PURIFICATION OF PLANT NUCLEI USING COLLOIDAL SILICA*

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1. Introduction

In connection with the studies in our laboratory on crown gall (plant tumors) and phytohormones (cf. [1, 2]) we are interested in the properties of DNA from plant nuclei and other organelles and the binding of a phytohormone analogue (α-naphthalene-acetic acid, NAA) in nuclei. Using the different existing methods to isolate nuclei [3–5] the resulting nuclear fraction always was heavily contaminated with crystals, starch particles and cellular debris (cf. fig. 1).

For this reason we developed a new method to obtain highly purified plant nuclei. Following the suggestion of Lyttleton [6] we tested a sample of LUDOX TM, having a density of 1.38 g/cm^3 and most probably suitable for banding nuclei with an estimated banding density of about 1.30 g/cm^3 in CsCl [7]. Our results indicate, that a much lower density (about 1.10 g/cm^3) is sufficient for banding, contaminants being pelleted on the bottom of the centrifuge tube. A lower density than expected was also found by several authors [8, 9] when purifying viruses or cell organelles.

2. Materials and methods

Leaves of tobacco (*Nicotiana tabacum* L. *var.* White Burley) with a length of 10–20 cm were harvested, washed with tap water and after removal of the midribs, cut into pieces. Subsequently the pieces were infiltrated in vacuo with cold Honda medium [10] (2.5% Ficoll, 5% Dextran T40, 0.25 M sucrose, 25 mM Tris-HCl, pH 8.0, 2 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM mercaptoethanol) in a ratio of 3 ml for 1 g tissue. In the same way crown gall tissue from tissue culture [1] and tobacco stems were treated with Honda medium. In the experiments with phytohormones we used 10 mm long, 7 days old etiolated pea stems, which were infiltrated with 4% pectinase in Honda medium [2, 3]. The infiltrated tissue was then homogenized in a Waring blendor or a Sorvall Omnimixer operated at top speed for 3 periods of 10 sec.

The resulting homogenate was filtered over stainless steel sieves (60–200–400 mesh) and the filtrate was centrifuged in a Spinco J 21 (JA 10 rotor) for 10 min at 3000 rpm (for small quantities a MSE super minor table centrifuge was used). The pellet, containing nuclei, chloroplasts, crystals and cellular debris was resuspended in 2% Triton X-100 in Honda medium (1 ml for 5–6 g tissue) to destroy chloroplasts. After a 5 min incubation the suspension was centrifuged for 5 min at 3000 rpm in a Homef or MSE table centrifuge to pellet the nuclei, chloroplast fragments remaining in solution.

The pellet was resuspended in Honda medium containing 5% LUDOX TM† (1 ml is used for 10 g tissue). This suspension is layered on a two step gradient consisting of 20 ml 25% LUDOX TM in Honda medium and 10 ml 10% LUDOX TM in Honda medium. The gradients were centrifuged in a SW 27 rotor for 30 min at 10,000 rpm in a Spinco L2 preparative ultracentri-

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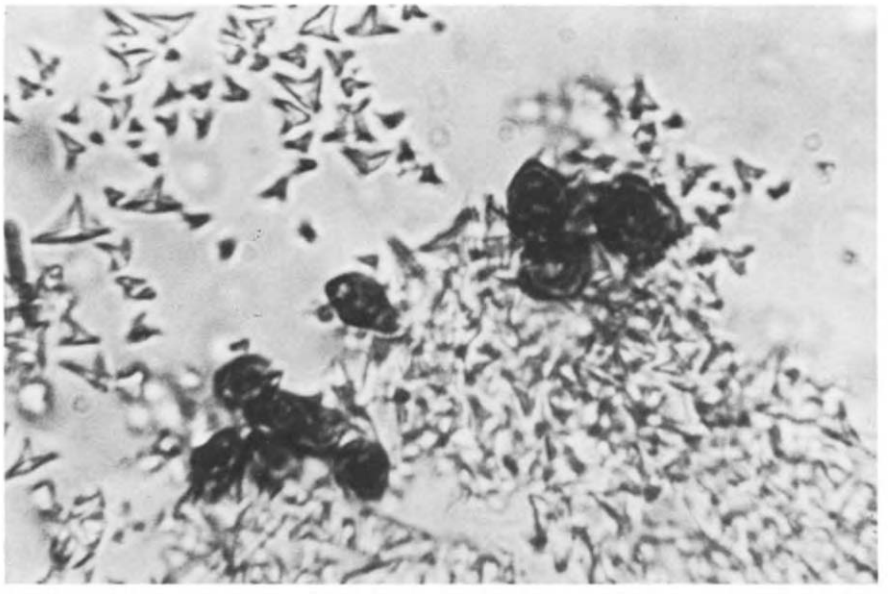

Fig. 1. Tobacco leave nuclei after centrifugation in sucrose–Honda media according to [5]. Magnification 500×.

fuge. We used 6 tubes for a suspension of nuclei from about 300 g leaves. After the run the layer between 10% and 25%, containing the nuclei, was removed with a pipette, diluted with at least an equal volume of Honda medium and mixed. The nuclei were spun down for 10 min at 3000 rpm in a Homef table centrifuge.

