Indian Res. J. Genet. & Biotech. 9(1) : 61-68 (2017)

A modified method for maize genomic DNA extraction without liquid nitrogen and phenol

Punya*, V.K. Sharma and Pankaj Kumar

Department of Agricultural Biotechnology and Molecular Biology, Dr. Rajendra Prasad Central Agricultural University, Pusa, 848125, Bihar, India
*Corresponding author: Email- punyamsingh@gmail.com
(Received : December, 2016 : Revised : January, 2017; Accepted : January, 2017)

Abstract

A reasonably good quality of extracted DNA sample is required for most molecular studies which can only be obtained using standard DNA extraction protocols. The present study describes a relatively quick, inexpensive and consistent protocol for extraction of DNA from maize leaf without using liquid nitrogen and phenol. Using this method, high quality of DNA was obtained from 0.2g of maize leaves and isolated genomic DNA was amplified by employing microsatellite markers which produced reproducible results.

Keywords- Genomic DNA, Maize, Microsatellite marker.

Introduction

Genomic DNA extraction is the first step for studying the molecular and genetic aspects of any organism. Maize is mostly used in genetic studies and there are many researchers which work on specific targets like flowering time[Remington *et al.*, 2001; Thornsberry., 2001; Pressoir *et al.*, 2009], kernel composition and starch pasting properties[Wilson *et al.*, 2004], and carotenoid contents[Harjes *et al.*, 2008; Yan *et al.*, 2010b]. Rapid DNA extraction with expectable quality is difficult due to the large amount of polysaccharides and polyphenols which become problematic during PCR reaction. CTAB is a frequently used surfactant in DNA extraction involves the lyses of all membranes (cell wall and cell membrane) which release the genetic material into the extraction buffer. The method involves a modified CTAB extraction, employing high salt concentrations to remove polysaccharides, the use of polyvinyl pyrrolidone (PVP) to remove polyphenols,

Corresponding author's e-mail : punyamsingh@gmail.com.
Published by Indian Society of Genetics, Biotechnology Research and Development,
5, E Biotech Bhawan, Nikhil Estate, Mugalia Road, Shastripuram, Sikandra, Agra 282007 Online management by www.isgbrd.co.in , www.irjgbt.org

The liquid nitrogen is used to freeze the plant tissue and blend it to powder, and minimize DNA degradation when grinding samples [Dellaporta *et al.*, 1983; Saghai-Maroof *et al.*, 1984]. However, liquid nitrogen is not only dangerous that needs particular attention when using it but also volatile, which will add the cost of experiment. If plant tissue could be ground in room temperature instead of in liquid nitrogen, then the DNA could be extracted in a shorter time at a lower cost, and molecular studies could be performed as usual even when liquid nitrogen is unavailable. Therefore, many researchers have reported some DNA isolation methods without liquid nitrogen [Ouenzar *et al.*, 1998; Sharma *et al.*, 2003; Rajendrakumar *et al.*, 2011].

Materials and methods

The experimental materials of the present investigation comprised eighteen inbred lines obtained from AICRP, Dholi Centre. The eighteen inbred lines involved in present investigation were CML467, CML468, CML469, CML470, CML471, CML373, CML115, CML196, CML465, LM13, Dholi2012, HKI 323-B, HKI586, HKI1105, CML161, CML165, CML163.The leaf samples were harvested from 4-5 leaf seedlings which were grown in pots under green house. From each plant 1.5-2 cm of leaf was taken for DNA extraction, using a modified CTAB method.

Reagents and Chemicals

CTAB stock solution(100ml): 1M Tris HCL (pH 8.0), 0.5M EDTA, 5M NaCl, 2% (w/v) CTAB, Final volume made upto 100ml by adding double distilled water.

CTAB Buffer: 20ml CTAB stock, 0.8g PVP, 100µL β-mercaptoethanol.

The β- mercaptoethanol should be added immediately in extraction buffer before use to avoid possible oxidation.

