

Short communication

Isolation of mitochondrial DNA from cytoplasmic male sterile and maintainer lines of pearl millet, *Pennisetum americanum* (L.) Leeke

B. B. Singh^{1,*}, H. Rathburn² and C. Hedgcoth²

¹ ICRISAT, BP 260 Maradi, Republic of Niger

² Department of Biochemistry, Kansas State University, Manhattan, KS 66506, USA

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Summary. Mitochondrial DNA has been isolated from paired lines of pearl millet maintainer and cytoplasmic male sterile plants. Evaluation of the DNA by agarose gel electrophoresis shows that good quality DNA of high molecular weight can be obtained from mitochondria of both maintainer and male sterile pearl millet.

Key words: Mitochondrial DNA – Cytoplasmic male sterile – Pearl millet – *Pennisetum americanum*

Introduction

Cytoplasmic male sterile line 'Tift 23A', developed at Tifton, Georgia, USA (Burton 1958), has been extensively used in Indian pearl millet improvement programs since 1962. Two other sources of cytoplasmic male sterility designated as 'L66A' and 'L67A' were identified and developed at Ludhiana, Punjab in India (Burton and Athwal 1967). The only method of distinguishing different sources of sterile cytoplasms has been to test the reciprocal maintainer-restoration ability among crosses of sterile and maintainer lines (Burton and Athwal 1967).

With the increasing number of discoveries of new male sterility sources and diversification of established sources of sterile cytoplasm by incorporating desirable nuclear genomes (Anand Kumar and Andrews 1984), the use of rapid assay and biochemical techniques to identify and classify different sources of male sterility needs to be explored. Investigations in maize by Leving and Pring (1976, 1977) and by Pring and

Leving (1978) have clearly demonstrated that this trait is not inherited in a Mendelian fashion, therefore, it is not under the control of nuclear genes. Pring et al. (1979) and Kemble et al. (1980) have suggested that the factors responsible for cytoplasmic male sterility in maize are located in mitochondrial DNA (mtDNA). Dixon and Leaver (1982) have observed that variation in sorghum mitochondrial translation products has enabled fertile (Kafir) cytoplasm to be distinguished from Milo cytoplasmic male sterile cytoplasm and from three alternative sources of cytoplasmic male sterile cytoplasm in this crop. Work along this line has not been reported in pearl millet.

We have attempted to standardize the biochemical procedure for isolating mtDNA from pearl millet lines. Electrophoretic analysis of mtDNA from A (cytoplasmic male sterile) and B (maintainer male fertile line) lines has been done.

Materials and methods

Growth of millet shoots

The growing of pearl millet seedlings was undertaken by a modification of the procedure of Speakman and Krüger 1983. An autoclavable container was fitted with wire mesh as a platform which was covered with cheesecloth and then another piece of wire mesh. The container, covered with aluminum foil, was autoclaved twice. Forty grams of clean and healthy seed of a pearl millet line was treated with 100 ml of 1 ppm terramycin (oxytetracycline hydrochloride) solution in sterile deionised water at room temperature for 14 h. After draining off the terramycin solution, 100 ml of 0.1% AgNO_3 , prepared with sterile deionised water, was added to the seed. After 10 min the AgNO_3 solution was replaced by 0.5% NaCl in sterile deionised water. The mixture was shaken at 150 RPM for 10 min at room temperature. Seeds were rinsed three times with 100 ml of sterile deionised water.

Under a bacteriological hood seeds were spread on the cheesecloth with a sterile glass rod. Seeds were sprayed liberally with terracote solution (0.13 g Terraclor Super-X, 20-5 in 250 ml of sterile water). The covered container was stored

* Present address: Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221 005, India

away from light. Shoots were harvested from one-week old etiolated seedlings.

Isolation of mitochondrial DNA

Isolation of millet mitochondrial DNA was adapted and modified from the procedure described by Day and Hanson (1977) and Kemble et al. (1980).

Etiolated seedlings, about 50 g wet weight, were harvested onto cold aluminium foil placed on a tray with ice. Shoots were ground gently in the cold room in 3 vol of grinding medium (based on tissue weight) using a mortar and pestle for about 40 s. The grinding medium consisted of 0.5 M mannitol in 10 mM TES, 1 mM EGTA, pH 7.2; 0.2% BSA fatty acid free, and 0.25% cysteine. The mixture was filtered through four layers of cheesecloth and one layer of miracloth directly into centrifuge tubes. After centrifuging for 10 min at 1,000×g at 4°C, the supernatant was decanted and again centrifuged at 12,000×g for 10 min. Pellets were resuspended gently with a small paint brush into 10 ml of grinding medium and centrifuged for 10 min at 1,000×g at 4°C. The supernatant was treated with 100 µl of 1 M Mg(OAc)₂ and 50 µl of a solution of DNase I (10 mg/ml). After 1 h at 0°, the solution was layered onto 20 ml of 0.6 M sucrose, 10 mM TES, 20 mM EDTA, pH 7.2, and centrifuged for 20 min at 10,000×g at 4°C. The pellet was resuspended with a paint brush in 10 ml of 0.6 M sucrose solution, 10 mM TES, 20 mM EDTA, pH 7.2, and centrifuged for 10 min at 10,000×g at 4°C. This step was repeated one more time. The pellet was resuspended in 2.0 ml of 50 mM Tris, 10 mM EDTA, pH 8.0. Then, 30 µl of 10 mg/ml Proteinase K and 0.5 ml of 10% Sarkosyl in 50 mM Tris, 10 mM EDTA, pH 8.0 were added. The mixture was incubated for 1 h at 37°C with gentle shaking. The lysate was transferred to a screw top culture tube and shaken gently with 0.3 ml 2 M NH₄OAc. The lysate was treated with 3.0 ml of water saturated