Starting with the infiltrated tissue the whole procedure was carried out at 4–8°. After resuspension of the nuclei in Honda medium, an aliquot was mixed with an equal volume of 0.5% Trypan Blue (in Honda medium) and examined and counted in a haemacytometer with a light microscope (Leitz Orthoplan) at a magnification of 250×.

3. Results and discussion

With the existing methods nuclei are pelleted through a gradient or separated from contaminants by rate zonal sedimentation in heavy sucrose solutions. In our hands these methods were not successful for most of the plant tissues used. Only rather pure nuclei from pea stems could be obtained using gradients of arabic gum according to Tautvydas [4] (cf. fig. 2).

The disadvantages of concentrated sucrose solutions are clear: difficult to handle because of their high viscosity and possibly damaging the nuclei by a high osmotic pressure. These disadvantages are eliminated by using LUDOX TM. The viscosity is much lower and the osmotic factor is negligible. To prevent gel forming when the LUDOX solutions are prepared we used the following technique: a 2-fold concentrated Honda medium with an adjusted pH of 8.0 was mixed with an equal volume of LUDOX TM under vigorous stirring, the pH being readjusted with 4 N HCl to 8.0. This final stock solution of 50% LUDOX TM in Honda medium has a density of 1.26 g/cm^3 at 25° and is stable for several weeks at 4°. The appropriate LUDOX TM solutions were prepared from it by diluting with Honda medium.

A buoyant density of 1.08–1.10 g/cm^3 (10–18% LUDOX TM in Honda medium) was found for plant nuclei when centrifuged on a 5–35% linear gradient (fig. 3). This is considerably lower than we experienced with nuclei in sucrose solutions, where the nuclei sedimented through 66 w/w % sucrose, with a density of 1.32 g/cm^3 at 25°.

Anderson [7] and Dobrota [11] found a banding density of nuclei of about 1.30 g/cm^3. Comparable

Fig. 2. Subapical pea stem nuclei after centrifugation in arabic gum solutions according to [4]. Magnification 300×.

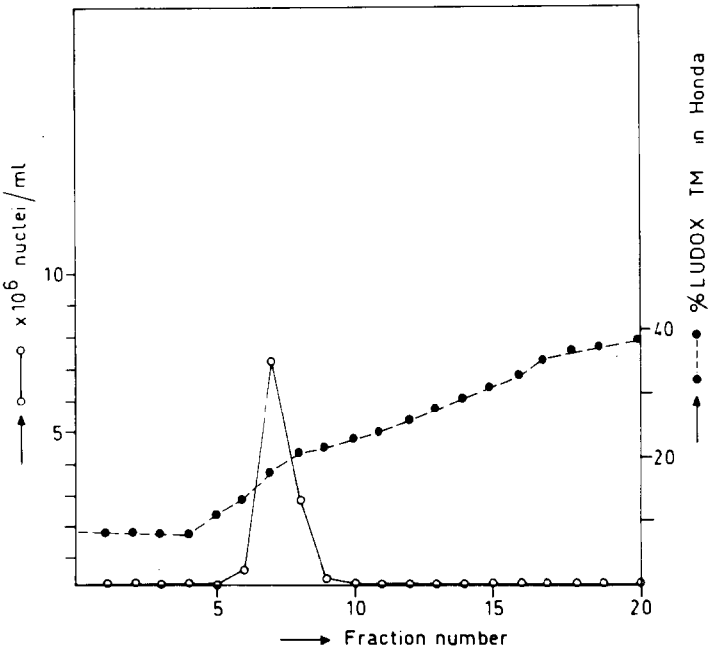

Fig. 3. Distribution pattern of nuclei (tobacco leaves) after centrifugation in a linear gradient from 5–35% LUDOX TM in Honda medium. Fractions are 2 ml. The percentage of LUDOX TM was determined from the refraction.