- 7.5M Ammonium acetate
- Chloroform: Isoamyl alcohol (24:1)
- Isopropanol (Ice cold)
- 70% Ethanol
- TE buffer [10mM Tris-HCl (pH=8.0), 1mM EDTA (pH=8.0)]
- RNase (10mg/ml)
- 5X TBE buffer: 54 gm of Tris base, 27.5 gm of Boric acid and 20 ml of 0.5 M EDTA (pH=8.0) was dissolved in double distilled water and volume was maintained one liter.

Procedure of DNA isolation

i. 0.2g of leaf tissue was ground with 800µl of CTAB buffer using glass rod in 2 ml centrifuge tube.

ii. CTAB/plant extract mixture was incubated for about 15 min at 55^0C in a circulating water bath.

iii. After incubation mixture was centrifuged at 12000 rpm for 10 min at room temperature.

iv. The supernatant was transferred into new 1.5 ml tubes and to each tube 400µl of Chloroform:Isoamyl Alcohol(24:1) was added..

v. The solution was mixed by inversion. After mixing, tubes were centrifuged at 12000 rpm for 10 min.

vi. 233µl cold isopropanol and 32µl of 7.5M ammonium acetate were added in new 1.5 ml tube. The upper aqueous phase only (contains the DNA) transferred to microfuge tube containing isopropanol and ammonium acetate solution.

vii. Tubes were incubated for 15 min at -20^0 C for precipitation of DNA and centrifuged at 12000 rpm for 10 min.

viii. The supernatant was removed and DNA pellet was washed with cold 70% ethanol.

ix. Again the supernatant was discarded and the pellet was air dried and resuspended in 30µl TE buffer.

x. Finally 2µl RNase (10mg/ml) was added and incubated at 37^0 C in water bath for 1hr. the DNA was stored at -20^0 C for further use.

DNA quality confirmation

The confirmation of quality of isolated DNA is essential for PCR based amplification. The isolated DNA samples were subjected to electrophoresis through agarose gel to assess the quality of DNA isolated from the leaves of young seedlings obtained from entries under evaluation in the present study. The location of DNA within the gel was determined directly by staining with low concentration of ethidium bromide which acts as fluorescent intercalating dye. The following steps were involved to check the quality of genomic DNA extracted from the leaves of maize seedling:

i. 0.8% solution of agarose was prepared by melting 0.8g of agarose in 100 ml of 0.5x TBE buffer in a microwave and then allowing it to cool around 60^0 C.

ii. Ethidium bromide was then added and the solution was poured into sealed gel casting plate arranged in a gel casting unit. The comb was placed at the top of the gel and allowed to solidify at room temperature for 30 min.

iii. After solidification, the comb was removed carefully and the gel plate (along with gel) was placed in a

migration chamber containing 0.5X TBE buffer.

iv. 5µl of DNA sample and 2µl of 6x loading dye was carefully loaded in separate well. Electrophoresis was carried out at 80 V for 30 min so that the dye moved approximately 5cm from the well.

v. The gel was viewed under gel documentation system (UV light) to detect the quality of DNA.

Presence of highly resolved high molecular weight band indicated good quality DNA, presence of a smeared band indicated DNA degradation. All those samples which showed single sharp band of high molecular weight were selected for amplification, while the DNA samples showing smearing and bands of low intensity were discarded.

