ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 12, pp. 22747-22750, December, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

A LOW COST AND EFFICIENT METHOD OF GENOMIC DNA EXTRACTION FROM TOMATO (*SOLANUM LYCOPERSICUM*)

Osman Basha P*[1]., Sudarsanam G[2]., MadhuSudhan Murthy J[1] and Reddaiah B[2]

[1]Department of Genetics and Genomics, Yogi Vemana University, Kadapa, A.P., India
[2]Department of Botany, Sri Venkateswara University, Tirupati, A.P., India

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1329

ARTICLE INFO

Article History:

Received 17th September, 2017
Received in revised form 21st October, 2017
Accepted 05th November, 2017
Published online 28th December, 2017

Key Words:

DNA isolation, Phenolic compounds, *Solanum lycopersicum*

ABSTRACT

The DNA isolation protocol we describe in the present investigation is simple, stable, adaptable, versatile, cost effective and with sufficient quality for PCR and enzymatic reactions. Here we standardized a low cost and high quality genomic DNA isolation from leaf tissues of *Solanum lycopersicum* as well as from medicinal plants with excluding the step of grinding the tissue using liquid nitrogen. SDS method of DNA isolation was altered at changing concentration of Tris, β-mercaptoethanol, NaCl, and PVPP to attain maximum yield with less phenolic contamination. Approximately 1.5 µg to 3.5 µg of DNA concentration was attained from the standardized protocol. The purity of the isolated genomic DNA was excellent as evident by A260/A280 ratio ranging from 1.75 to 1.87. The isolated DNA is free of phenolic compounds and the quality is sufficient for PCR and restriction digestion reactions. The DNA obtained from the standardized protocol is consistent and suitable for long storage purpose as well as the protocol can be applied for high throughput DNA isolation procedures.

INTRODUCTION

One of the ultimate goals of agricultural research is to increase yield, quality, and improved resistance to diseases and pests, to meet demands from industries and these have been targeted by breeders and plant biologists. Understanding the complex pathways controlling different physiological processes and identification of the genes involved in different stages of these pathways in plants is a challenging task requiring several genetic resources and techniques. Over the past decades research in the field of molecular biology such as marker-assisted selection, diversity analysis, fingerprinting of cultivars and clones, population studies and transgenic has been increasing popularity. For this the DNA isolation phase is a time taking and costly component of overall time necessary for dealing out (Dilworth and Frey, 2000). To overcome this problem several procedures of DNA extraction protocols have been reported for several crop species (Dilworth and Frey, 2000) as well as numerous number of costly commercial products are available (DNeasy 96 Plant Kit [QIAGEN], Wizard Magnetic 96 DNA Plant System [Promega]. In the literature many different protocols are available concerning the DNA extraction from numerous plant species; however, these methods produce either insufficient amounts or inconsistent quality of DNA (Xin *et al*., 2003; Geuna *et al*., 2000; Mace *et al*., 2003; Flagel *et al*., 2005). Commercial kits are also available to extract genomic DNA from plant tissues with adequate quality (Xin *et al*., 2006), but the quantity of DNA produced is less from commercial kit DNA isolation and the cost can be expensive for small laboratories.

Tomato (*Solanum lycopersicum*) tissues also contain high levels of polyphenolic compounds like many other medicinal plant species. When cell disrupts, the oxidized form of phenolic compound covalently bind to DNA make it useless for most research applications (Katterman and Shattuck, 1983; Guillemaut and Maréchal-Drouard 1992). One of the typically procedure used to avoid covalent binding of phenols to DNA is freezing and grinding of tissue in the liquid nitrogen (Leutwiler *et al*., 1984). Researchers have been tried to exclude the use of hazardous chemicals and the each protocols have its own advantages and disadvantages such as phenolic contaminations, low recovery, and unable to use to PCR and restriction digestions (Ikeda, 2001).

Endonucleases are one of the factors that cause DNA degradation during isolation and purification of high molecular weight DNA from plants. Polysaccharides are another problematic factor when present in isolated DNA samples, as

Corresponding author:* **Osman Basha P
Department of Genetics and Genomics, Yogi Vemana University, Kadapa, A.P., India

their presence may also inhibit Taq polymerase activity and restriction enzyme activity (Fang, 1992; Pandey, 1996) and the polysaccharides in the DNA sample is identified observation of highly viscous solution (Do and Adams, 1991). The DNA was bind with the oxidized form of polyphenols covalently and gives a brown colour and it useless for molecular studies and considered as important factor that determined the quality of the DNA (Katterman and Shattuck 1983).

