Plant Molecular Biology Reporter
Volume 9(3) 1991

pages 262-266

Protocol

A Modified CTAB DNA Extraction Procedure for *Musa* and *Ipomoea*

N.J. Gawel and R.L. Jarret

(NJG) Department of Plant Introduction, and (RLJ) USDA-ARS Regional Plant Introduction Station, Georgia Station, 1109 Experiment St., Griffin, GA 30223, USA

Key Words: DNA extraction, CTAB, *Ipomoea*, *Musa*, sweetpotato, banana, okra, pigeon pea, cotton.

Abstract: The utilization of current nucleic acid technologies in crop improvement and phylogenetic studies require the development and application of efficient DNA extraction procedures from plants. This paper describes efficient, reliable DNA extraction procedures for *Ipomoea* and *Musa*. These procedures are combinations and modifications of the techniques described by Murray and Thompson (1980) and Saghai-Maroof et al. (1984) and are applicable, without further modification, to other plant genera.

The applications of current nucleic acid technologies in crop improvement are numerous: gene introgression, gene mapping, genetic fingerprinting, phylogenetic analyses, etc. (Burr et al., 1986). These techniques have numerous applications to improvement of *Musa* (Jarret, 1990; Gawel and Jarret, 1990) and *Ipomoea* research (Jarret and Florkowski, 1990). The development of efficient DNA extraction techniques, which yield DNA of a purity adequate for restriction enzyme digestion, has proven difficult from plant materials in these genera. After experimenting with a variety of techniques, our lab has developed

---

Abbreviations: RT, room temperature; CTAB, hexadecyltrimethylammonium bromide.

simple, efficient procedures for the extraction of *Musa* and *Ipomoea* DNA. We have, on occasion, received inquiries from other laboratories concerning these techniques. Therefore, with the hope of helping others to avoid the frustrations we initially encountered, we present our protocol for isolation of high molecular weight DNA from *Musa* and *Ipomoea* leaf tissue. These procedures are a combination of modified extraction techniques of Murray and Thompson (1980) and Saghai-Maroof et al. (1984).

Materials and Methods

Reagents

Extraction buffer (EB): [1% (*Ipomoea*) or 2% (*Musa*) (w/v) CTAB, 100 mM Tris.HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.1% (*Musa*) or 1.0% (*Ipomoea*) (v/v) mercaptoethanol (ME added immediately prior to use)]
TE: 10 mM Tris , 1.0 mM EDTA, pH 8.0
Precipitation buffer (PB): EB without ME or NaCl
EtOH: 100% and 70%
Isopropanol
Chloroform: Isoamyl alcohol (24:1, v/v)
RNAse

DNA Extraction from *Musa*

- Grind lyophilized leaf tissue (500 mg) in liquid N_2 with a mortar and pestle or with a grinding mill.
- Add the powdered tissue to 20 mL pre-heated extraction buffer and incubate (65 °C) for 30 min with occasional mixing.
- Add 15 mL of chloroform:isoamyl alcohol (24:1, v/v), and mix by inversion for 15 min.
- Centrifuge 5 min at 5,000 x g at room temperature (RT) and filter the aqueous phase through Miracloth (or equivalent).
- Add an equal volume of ice-cold isopropanol. Mix by inversion until DNA precipitates (10 to 20 seconds).
- Hook the DNA onto a bent Pasteur pipet or spool onto a glass rod and rinse in a beaker of 70% ethanol.
- Blot the DNA dry on a Kimwipe or paper towel.
- Dissolve the DNA in 250 to 500 µL TE buffer (Extracts may need to be heated at 65 °C for a short time to completely dissolve.)
- Add RNAse (final concentration 10 µg/mL) and incubate 30 min at room temperature.
- Add 1/10 vol 3 M NaOAc (pH 6.8) and 2 volumes of 95% ethanol.

- Hook DNA on a bent Pasteur pipet, rinse in 70% alcohol and blot dry.
- Dissolve in 250 to 500 µL TE.

DNA Extraction from *Ipomoea*

- Add 200 mg powdered lyophilized leaf tissue to 8 mL EB containing 1% (w/v) CTAB and 1% (v/v) ME (see above).
- Incubate the mixture for 30 min at 65°C.
- Extract with an equal volume of chloroform:isoamyl alcohol.
- Centrifuge at 2,500 x g for 5 min at room temperature to separate the phases.
- Draw off the aqueous phase and re-extract with an equal volume of chloroform:isoamyl alcohol.
- Centrifuge at 5,000 x g and transfer the upper phase to a 15 mL Corex tube.
- Add an equal volume of PB and mix by quick inversion several times.
- Allow to sit at room temperature for 1 hour.
- Pellet the nucleic acids by centrifugation at 5,000 x g at RT.
- Redissolve the pellet in 450 µL 1M NaCl with heat (1 hr at 65°C).
- The DNA is precipitated by addition of 2 volumes of 100% EtOH, collected by centrifugation, washed with 70% EtOH and resuspended in 200 µL TE.