Primer directed amplification

The amplification of DNA was carried out using standard protocol of polymerase chain reaction (PCR) with the help of microsatellite sited based 4 pairs of forward and reverse microsatellite primers namely, nc130,phi084,umc1161, phi034(Table 1) present in genome of maize. The amplification was carried out in a thermal cycler using 15µl of reaction mixture containing 5X PCR buffer, 200µM each deoxynucleotide triphosphate (dNTPs), 10mM of MgCl2, 5 µM of each forward and reverse primer, 1 unit Taq DNA polymerase (Fermentas) and 2µl of template DNA in a sterile 0.5 mm thin walled PCR tubes . The amplification was carried out in a thermo-cycler (Eppendorf). The amplification was carried out using the program 1 cycle of 5 minutes at 94 ^{0}C as an initial denaturation step. This was followed by 30 cycles of 1 minute at 94 ^{0}C for denaturation, 1 minute for annealing temperature depending on the marker used (52 ^{0}C-60 ^{0}C) and 2 minute at 72 ^{0}C for primer elongation. Finally, 7 min at 72 ^{0}C was used for final extension then PCR was hold at 4 ^{0}C. Amplified products were stored at -20 ^{0}C for further use. The amplified products were electrophoretically resolved on a 2.0% agarose gel in 0.5X TBE buffer at 110V for 1.5 hrs. 50bp ladder was used for estimation of size of fragments. The amplified products were visualized with the help of a gel documentation system (Alpha Innotech, USA) and the size of fragments was estimated with the help of 50bp ladder (Fermentas).

Results

The superior quality of DNA sample was obtained from young leaf of eighteen maize inbred lines(Fig.1) and amplified using microsatellite markers namely, nc130, phi084, umc1161, phi034. The amplification was observed in all samples tested with little variation in intensity of the amplified bands. After amplification nc130

produced 150-171bp, phi084 produced 157-195, umc1161 produced 63-180bp and phi034 produced 138-172bp polymorphic product (Fig. 2).

Discussion

The present study is concerned with modification of CTAB method of genomic DNA extraction without using liquid nitrogen and phenol.The concentration of NaCl was high(5M) which extracted high quantity of DNA.The main action of NaCl in extraction buffer is the removal of proteins and carbohydrates which are attached to the DNA and use of polyvinylpyrrolidon to remove polyphenols[Porebski *et al.*, 1997].The DNA obtained using this extraction protocol is suitable for polymerase chain reaction (PCR) genotyping, which can be employed for the identification of alleles in diverse genetic and breeding approaches, such as marker-assisted selection, genetic fine mapping, and mutant introgression. All four microsatellite markers show very good amplification and polymorphism with the DNA extracted through this method. Therefore, this method should be recognizing as a good rapid and inexpensive method for DNA extraction from maize leaves.

Acknowledgements

Authors are grateful to the Department of Agricultural Biotechnology & Molecular Biology,Faculty of Basic Sciences & Humanities (FBS&H),Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India for providing laboratory facilitation for this study.

Table 1. Microsatellite primers used during amplification reaction.

Sl. No.	Locus	Primer sequences(5'-3')	Repeat Motif
1.	nc130	(F) GCACATGAAGATCCTGCTGA (R) TGTGGATGACGGTGATGC	AGC
2.	phi084	(F)AGAAGGAATCCGATCCATCCAAGC (R) CACCCGTACTTGAGGAAAACCC	GAA
3.	umc1161	(F) GGTACCGCTACTGCTTGTTACTGC (R) GCTCGCTGTTGGTAGCAAGTTTTA	(GCTGGG)5
4.	Phi034	(F) TAGCGACAGGATGGCCTCTTCT (R) GGGGAGCACGCCTTCGTTCT	CCT