phenol and 3.0 ml of 24:1 chloroform:isoamyl alcohol with gentle shaking for 10 min. The tube was centrifuged for 5 min. The aqueous phase was saved and the organic phase was re-extracted with 0.25 ml of 0.2 M ammonium acetate in Tris/EDTA, pH 8.00 (270 µl of 50 mM Tris, 10 mM EDTA, pH 8 + 30 µl of 2 M ammonium acetate). The aqueous phases were combined and phenol chloroform extractions were repeated two more times. The final aqueous phase was transferred into a 15 ml corex tube and precipitated with 2.5 vol of cold 100% ethanol at -20°C, overnight. DNA was pelleted by centrifugation at 15,000×g for 10 min at 4°C. The pellet was resuspended in 70% ethanol, transferred to a 1.5 ml conical tube, and centrifuged for 5 min in an Eppendorf micro centrifuge. The mtDNA pellet was washed with 1 ml cold 70% ethanol. The mtDNA pellet was dried in a vacuum dessicator for 15–20 min and dissolved in 2.5 µl 50 mM Tris, 2.5 mM EDTA, pH 8.0, and 22.5 µl sterile deionized water.

Agarose gel electrophoresis of mtDNA

In one mtDNA sample, 1 µl was mixed with 4 µl of 50 mM Tris, 2.3 mM EDTA, pH 8, and 2.0 µl of 50% glycerol with 0.1% bromophenol blue. DNA was electrophoresed at room temperature in a 1% (wt/vol) agarose gel (10 cm×6 cm) for 2 h. After electrophoresis the gel was soaked in 10 µl of 10 mg/ml ethidium bromide per 100 ml water. The gel was photographed with a short wave length ultraviolet light with polaroid type 55 P/N film using a wratten No. 09 filter.

Results and discussion

Using the procedure developed by Kemble et al. (1980) to isolate mitochondrial DNA from maize, and wheat (Rathburn and Hedgcoth, unpublished observations), high molecular weight mitochondrial DNA was obtained from a line of fertile millet ('23 BE₁') and a line of cytoplasmic male sterile millet ('23 AE₁') (Fig. 1). These findings illustrate that the investigation of millet cytoplasmic male sterility at the molecular level can be pursued in a manner analogous to studies of other plants with cytoplasmic male sterile traits.

The spectrophotometric estimation indicated 3 to 7 µg of mitochondrial DNA per gram wet weights of plant tissue.

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References

- Anand Kumar K, Andrews DJ (1984) Cytoplasmic male sterility in pearl millet (*Pennisetum americanum* (L.) Leeke): a review. *Adv Appl Biol* 10:113–143
- Burton GW (1958) Cytoplasmic male sterility in pearl millet (*Pennisetum glaucum*). *Agron J* 50:230
- Burton GW, Athwal DS (1967) Two additional sources of cytoplasmic male sterility in pearl millet and their relation to 'Tift 23A'. *Crop Sci* 7:209–211

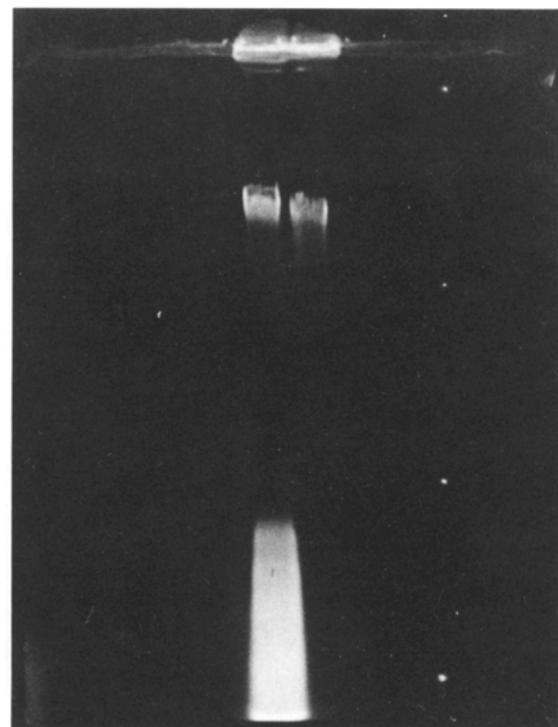


Fig. 1. Electrophoresis on a 1% agarose gel of mtDNA isolated from pearl millet lines '23 BE₁' (left) and '23 AE₁' (right)

- Day DA, Hanson JB (1977) On methods for the isolation of mitochondria from etiolated corn shoots. *Plant Sci Lett* 11: 99–104
- Dixon LK, Leaver CJ (1982) Mitochondrial gene expression and cytoplasmic male sterility in Sorghum. *Plant Mol Biol* 1:89–102
- Kemble RJ, Gunn RE, Flavell RB (1980) Classification of normal and male sterile cytoplasm in maize. 2. Electrophoretic analysis of DNA species in mitochondria. *Genetics* 95:451–458
- Levings III CS, Pring DR (1976) Restriction endonuclease analysis of mitochondrial DNA from normal and 'Texas' cytoplasmic male-sterile maize. *Science* 193:158–160
- Levings III CS, Pring DR (1977) Diversity of mitochondrial genomes among normal cytoplasms of maize. *J Hered* 68: 350–354
- Pring DR, Levings III CS (1978) Heterogeneity of maize cytoplasmic genomes among male sterile cytoplasms. *Genetics* 89:121–136
- Pring DR, Conde MF, Levings III CS (1979) Heterogeneity with the C group of male sterile cytoplasms. *Maize Genet Coop Newslett* 53:42
- Speakman JB, Krüger W (1983) A comparison of methods to surface sterilize wheat seeds. *Trans Br Mycol Soc* 80: 374–376