Discussion

Musa

With this technique, we generally obtain 100 to500 µg DNA (A_{260}/A_{280} = 1.85-1.95) per gram fresh weight leaf tissue (Table I). Best results are obtained when the leaf tissue is harvested as young as possible, preferably while still tightly furled. The midrib should be removed prior to lyophilization. If lyophilization is not possible, fresh tissue may be used if the CTAB concentration is increased to 4% (w/v). When using fresh tissue, care should be taken to assure that the ground tissue does not thaw before it is added to the extraction buffer. The use of 0.1% mercaptoethanol does not completely inhibit oxidation in all cases. However, the oxidation which does occur does not appear to affect the activity of restriction enzymes. If more than 0.1% mercaptoethanol is used, DNA recovery is greatly reduced. In addition, care should be taken to hook out the DNA immediately after the isopropanol precipitation in order to avoid the co-precipitation of contaminating proteins.

Table I. Yields and A_{260}/A_{280} values for DNA extracted from various plant genera.

Plant	Lyophilized tissue	DNA Yield	A_{260}/A_{280}
Musa acuminata	500 mg	393.4 μg*	1.87*
Ipomoea batatas	200 mg	249.2 μg*	2.08*
Gossypium hirsutum	500 mg	276.6 μg*	2.01*
Juglans nigra	1000 mg	380.0 μg*	1.97*
Abelmoschus manihot	250 mg	198.8 μg**	2.10**
Cajanus cajan	250 mg	445.0 μg**	2.02**

*Mean of 10 extractions.
**Single extraction.

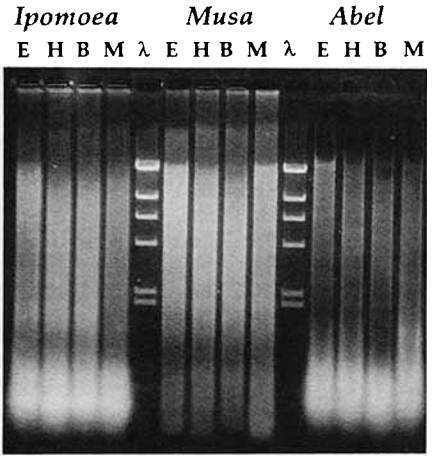

Fig. 1. Restriction enzyme digestion of DNA extracted from *Ipomoea batatas*, *Musa acuminata*, and *Abelmoschus manihot*. Restriction enzymes used are *Eco* RI (*E*), *Hind*III (*H*), *Bam* HI (*B*) and *Msp*I (*M*). Molecular weight markers are *Hind* III-digested λ. The *M. acuminata* DNA has been treated with RNAase.

Ipomoea

We routinely separate the DNA resuspended in TE from insoluble material by centrifugation in a microcentrifuge for 30 to 60 sec. This improves the efficiency of the enzyme digestion. Yields range from 100 to 500 µg DNA/200 mg lyophilized leaf tissue following this procedure. However, yields were noticeably higher when using sweetpotato (*Ipomoea batatas*) as compared to other *Ipomoea* species.

Yields of DNA may vary with the physiological age of the plant material, the period of time that the material is stored prior to extraction, etc.; hence, modifications may be necessary to increase yields in specific instances. Treatment with RNAase is not essential for complete digestion by restriction enzymes. The extraction procedures described here are relatively simple and efficient. These procedures yield high-quality DNA which is readily digested by restriction endonucleases. We have adapted the Murray andThompson (1980) CTAB technique to other plant genera (*Cajanus, Gossypium, Juglans, Abelmoschus*)and recommend this technique as a starting point when developing a DNA extraction procedure for plants.

References

Burr B., S.V. Evola and F.A. Burr. 1986. The applications of restriction fragment polymorphisms to plant breeding. In: *Genetic Engineering: Principles and Methods.* New York, Plenum Press.

Gawel, N.J. and R.L. Jarret. 1990. Cytoplasmic genetic diversity in bananas and plantains. Euphytica 52:19-23.

Jarret, R.L. 1990. Molecular methods for detection genetic diversity in *Musa*. In: *Identification of Genetic Diversity in the Genus* Musa. R.L. Jarret, ed. INIBAP, Montpellier.

Jarret, R.L. and W.J. Florkowski. 1990. *In vitro* vs. field gene bank maintenance of sweetpotato germplasm: Major costs and considerations. HortScience 25(2):141-146.

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight plant DNA. Nuc. Acids Res. 8:4321-4325.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen and R.W. Allard. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc. Natl. Acad. Sci. (USA) 81:8014-8018.