov M, Friedt W. 2005. Intergeneric hybrids between cultivated sunflower (*Helianthus annuus* L.) and *Verbesina helianthoides* (genus Verbesina): RAPD analysis. Heila **28(42),** 37-44.

Horne EC, Kumpatla SP, Patterson KA, Gupta M, Thompson SA. 2004. Improved high-throughput sunflower and cotton genomic DNA extraction and PCR fidelity. Plant Molecular Biology Reporter **22(1),** 83-84.

Iqbal M, Sadaqat H, Khan I. 2008. Estimation of genetic diversity among sunflower genotypes through random amplified polymorphic DNA analysis. Genet. Mol. Res. **7(Suppl 4),** 1408-1413.

Isaacs MS, Manivannan N, Muralidharan V. 2003. Genetic diversity analysis using RAPD marker in inbred lines of sunflower *Helianthus annuus* L. Helia **26(39),** 59-65.

Li J, Yang J, Chen D, Zhang X, Tang Z. 2007. An optimized mini-preparation method to obtain high-quality genomic DNA from mature leaves of sunflower. Genet. Mol. Res **6(4),** 1064-1071.

Zhizhong X. 2008. DNA extraction from oil sunflower seeds and the optimization of its RAPD reaction system. Journal of Anhui Agricultural Sciences **36(6),** 2259.