We tried several published protocol for the isolation of the genomic DNA without polyphenolic contamination and failed repeatedly. In this paper we standardized low cost and high yield DNA isolation protocol from tomato (*Solanum lycopersicum*) as well as can be applied to any plant tissues. The method combines traditional SDS method excluding the freezing and homogenization protocol using liquid nitrogen that reduced significantly cost of the experiment while producing high yield and high quantity of genomic DNA. The resulting optimized protocol allows the isolation of high quality genomic DNA amenable to use in molecular biology studies.

MATERIAL AND METHOD

Isolation of Genomic DNA

Genomic DNA was isolated from young juvenile leaves as well as old leaves in 2 ml Eppendorf tube. ~100 mg of leaf tissue was collected from tomato plants. Theleaftissue was grinded in the 1 ml of DNA extraction buffer (65 °C preheated extraction buffer 0.1 M Tris-HCl (pH 7.5); 0.5 M EDTA (pH 8.0); 1.5% (w/v) SDS; 0.2 M β-mercaptoethanol; 15 mg of insoluble polyvinylpolypyrrolidone) usingmotor and pestle. Polyvinylpolypyrrolidone was added to the buffer just before grinding of the leaf tissue and β-mercaptoethanol was added after grinding of the sample. Grinded tissue was transferred in to 2 ml Eppendorf tubes and then incubated at 65°C in water bath for 1 hr for cell lysis. After incubation, tubes were cooled down to room temperature and 500µl of cold 6 Mammoniumacetate was added and mixed well and then incubated at 4°C for 15 min followed by 30 min centrifugation at 10,000 RPM at 4°C. The clear supernatant containing DNA in aqueous phase was transferred to a new Eppendorf tube and added equal volume of Phenol:Choloroform:Isoamylalcohol (25:24:1) and mixed well by repeated inverting then followed by 30 min centrifugation at 10,000 RPM at 4°C. The upper layer was separated and to this equal volume of Choloro form: Isoamylalcohol (24:1) was added and mixed by inverting then centrifuged at 10,000 RPM at 4°C. The upper layer was separated and to this equal volume of ice cold isopropanol was added and incubated at -20 °C for at least 1 hr to precipitate DNA and the tubes were centrifuged at 10,000 rpm at 4 °C for 30 min. The pellet was washed twice with 70% (v/v) ethanol and air dried the pellet. The pellet was dissolve in 150 µlof TE (10mM Tris, pH7.5, 1mM EDTA pH 8.0 supplemented with 3.2 µg/ml RNase).

Quantification of genomic DNA

The samples quality and quantity was determined by running the samples on 0.8% (w/v) agarose gel in 1X TAE buffer at 50 V and measured at 260 nm and 280 nm.

PCR conditions used to check DNA quality

Isolated tomato DNA was used to PCR amplification as per the following procedure. PCR was carried out using a programmable thermal cycler. The reaction was performed in total volume of 15µl and the components are 1X PCR buffer (10 mM Tris, 5 mM KCl, 1.5 mM $MgCl_2$, 0.1% (w/v) gelatin, 0.005% (v/v) Tween-20, 0.005% (v/v) Np-40, pH 8.8), 0.25 mM dNTPs, 1 U of *Taq* polymerase, 5 pmoles of each of forward and reverse primers (Simple Sequence Repeats) and 20 ng of genomic DNA. The following PCR cycling conditions included an initial denaturation for 4 min at 94^{o}C, followed by 35 cycles of denaturation at 94^{o}C for 30 sec, annealing at 55^{o}C for 45 sec followed by final extension at 72^{o}C for 1 min, and followed by a final extension at 72^{o}C for 8-10 min. RAPD amplification was carried out using three arbitrary 10 bp primers. Approximately 20 ng of the isolated DNA were used for PCR amplification. The PCR reaction was performed in a final volume of 20 µl reaction containing 1X Taq polymerase buffer (Himedia-MBT060), 0.5 U of TaqPolymerage (Himedia) 200 µl of each of dNTPs (Thermo Scientific), 15 ng of primers (Operan Technologies), 2.5 mM MgCl2. The PCR was carried out using the following conditions; initial denaturation for 4 min at 94^{o}C, followed by 40 cycles of denaturation at 94^{o}C for 1 min sec, annealing at 35^{o}C for 2 min and extension at 72^{o}C for 2.5 min was followed by a final extension at 72^{o}C for 8 min and stored at 4^{o}C until electrophoresis. The amplified PCR products were resolved on agarose gel in 1X TAE buffer in an electrophoresis unit at 50 V and gel images were collected with Alpha Imager™ gel documentation system (Alpha Innotech, USA).