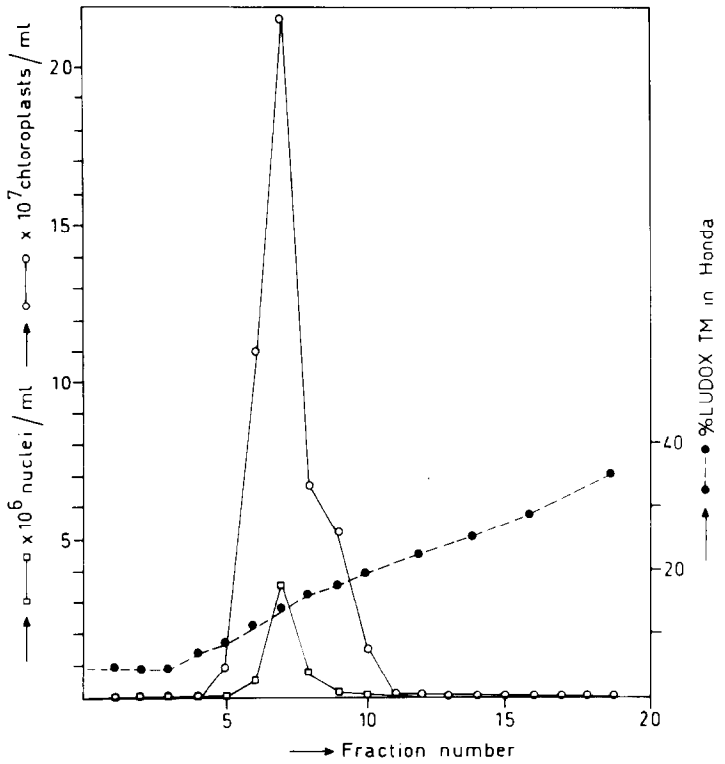

Fig. 4. Distribution pattern of chloroplasts and nuclei, centrifuged as in fig. 3. Further explanation in the text.

results are found by working with viruses or other organelles [8, 9]. In the same experiment as shown in fig. 3 a linear gradient was loaded with a suspension of a nuclear fraction, not treated with Triton X-100. Surprisingly we found that chloroplasts band at the same density (fig. 4).

Light microscope examination of the nuclei, obtained in the procedure with Triton X-100 treatment, revealed that they were highly purified, had very distinct nucleoli and showed no shrinkage (fig. 5). This last fact might explain that the buoyant density of the nuclei in LUDOX TM is lower than in sucrose. As already mentioned by several authors [6, 9, 12] it is possible that organelles will shrink in hypertonic solutions and therefore increase in density. The fact that the buoyant density of chloroplasts and nuclei in sucrose (in Honda medium) is about 1.20 g/cm^3 and 1.30 g/cm^3, respectively, indicates that shrinkage of these cell organelles is different in sucrose (in Honda medium). In the LUDOX TM medium, however, both are found at the same density (1.10 g/cm^3).

We conclude that the purification of plant nuclei in LUDOX TM in Honda medium has advantages over purification in sucrose media for the following reasons:

1) Highly purified nuclei can be obtained by banding in a gradient;

2) no shrinkage occurring, the nuclei resemble better the *in vivo* state;

3) simple procedures, as the LUDOX media are very easy to handle.

It should be mentioned that LUDOX is also a suitable gradient material for other plant organelles such as chloroplasts [6] and mitochondria [13].

Acknowledgements

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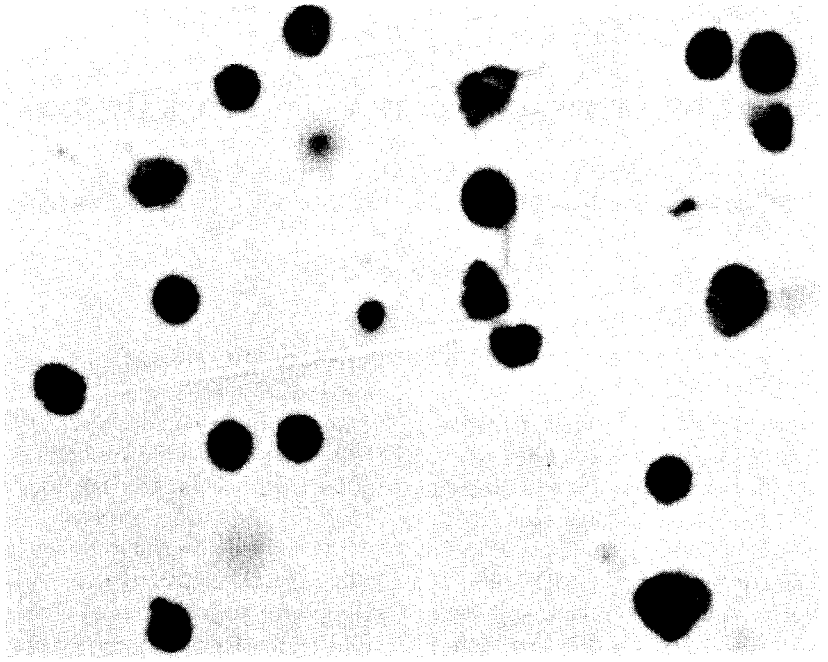

Fig. 5. Tobacco leaf nuclei after centrifugation in LUDOX TM–Honda media as described in the text. Magnification 500×.

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