Indian J. Hort. 70(4), December 2013: 584-586

Short communication

An efficient method of genomic DNA isolation from pomegranate

Kanupriya, Nischita P. and K.V. Ravishankar*

Division of Biotechnology, Indian Institute of Horticultural Research, Hessaraghatta Lake PO, Bengaluru 560089

ABSTRACT

Protocol for extracting intact genomic DNA of reasonable quality and purity from minor and underexploited and secondary metabolite rich crops like pomegranate is not available. The presence of large amount of interfering components like polyphenols, polysaccharides, and other secondary metabolites make DNA isolation from pomegranate challenging. The method described involves a modification of the available CTAB method employing silica matrix which yielded high quality DNA. The protocol has been successfully tested with a wild pomegranate genotype and *Hind* III enzyme digestion. Results show that the DNA isolated is good enough for downstream analysis.

Key words: DNA isolation, silica matrix, pomegranate, polysaccharides, phenols.

Pomegranate also called *anar* (*Punica granatum* L.) belonging to Punicaceae family is a favourite table fruit of tropical and sub-tropical countries. The pomegranate fruit has therapeutic value, good keeping quality and export potential. In the global functional food industry, pomegranate is included among a novel category of exotic plant sources called super fruits. The extracts from this plant (juices, seed oil and peel) have been reported to exhibit strong antioxidant activity (Lanskey, 5).

Pomegranate like several other medicinal and fruit crops is considered to be a "difficult" plant for DNA isolation due to its high polyphenol content, which interfere with the DNA purity especially for subsequent manipulations. In our lab, we are working on isolation of microsatellites from pomegranate which involves digestion of genomic DNA with enzymes and cloning. We found that the isolation of good quality DNA from this crop was difficult with standard CTAB method. Antioxidants like 2 β-mercaptoethanol, ascorbic acid, bovine serum albumin, sodium azide and PVP are commonly used to deal with problems related to phenolics (Dawson, 2; Clake, 1). Phenol extractions when coupled with SDS are also helpful. However, with plants having a high content of polyphenolics, SDS-phenol tends to produce low yields of DNA (Rezaian, 6). Although there are several published protocols on plant DNA isolation (Dellaporta, 3; Rogers, 7; Draper, 4) the production of large quantities of purified pomegranate genomic DNA is still difficult. Hence, the present study was undertaken in a wild pomegranate genotype called *Daru*, which grows on the Himalayan foothills and is known to be an important source of resistance for several diseases.

*Corresponding author's E-mail: kvravi@iihr.ernet.in

About hundred milligram sample of young, tender leaves from mother plants was collected from the fruit block of IIHR, Bengaluru. The chemicals and reagents used in the isolation of DNA were: CTAB extraction buffer (2% CTAB, 20 mM Na EDTA, pH 8.0; 100 mM Tris-HCl, 1.4 mM NaCl); 0.2% β-mercaptaethanol; sodium metabisulphate; PVP; chloroform : isoamyl alcohol (24:1); 2.5 mM potassium acetate; isopropanaol; absolute alcohol; 75% ethanol; RNase (10 mg/ ml) ; TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0); washing solution (50% ethanol, 10 mM Tris-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA).

Plant material frozen in liquid nitrogen (-196°C) was ground to fine powder in sterile mortar, sodium metabisulphate (50 mg) and PVP = Polyvinyl pyrollidone (50 mg) were added before transferring to 1 ml of pre-heated (65°C) CTAB-DNA extraction buffer in sterile 2 ml Eppendorf tubes. The samples were given gentle inversions for 5 min. The homogenates were incubated in a warm water bath maintained at 60°C for 1 h with intermittent swirling. After incubation, the homogenates was brought to room temperature and 1 ml of chloroform: isoamylalcohol (24:1; v/v) was added to the homogenate and mixed gently by inversions, followed by centrifugation at 10,000 rpm for 10-15 min. The supernatant was collected and an equal volume of potassium acetate and chloroform: isoamylalcohol (24:1, v/v) was added. The homogenates were mixed gently by inversions, followed by centrifugation at 10,000 rpm for 10-15 min. The aqueous phase was transferred to fresh tubes and equal volume of isopropanol was added, and the mixture placed in -20°C for 1 h. Thereafter, the samples were centrifuged at 10,000 rpm for 10 minutes, the supernatant was discarded, and the DNA pellet washed twice with 1 ml

absolute ethanol and kept at -20°C for half an hour. It was then centrifuged at 10,000 rpm for 5-10 min. to obtain a pellet. Further washing was carried out with 200 µl of 76% ethanol followed by spinning at 10,000 rpm for 5-10 min. To remove ethanol and dry the pellet it was inverted and kept at 37°C for 30-60 min. Finally it was dissolved in 30 µl TE buffer, incubated at 37°C till completely dissolved before adding RNase to the final concentration of 10 µg/ml. 20 µl of silica dioxide was added to the sample and kept undisturbed at room temperature for 5 min. The matrix was then pelleted by centrifugation at 16,000 rpm for 10 second; the supernatant was removed by pipetting. The matrix was washed by resuspending with 1 ml washing solution and vigorously vortexed. The matrix was again pelleted by centrifugation at 16,000 rpm for 10 sec and pellet was retained. This step was repeated twice. The pellet was centrifuged for another 10 sec and the residual liquid was carefully pipetted off. To the pellet 40 µl of sterile water/ TE buffer was added and the pellet was resuspended by brief vortexing. The microfuge tube was placed at 70°C for 2 min. followed by centrifugation at 16,000 rpm for 2 min. and the supernatant containing the eluted genomic DNA was transferred to a fresh tube. The yield of DNA per gram of leaf tissue extracted was measured using a UV spectrophotometer (GeneQuant , GE Health Care Bio-Sciences Ltd., England). The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm. DNA samples from the leaf tissues were digested with *Hind*III and electrophoresed on a 0.8% agarose gel.