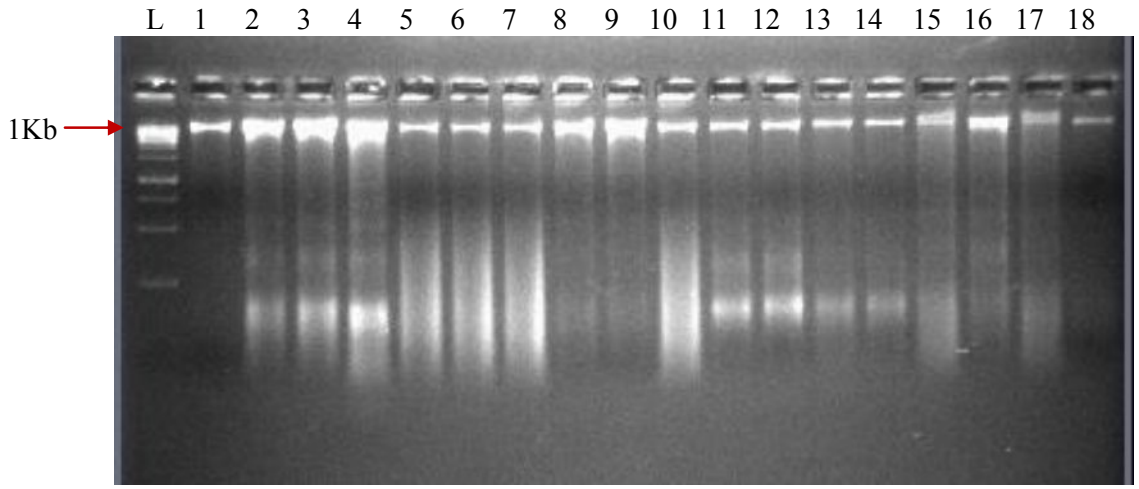

Fig.1. Genomic DNA extracted from the leaves at seedling stage in different maize genotypes used in the study.

1. CML 467	4. CML470	7. CML115	10. LM13	13. HKI323B	16. CML161
2. CML468	5. CML471	8. CML 196	11. DH2012	14. HKI586	17. CML165
3. CML469	6. CML373	9. CML465	12. HKI162	15. HKI1105	18. CML163