RESULTS AND DISCUSSION

The selection of suitable DNA isolation protocols depends on numerous factors such as quality of starting material, cost, time, labor requirement, quantity and quality of the genomic DNA. The standardized protocol in this study can be applicable to wide range of plant material. The isolated DNA would be stored at -20°C for over one year without any seeming effect on the yield and quality of the DNA (Fig 1). Commercial DNA isolation methods and high-throughput DNA extraction methods yielded good quality and quantity but these methods require expensive robotics, reagents and the minimum cost for DNA isolation per sample is relatively more. Therefore their usage by many small laboratories was not economical.

We tried several published protocol for the isolation of the intact and pure genomic DNA from tomato leaf samples and these protocols had its own advantages and disadvantages. Few protocols required liquid nitrogen for grinding of the tissue and some protocols does not produce sufficient quality and quantity of the DNA and making the isolated DNA not acquiescent for molecular studies. Hence, we modified the SDS method of DNA isolation at varying concentration of Tris, β-mercaptoethanol, NaCl, and PVPP with maximum yield with less phenolic contamination. We excluded the step of using expensive liquid nitrogen in the protocol, because we were interested in isolation of genomic DNA from large number of samples to reduce the cost per sample. We successfully subsidiary the liquid nitrogen usage by pre-chilled mortar and pestle and −40°C/−80°C stored leaf sample. It is likely, that standardized DNA isolation protocol for tomato leaf sample

used to isolate genomic DNA from a variety of other plant species. 75 g of leaf tissue was used as starting material and 1.5 to 3 mg of genomic DNA was achieved. The DNA obtained after PCI step followed by precipitation of solution, the DNA is clear and transparent i.e., there is no visible coloration.

The purity of the isolated DNA was good as evident by A260/A280 ratio ranging from 1.78 to 1.84 and the ration suggesting that the DNA was sufficiently free of proteins and polyphenolics (Fig 1). The isolated Genomic DNA was high molecular weight, intact and without RNA contamination (Fig 1). The isolated tomato DNA is absolutely suitable for molecular studies and is proved by setting PCR reaction with SSR molecular marker and RAPD marker (Fig 2). The isolated DNA exhibits long storage capacity at -20^{0}C without much damage (lane number 4 Fig 1).

Fresh and young leaf materials are the excellent choice to obtain good-quality DNA and mature leaves contain higher polyphenols and polysaccharides (Porebski, *et al*., 1997). The weight of leaf material used for the DNA isolation is another important factor that affects the quality and quantity of the DNA. Taking too much leaf material for DNA isolation leads to accumulation of secondary components/phenolic contaminations. The phenolic contamination affects the success rate of the PCR reaction. The isolation of high quality, pure and intact DNA is very crucial for numerous applications but DNA isolation from plants is typically conceded by excessive contamination by secondary metabolites (Tan and Yiap, 2009). Unlike animals and microbes the DNA isolation protocols need to be adjusted to each plant species and even to each plant tissue because of presence of the secondary metabolites (Sangwan, 1998). Several scientist and researcher examines for a more efficient DNA extraction protocol from plants containing high levels of secondary metabolites (Peterson, 1997; Porebski, 1997; Cheng, 2003; Michiels, 2003; Xu *et al* 2004). The brown colored DNA pellet contained polyphenols contamination and interfere in molecular biology studies. It is well known that phenolic compound are major secondary metabolites in plants which get oxidized during homogenization of samples and covalently binds to DNA giving a dark (brown/black) color and making it useless for molecular studies [impart brown or black dark colors to DNA pellet (Peterson, 1997;Katterman and Shattuck 1983). We therefore assessed numerous possible modifications (inclusion of PVP, PVPP, b-mercaptoethanol and extraction with phenol: chloroform: isoamyl alcohol), that could eliminate the phenolic compounds at the stage of tissue homogenization.

