Plant Molecular Biology Reporter
Volume 8(1) 1990

pages 3-7

Protocol

A Rapid and Efficient Method for the Isolation of Restrictable Total DNA from Plants of the Genus *Abelmoschus*

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Keywords: *Abelmoschus*, total DNA, purification, restriction.

Abstract: A rapid, simple and efficient protocol is given for the extraction of restrictable total DNA from plants of the genus *Abelmoschus*, for which the main obstacle is the stickiness of the solution after grinding of green leaves. This problem is resolved using cotyledons of dark-grown seedlings.

The genus *Abelmoschus* (okra) is composed of numerous species growing in tropical and subtropical areas (Charrier, 1984). Two are widely cultivated: *A. esculentus*, found throughout the tropics,

addition, the number of chloroplasts is lower in these leaves and hence the proportion of cpDNA is reduced.

Solutions required

Extraction buffer
100 mM Tris-HCl pH 8
50 mM EDTA
500 mM NaCl
10 mM 2-mercaptoethanol

TE
50 mM Tris-HCl pH 8.0
10 mM EDTA

Methods

Seeds were sown in sterile soil in the dark at 30°C. Five to seven days later, about 1 g of cotyledons, yellow and unopened, was harvested. For most varieties of cultivated species, leaves from seven to ten seedlings sufficed.

The leaves were then washed with tap water and air dried on filter paper. Thereafter a purification process adapted from Dellaporta (1983) was followed:

- The cotyledons were quickly ground in liquid nitrogen in a mortar cooled previously in a freezer.
- The resulting powder was placed in a 30-ml tube; 15 ml of extraction buffer and 1 ml of 20% SDS were added. The powder had to be kept frozen until the buffer was added.
- After vigorous shaking by hand, the tube was heated for 10 min at 65°C and shaken gently.
- 5 ml of 5 M potassium acetate was added and after further shaking the tube was placed in ice for 20 min.
- The tube was centrifuged at 13,000 g for 20 min.
- The supernatant was decanted through a nylon membrane into a clean 30-ml tube containing 10 ml of isopropanol. After several inversions of the tube to homogenize the contents, it was kept at -20°C for a minimum of 30 min.
- A further centrifugation at 13,000 g for 15 min produced a pellet rich in nucleic acids.

- This pellet was air dried for 10 min and then resuspended in 700 µl

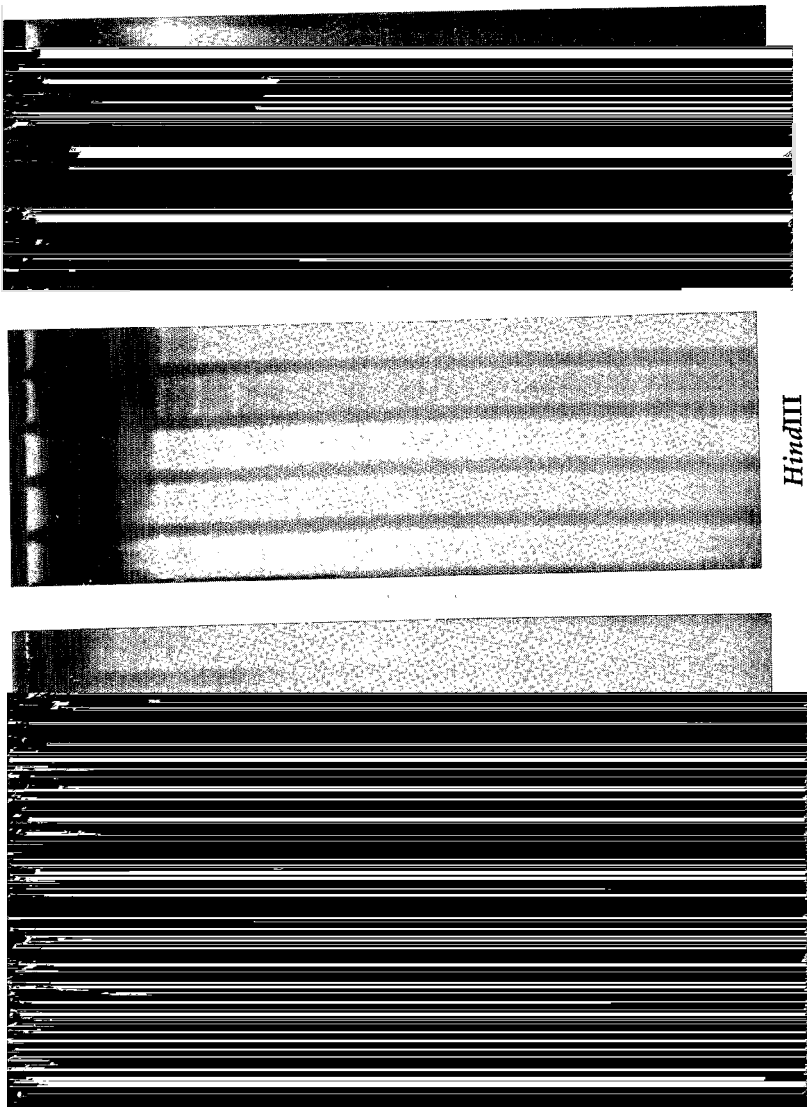
*Hind*III

Fig. 1. (opposite page) **Agarose gel electrophoresis of *Abelmoschus* DNAs samples** digested by: *Eco*RI, *Hind*III, and *Sal*I.

This method requires only a few seeds of each variety for analysis and can be applied successfully to frozen material, yielding an amount of DNA comparable to that obtained using fresh material. The only drawback to our method is that it involves the destruction of the analyzed plants and it is not applicable if there is intravarietal variability.

In order to solve this problem, we tried to isolate total DNA using young leaves from two-week-old plants grown in light, but placed in darkness 48 hours before extraction. With sufficient precautions, the plants will survive under these circumstances. Theoretically there is enough fresh material in one plant to obtain sufficient DNA for several digests. It proved impossible, however, to recover the DNA which was lost in one of the pellets. Keeping the plants in darkness for more than 48 hours does not improve yield since their condition visibly deteriorates.

The technique described here can be used with other higher plants if fresh green leaves are used in place of cotyledons. We have tested it successfully on rice.

Acknowledgements: The authors are very grateful to Dr. L. Fishpool for correcting the English. We thank the IBPGR for the financial support.

References

Charrier, A. 1984. Genetic resources of *Abelmoschus* (okra). IBPGR ed. Rome, 61p.

Dellaporta, S.J., J. Wood and J.B. Hicks. 1983. A plant minipreparation: Version II. Plant Mol. Biol. Reporter. 1(4):19-21.

Grubben, G.J.H. 1977. Tropical vegetables and their genetic resources. (H.D. Tindall & J.T. Willams, eds.) IBPGR, Rome, 197p.

Hamon, S. 1987. Organisation génétique du genre *Abelmoschus* (gombo): co-évolution de deux espèces cultivées de gombo en Afrique de l'Ouest (*A. esculentus* et *A. caillei*). Thèse

PLANT MOLECULAR BIOLOGY REPORTER

Volume VII, Number 1, February 1990

ISPMB