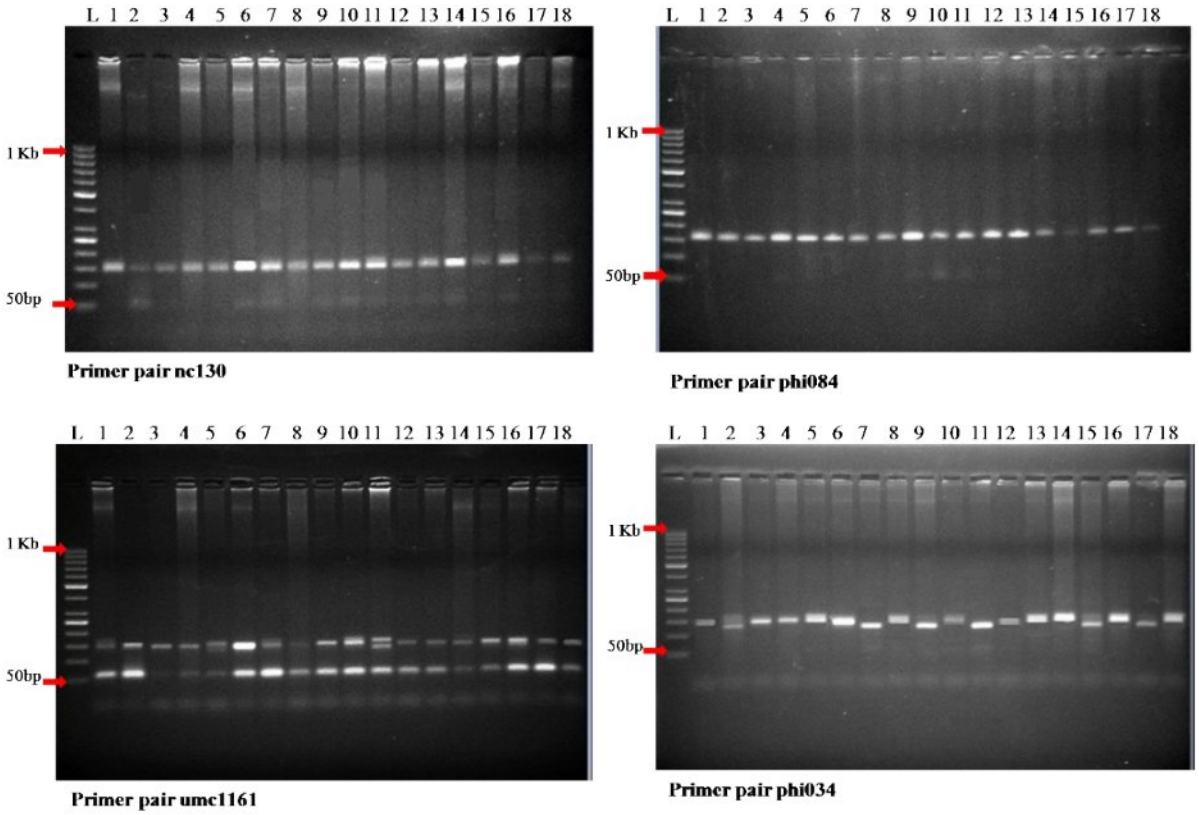

Fig. 2. PCR amplification of DNA isolated from leaves of 18 maize inbred lines.

References

1. **Remington D.L., Thornsberry J.M., Matsuoka Y., Wilson L.M., Whitt S.R., Doebley J., Kresovich S., Goodman M.M. and Buckler E.S.** 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc. Nat1 Acad. Sci*., **98** : 11479-11484.

2. **Thornsberry J.M., Goodman M.M., Doebley J., Kresovich S., Nielsen D. and Buckler E.S.** 2001. Dwarf8 polymorphisms associated with variation in flowering time. *Nat. Genet.*, **28** : 286-289.

3. **Pressoir G., Brown P.J., Zhu W.Y., Upadyayula N., Rocheford T., Buckler E.S. and Kresovich S.** 2009. Natural variation in maize architecture is mediated by allelic differences at the PINCODE cortholog barren inflorescence. *Plant J.*, **58** : 618-628.

4. **Wilson L.M., Wllitt S.R., Ibanes A.M., Rocheford T.R., Goodman M.M. and Buckler E.S.** 2004. Dissection of maize kernel composition and starch production by candidate associations. *Plant Cell*, **16** : 2719-2733.

5. **Harjes C.E., Rocheford T.R., Bai L., Brutnell T.P., Kandianis C.B., Sowinski S.G., Stapleton A.E., Vallabhaneni R., Williams M., Wurtzel E.T., Yan J.B. and Buckler E.S.** 2008. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. *Science*, **319** : 330-333.

6. **Yan J.B., Kandianis C.B., Harjes C.E., Bai L., Kim E., Yang X.H., Skinner D., Fu Z.Y., Mitchell S., Li Q., Fernandez M.G.S., Zaharieva M., Babu R., Fu Y., Palacios N., Li J.S., DellaPenna D, Brutnell T., Buckler E.S., Warburton M.L. and Rocheford T.** 2010b. Rare genetic variation at zea mays crtRBI increases b-carotene in maize grain. *Nat Genet*, doi:10.1038/ng.551.

7. **Dellaporta S.L., Wood J. and Hicks J.B.** 1983. A plant DNA mini preparation version II. *PMB Reporter*, **4** : 19-21.

8. **Saghai-Maroof M.A., Soliman K.M., Jorgensen R.A. and Allard R.W.** 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc. Natl. Acad. Sci.* USA, **81** : 8014–8019.

9. **Ouenzar B., Hartmann C., Rode A. and Benslimane A.** 1998. Date Palm DNA Mini-Preparation without liquid nitrogen. *Plant Mol. Biol. Rep*, **16** : 263-269.

10.**Rajendrakumar P., Sujatha K., Rao K.S., Nataraj K.P., Viraktamath B.C., Balachandran S.M., Biswal A.K. and Sundram R.M.** 2011. A protocol for isolation of DNA suitable for rapid seed and grain purity assessments in rice. *Rice Genetics Newsletter*, **23**: 92-95.

11. **Sharma R., Mahla H.R., Mohapatra T., Bhargava S.C. and Sharma M.M.** 2003. Isolating Plant Genomic DNA without Liquid Nitrogen. *Plant Mol. Biol. Rep.,* **21**: 43-50.

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