The β-mercaptoethanol was added in the pre heated extraction buffer before stating the grinding of the tissue. It has been reported that high level of β-mercaptoethanol successfully removes the polyphenols (Suman, *et al* 1999) by acting as a strong reducing agent by breaking disulphide bonds in proteins and with reduce the activity of peroxidases and polyphenols oxidases. We tried different concentration of β-mercaptoethanol and finally 0.2 M β-mercaptoethanol was used which made the protocol good for DNA isolation. The polyvinylpolypyrrolidone (PVPP) was included in the extraction buffer and the amount of PVPP optimized as it is essential to remove polyphenol contaminates. Polysaccharides may be mostly problematic and may also inhibit Taq polymerase activity and restriction enzyme activity (Fang, 1992; Pandey, 1996).

The addition of 400 µl of cold 6 M ammonium acetate was done for precipitation of the proteins and in further steps higher molecular weight DNA was precipitated with isopropanol. Compared to ethanol, isopropanol required less volume and it also separates polysaccharides. Treatment with Phenol: chloroform: isoamylalcohol (25:24:1) and followed by chloroform: isoamylalcohol (24:1) treatment ensured removal of chlorophyll, pigments, and dyes. We found that DNA suspended in TE (10 mM Tris pH 7.5 and 1 mM EDTA pH 8.0) supplemented with RNase helps to remove contaminating RNA in DNA preparations. This protocol could also be useful for the isolation of the DNA from other plant species with high polysaccharide and secondary metabolites.

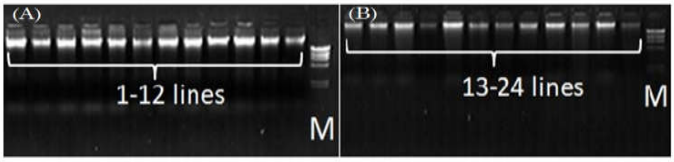

Figure 1 Gel electrophoresis separation of genomic DNA in 0.8% agarose-ethidium bromide gel, (A) After immediate isolation of genomic; (B) After one year storage at 4^{0}C.

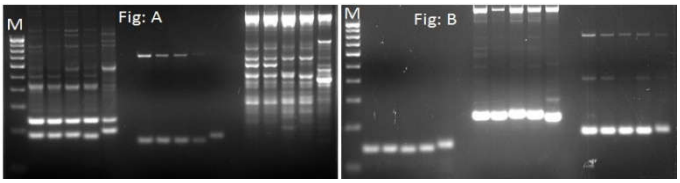

Figure 2 Assessment of quality of genomic DNA by PCR amplification using RAPD markers (Fig A) and SSR markers (Fig B) M- 100bp ladders.

CONCLUSION

Here we have standardized a simple, dependable, and cost-efficient DNA isolation protocol for molecular and breeding studies. This protocol removes the step of using liquid nitrogen to attain good quality genomic DNA ranging from 1.5 µg to 3.5 µg. The resulting optimized protocol permits the isolation of high quality genomic DNA suitable to cloning and breeding studies and it is suitable to even in small laboratories.

Acknowledgements

We express sincere thanks to Council of Scientific & Industrial Research ***(CSIR),*** R&D organization (Sanction letter No. 38(1329)/12/EMR-II, dated 03-04-2012), India for the financial support.

References

Cheng, Y.J., Guo, W.W.U., Hua-Lin, Y.I., Pang X.M. and Deng, X. 2003. An efficient protocol for genomic DNA extraction from citrus species. *Plant Molecular Biology Reporter.*, 21(2): 177-178.

Dilworth, E. and Frey J.E. 2000.A rapid method for high throughput DNA extraction from plant material for PCR amplification. *Plant Molecular Biology Reporter.*, 18(1): 61-64.

Do, N. and Adams R.P. 1991. A simple technique for removing plant polysaccharide contaminants from DNA. *Bio Techniques.*, 10(2): 162-166.

