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Protocol

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

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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, and *A. caillei*, which is cultivated exclusively in Africa (Hamon, 1987; Hamon and Van Slotten, 1989). Interest in the crop is due principally to the high protein and mineral salt content of the pods, making okra a very good vegetable. Studies have shown that the daily consumption of 100 grams of okra provides 20% of the calcium, 15% of the iron, and 50% of the vitamin C of human dietary requirements (Grubben, 1977).

The main obstacle preventing the extraction of DNA from green leaves of all members of the genus is that, even after grinding in liquid nitrogen, the resulting suspension is very sticky and it is almost impossible to obtain DNA by a mini-preparation, such as the one described below. This consistency is principally due to large amounts of polysaccharides produced during photosynthesis. For this reason we chose to purify total DNA using cotyledons of seedlings grown in the dark. In

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 of TE. The suspension was then decanted into an Eppendorf tube.
- The sample was centrifuged for 10 min in a microfuge at maximum speed and the supernatant was put in a fresh tube.
- 1 µl of boiled RNAase (10 mg/ml) was added and the tube incubated for 1 h at 37°C.
- 77 µl of 3 M sodium acetate were added[1] and the solution purified by adding an equal volume of phenol-chloroform (1:1).
- The aqueous phase was re-extracted with an equal volume of chloroform.
- The 0.7 volume of cold isopropanol was added to the aqueous phase, the tube inverted several times, and centrifuged for 1 min. The supernatant was decanted carefully so as not to disturb the pellet, and the pellet washed with 80% (v/v) cold ethanol and centrifuged again for 30 sec.
- The supernatant was carefully removed, and the pellet dried in a vacuum dessicator.
- 100 µl of TE were then added and the pellet left to rehydrate on ice for at least one hour or preferably overnight.

Note

1. Addition of acetate at this stage, rather than just prior to precipitation, resulted in a better recovery of DNA in the aqueous phase.

Results and Discussion

This method, yields about 100 µg of total DNA from one gram of cotyledons from several samples and does not require the use of sophisticated equipment. The DNA obtained is clean and restrictable by all the enzymes we have tested (*Eco*RI, *Hind*III, *Sal*I, etc.) (Fig. 1).

The RNAase treatment during purification, followed by a phenol-chloroform extraction, produces pure DNA that is both RNA- and protein-free. It proved easier to perform a second extraction with pure chloroform, as this allows better cleaning of the DNA and avoids the necessity of removing traces of phenol in the aqueous phase using ether, which might itself be eliminated before DNA precipitation. We also tried precipitation using cetyl trimethyl ammonium bromide. Apart from the fact that it is more difficult to obtain and preserve, it also gives a lower yield than isopropanol.

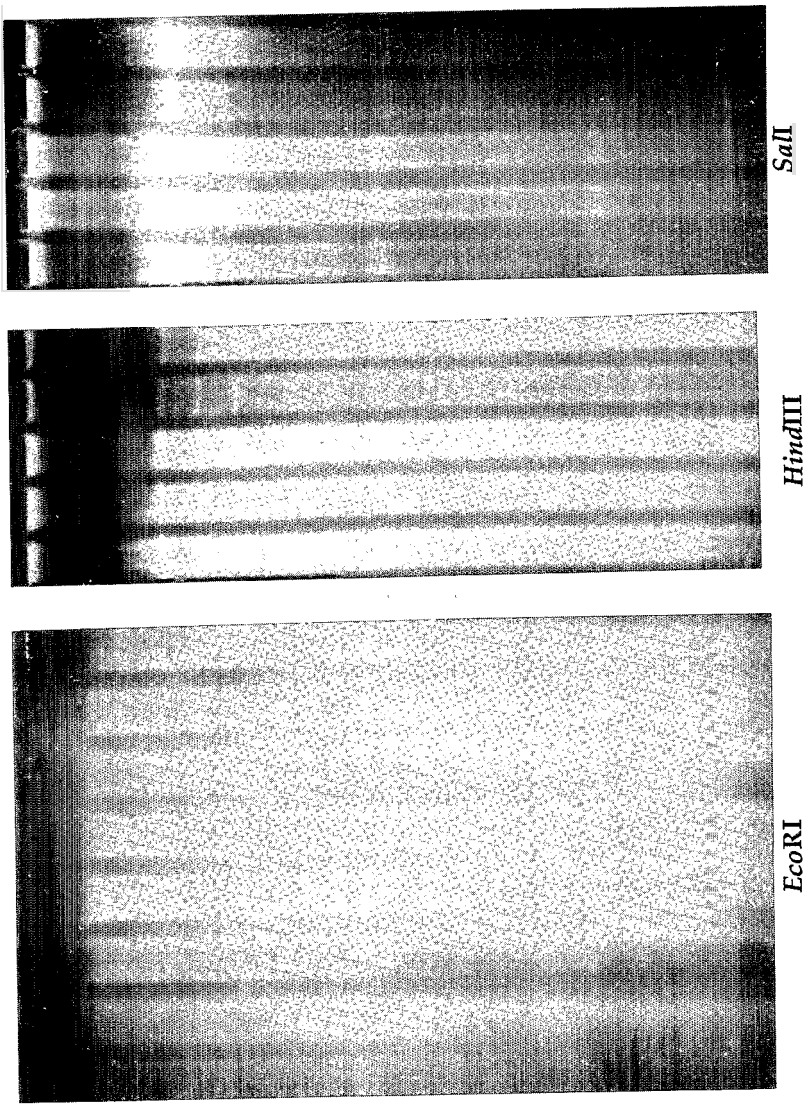
SalI
HindIII
EcoRI

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.

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