Isolation treatment where the standard CTAB method of extraction without modification was used, gave a DNA yield of 1800 ng per 2 g of leaf material. However, it was contaminated with polysaccharides and phenolics as shown by the spectrophotometer readings A_{260} nm / A_{280} nm ratio of 1.48. This ratio is highly deviated from the ideal ratio of 1.8-2.0 due to presence of contaminants. The sample was very viscous and pinkish in colour (Fig.1A). Isolation of genomic DNA using the described method was quite easy, completed in a day and did not take more than 100 mg of leaf tissue. The yield of DNA was reduced following purification with silica matrix and it was 106 ng per 100 mg of leaf material. This problem was overcome by pooling the DNA after the purification step. The quality of DNA was good as concluded from spectrophotometer readings A_{260} nm/A_{280} nm ratio was 1.90 and enzyme digestion. From the different steps followed and modifications made, it was found that using younger leaves instead of older ones reduced the nucleic acid contamination by plant metabolites. The presence of sodium metabisulphite, PVP and potassium acetate helped to remove polyphenols and secondary metabolites. Potassium acetate with isopropanol can efficiently precipitate DNA of high molecular weight free of polyphenols and other secondary metabolites. The silica matrix further purified the DNA based on the principle of separation of DNA molecules binding to silica surfaces in the presence of certain salts and under certain pH conditions. This principle is used in DNA isolation kits which have a silica column for this purpose. While the columns are expensive, silica dioxide can be used as an alternative to get similar results. After the DNA is adsorbed to the silica surface, all other molecules pass through in the supernatant during the wash steps and the DNA is eluted from matrix by water or TE buffer (Fig B). In this process, the concentration of DNA goes down but the quality improves and the DNA extracted can be digested with restriction enzymes such as *Hind*III (Fig. 1C). In conclusion, this protocol is expected to be preferable for DNA extraction for plants rich in secondary metabolites like pomegranate.

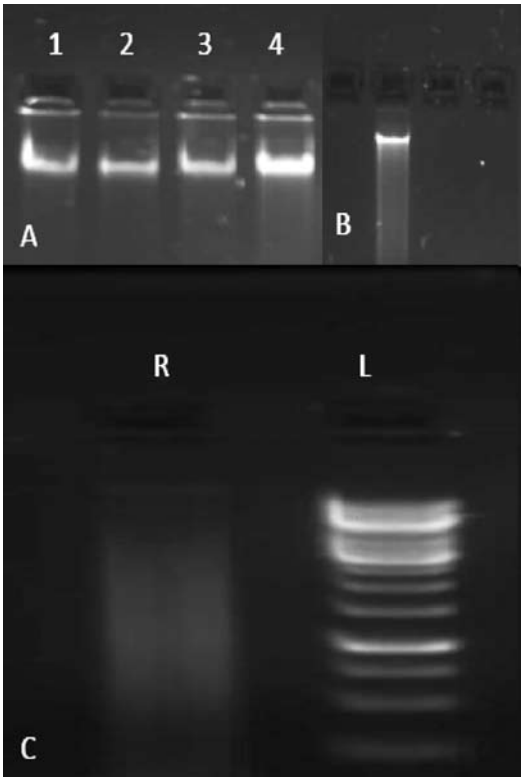

Fig. 1. DNA profile of pomergranate (A) Four replications of genomic DNA samples of Daru which were isolated by the standard CTAB method in pomegranate, (B). The pooled genomic DNA of pomegranate isolated by modified CTAB method using silica matrix, (C) Fig C. *Hind*III DNA digestion to check the purity of the isolated DNA R: *Hind*III digestion, L-50 bp ladder.

REFERENCES

1. Clark, M.S. 1997. **In**: *Plant Molecular Biology - A Laboratory Manual*, Springer-Verlag, Berlin, Heidelberg, New York, pp. 305-28.
2. Dawson, C.R. and Magee, R.J. 1995. Plant tyrosinase (polyphenol oxidase). *Methods Enzymol.* **2**: 817-27.
3. Dellaporta, S.L., Wood, J. and Hicks, J.B. 1995. A plant DNA mini-preparation: version II. *Plant Mol. Biol*. *Rep.* pp. 19-21.
4. Draper, J. and Scott, R. 1991. **In**: *Gene Transfer to Plants*, D. Grierson (Ed.), Plant Genetic Engineering, Chapman and Hall Pub., New York, pp. 40-43.
5. Lansky, E.P. and Newman, R.A. 2007. *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *J. Ethnopharmacol.* **109**: 177-206.
6. Rezaian, M.A. and Krake, L.R. 1987. Nucleic acid extraction and virus detection in grapevine. *J. Virol. Methods*, **17**: 277-85.
7. Rogers, S.O. and Bendich, A.J. 1988. Extraction of DNA from plant tissues. *Plant Mol. Biol. Manual.* **6**: 1-10.
8. Jian-Feng, L., Li, L., and Jen Sheen 2010. Protocol: a rapid and economical procedure for purification of plasmid or plant DNA with diverse applications in plant biology. *Plant Methods*, **6**: 1-5.

Received : December, 2012; Revised : August, 2013;
Accepted : September, 2013