

2. Isolation and purification of maize nuclear DNA.

A large number of methods have been published for extraction of nucleic acids from plant material. The variety of approaches used attests to the difficulties of plant material. Our own experience and discussions with people working with maize suggest that DNA extraction from maize is particularly difficult.

A method has been devised in our laboratory for preparing highly purified DNA from maize nuclei in high yield. The method borrows primarily from reports of Marmur (2) and Kirby (1) and incorporates modifications and/or suggestions from several other laboratories (3, 5).

Materials: Nuclei were prepared from 5-6 day old corn seedlings and mature leaf material with good results. Seedlings were obtained by germinating seeds on paper toweling moistened with 0.1 mM CaCl_2 after surface sterilization with 10% sodium hypochlorite. Leaf material from mature plants was harvested from field or greenhouse grown plants, washed and deribbed.

Buffers: Grinding Sucrose-Tris Buffer - 0.5 M sucrose; 0.01 M MgCl_2 ; 0.05 M Tris; 0.025 M KCl; 0.005 M mercaptoethanol; pH 8.2 (3).

Saline-EDTA Buffer - 0.15 M NaCl; 0.1 M EDTA; pH 8.0.

Standard Saline Citrate (SSC) - 0.15 M NaCl; 0.015 M Na citrate.

Nuclear isolation:

1. Wash and soak seedlings and deribbed leaves in 0.001 M NH_4OH for 1 to 2 hours.
2. Drain, blot, chop and infiltrate the tissue with grinding sucrose-tris buffer under full vacuum for at least 30 minutes and store at 2-4°C overnight.
3. Drain and grind the tissue in an equal-weight of fresh sucrose-tris buffer with ice-cold acid-washed sand, mortar and pestle.
4. Filter the homogenate through four layers of cheesecloth and four layers of Miracloth. Centrifuge at 1000 x g in an SS-34 head (Sorvall RC-2) for 10 minutes at 2°C.

5. Resuspend, wash and centrifuge the pellet, containing nuclei and chloroplast, as above for three successive times with half grinding volume of sucrose-tris buffer containing 3.5% Triton X-100 (Rohm and Haas) to remove the chloroplasts.

DNA extraction: 6. Resuspend the nuclear pellet in 1 ml NaCl-EDTA per 2.5 g of fresh tissue.

7. Lyse the nuclei by adding solid sodium dodecylsulfate (SDS) to make 2% SDS and shake gently for 0.5-1 hour at room temperature.

8. Add solid NaClO_4 to make the solution 1 M to dissociate proteins (solid NaCl to make 2.5 M also gives good results).

9. Extract and deproteinize the DNA three times with chloroform-n, octanol (19:1 by volume), and centrifuge at 10,000 rpm for 10 minutes.

10. Layer the aqueous portion (nucleic acids) with two volumes of 95% cold ethanol. The DNA is either spooled out with a glass rod or centrifuged at 5,000 rpm for 5 minutes. Suspend the pelleted DNA in 0.1 X SSC.

11. Further purify the DNA by digesting for 0.5-1 hour at 37°C with 50 ug/ml of pancreatic RNase (dissolved in 0.1 X SSC, pH 5, and pre-digested at 80°C for 10 minutes) and 50 units of T_1 RNase. Add self-digested pronase to a concentration of 50 ug/ml and incubate for an additional 30 minutes at 37°C. Adjust the NaCl concentration to 2.5 M by adding solid NaCl, 1.46 g per 10 ml of solution and reextract the solution twice with an equal volume of chloroform-n, octanol. Precipitate the DNA with two volumes of 95% cold ethanol and suspend in 0.1 X SSC.

12. Chromatograph the DNA suspension on a methylated-albumin-kieselguhr (MAK) column (4) and elute with a 0.2 to 1.2 M NaCl (0.05 M PO_4 , pH 6.8) buffer gradient.

Rationale: This procedure involves isolation of nuclei. This is necessary for our purpose because it eliminates chloroplast or mitochondrial DNA contamination. It has the additional advantage of eliminating chloroplast pigment contamination. Due to heavy cell walls, high nuclease content and heavy shearing forces required for disrupting the cells, vacuoles are disrupted and active nucleases released. In a number of plants the

vacuolar pH is also very low and this plus the nucleases quickly denature and degrade DNA. Vacuolar membranes are likely to be disrupted before cells are broken and Tris and/or other buffers may not penetrate rapidly enough to offset acidity or nucleases. Stern (5) suggested that carrying out the entire isolation at high pH would effectively neutralize nucleases. Thus it is possible that by removing the nuclei from the presence of cytoplasmic degradative enzymes (especially hydrolytic enzymes released from broken vacuoles) prior to lysis, additional protection is afforded the DNA. These enzymes are probably the major cause of poor results in DNA extraction from plants.

References:

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1. Isozyme pattern of catalase in the developing maize endosperm.

It is known that in maize endosperm the enzyme catalase is controlled by the Ct_1 locus (Scandalios, J.G. 1969). Furthermore, a second locus, Ct_2 , has been detected in the scutella acting to control catalase synthesis. The products of the Ct_2 and Ct_1 loci tend to aggregate and, as a result, at a certain stage of seed germination a catalase pattern emerges consisting of 5 isozymes. In this paper we report the isozyme pattern of catalase in the developing endosperm. The study was carried out on 32 inbred lines of maize and 22 interline hybrids. The endosperm was fixed in solid carbonic acid at 13, 16, 19 and 25 days after pollination.