Fang, G., Hammar, S. and Grumet R. 1992. A quick and inexpensive method for removing polysaccharides from plant genomic DNA.,*BioTechniques.*, 13(1): 52-56.

Flagel, L., Christensen, J.R., Gustus, C.D., Smith, K.P., Olhoft, P.M., Somers, D.A. Matthews, P.D. 2005. Inexpensive, High Throughput Microplate Format for Plant Nucleic Acid Extraction: Suitable for Multiplex Southern Analyses of Transgenes. *Crop Sci*., 45(5): 1985-1989.

Geuna, F., Hartings, H. and Scienza, A. 2000. Plant DNA extraction based on grinding by reciprocal shaking of dried tissue. *Anal Biochem*., 278(2): 228-230.

Guillemaut, P. and Maréchal-Drouard, L. 1992. Isolation of plant DNA: A fast, inexpensive, and reliable method. *Plant Molecular Biology Reporter*., 10: 60-65.

Ikeda, N., Bautista, N.S., Yamada, T., Kamijima, O. and Ishii, T. 2001. Ultra-simple DNA extraction method for marker assisted selection using microsatellite markers in rice. *Plant Molecular Biology Reporter.*, 19(1): 27-32.

Katterman, F.R. and Shattuck, V.I. 1983. An effective method of DNA isolation from the mature leaves of Gossypium species that contain large amounts of phenolic terpenoids and tannins. *Preparative Biochemistry.*, 13(4): 347-359.

Leutwiler, L.S., Hough-Evans, B.R. and Meyerowitz, E.M. 1984.The DNA of *Arabidopsis thaliana*. *Mol. Gen. Genet.,* 194: 15-23.

Mace, E.S., Buhariwalla, H.K. and Crouch, J, H. 2003. A high-throughput DNA extraction protocol for tropical molecular breeding programs. *Plant Molecular Biology Reporter.,*21: 459a-459h.

Michiels, A., Van Den Ende, W., Tucker, M., Van Riet, L. and Van Laere, A. 2003. Extraction of high-quality genomic DNA from latex-containing plants. *Analytical Biochemistry.*, 315:(1) 85-89.

Pandey, R.N., Adams, R.P. and Flournoy, L.E. 1996. Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. *Plant Molecular Biology Reporter.*, 14 (1) 17-22..

Peterson, D.G., Boehm, K.S. and Stack, S.M. 1997. Isolation of milligram quantities of nuclear DNA from tomato (*Lycopersiconesculentum*), a plant containing high levels of polyphenolic compounds. *Plant Molecular Biology Reporter.*, 15(2) 148-153. 1997.

Porebski, S., Bailey, L.G. and Baum, B.R. 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter.*, 15(1) 8-15.

Sangwan, N.S., Sangwan, R.S. and Kumar, S. 1998. Isolation of genomic DNA from the antimalarial plant *Artemisia annua*.*Plant Molecular Biology Reporter.*, 16(4): 1-9.

Suman, P.S.K., Ajit, K.S., Darokar, M.P. and Sushil, K. 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Plant Molecular Biology Reporter.*, 17: 1-7.

Tan, S.C. and Yiap, B.C. 2009. DNA, RNA, and protein extraction: the past and the present. *Journal of Biomedicine and Biotechnology.,* Article ID 574398: 1-10 pages.

Xin, Z. and Chen, J. 2006. DNA sequencing II: optimizing preparation and cleanup," in *Extraction of Genomic DNA from Plant Tissue*, K. J. Sudbury, Ed., pp. 47-59, Jones and Bartlett, Boston, Mass, USA, 2006.

Xin, Z., Velten, J.P., Oliver, M.J. and Burke, J.J. 2003.High-throughput DNA extraction method suitable for PCR. *Biotechniques.,* 34 (4):820-826.

Xu, Q., Wen, X. and Deng X. 2004.A simple protocol for isolating genomic DNA from Chestnut Rose (Rosa roxburghiitratt) for RFLP and PCR analyses. *Plant Molecular Biology Reporter*., 22 (3) 301-302.

How to cite this article:

Osman Basha P *et al*. 2017, A Low Cost And Efficient Method of Genomic Dna Extraction From Tomato (*Solanum Lycopersicum*). *Int J Recent Sci Res.* 8(12), pp. 22747-22750. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1329

****