

***Nicotiana tabacum* DNA : Isolation from Cell Culture**

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Abstract. The adaptation of Marmur's method, suitable for DNA isolation from plant cell culture, is described.

In the course of our experiments connected with mutagenesis and DNA repair in plants, we started to work also with plant cell cultures. Since among other things such studies require the analysis of DNA, it was necessary to find a suitable technique for routine DNA isolation from cultured plant cells. Marmur's method (MARMUR 1961) was chosen as starting procedure and adapted for this purpose. Modifications consisted mainly in ways of cell homogenization and the estimation of the proper ratios between the amount of starting material and the buffer volumes used during isolation procedures.

MATERIAL AND METHODS

Cells of *Nicotiana tabacum* were cultured up to the end of the exponential phase and harvested by filtration through a nylon cloth, then resuspended in NaCl — EDTA (0.15 M NaCl, 0.1 M EDTA, pH 8.5) and filtered again. The mass of cells on the cloth was squeezed very gently and weighed ("wet weight"). The cells were either used immediately for DNA isolation or stored at -20°C , even for some weeks.

During experiments, Marmur's original method (MARMUR 1961) was followed in principle. For cell homogenization the following techniques were used: Homogenizing in a laboratory homogenizer LHN 20 (Developmental Workshops of Czechoslovak Academy of Sciences, Praha), 0°C or room temperature, 30 s to 3 min; homogenizing with pestle and mortar, 0°C , 5 to 15 min; homogenizing with pestle and mortar, liquid nitrogen, 5 to 15 min; homogenizing with pestle and mortar with sand, 0°C , 5 to 15 min. Further, the numbers of deproteinization steps were changed and isopropanol DNA precipitation was excluded or used. Also the optimal ratio between the starting amount of cells and the volume of homogeni-

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TABLE 1

Differences between single adaptations of Marmur's method used for DNA isolation from *Nicotiana tabacum* cell culture

Procedure No.	Mode of homogenization	RNase action	Pronase action	Ethanol precipitation	Chloroform mixture	Isopropanol precipitation
1	LHN 20	+	+	2×	4×	—
2	Grinding	+	+	2×	4×	—
3	Grinding with liquid N ₂	+	—	2×	4×	—
4	Grinding with sand	+	—	2×	4×	—
5	Grinding with sand	+	—	2×	6×	—
6	Grinding with sand	+	±	2×	6×	+

zing buffer added before lysis was tested and the optimal volume of saline — citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) for dissolving DNA in single steps of the procedure was determined. In some cases, pronase (solution of 2 mg ml⁻¹ in water, before use incubated at 46 °C for 45 min) was applied (1/40 volume added, incubated at 37 °C for 2 h) immediately after RNase action.

DNA preparations were characterized spectrophotometrically, by determination of molecular mass [from $s_{w,20}$ according to the Eigner-Doty relation (EIGNER and DOTY 1965)] and by calculating DNA yield per "wet weight" of cells used for isolation. DNA concentrations in single preparations were calculated from A_{260} . T_m of DNA preparation No. 6 (see Tables 1—4) was found to be 82.5 °C.

RESULTS

Six exemplary procedures are shown in Table 1. Characteristics of the resulting DNA preparations are summarized in Tables 2—4.

The optimal procedure found is as follows:

Cells are homogenized with pestle and mortar with sand (0 °C, 10—15 min). To the homogenate, the same volume of NaCl — EDTA plus 1/40 volume of 25% dodecyl sulphate are added and mixed; the mixture is left at room temperature for 5 min. Then 5 M NaClO₄ is added up to the final concentration of 1 M and the mixture is shaken with the same volume of chloro-

TABLE 2

Spectrophotometric characterization of DNA preparations

Procedure No.	$\lambda_{\max}$	$\lambda_{\min}$	$\frac{A_{\max}}{A_{\min}}$	$\frac{A_{\max}}{A_{280}}$	Total amount of DNA [mg]
1	258	232	2.06	1.68	4.44
2	258	235	2.00	1.68	1.68
3	259	233	1.83	1.74	0.99
4	258	233	1.94	1.79	3.40
5	259	232	2.14	1.65	6.42
6	260	234	2.08	1.81	0.16

TABLE 3

Molecular mass of DNA preparations

No.	Sedimentation constant [S]	m. m.
1	< 5	—
2	< 5	—
3	22.1	8.85×10^6
4	18.4	5.63×10^6
5	—	—
6	22.2	8.95×10^6

form — isoamyl alcohol (24 : 1, v/v). Phases are separated by centrifugation and the water phase pipetted out. Treatment with chloroform — isoamyl alcohol is repeated once more; the last water phase is mixed with the same volume of 96 % ethanol and left at -20°C overnight. The precipitate is obtained by centrifugation and dissolved in the volume of saline — citrate corresponding in ml to 1/10 of grams of starting material. To the crude DNA solution, 1/40 volume of RNase solution (2 mg ml^{-1} in 0.15 M NaCl, pH 5.0; before use incubated at 80°C for 10 min) is added and incubated at 37°C for 30 min. The solution is then treated with chloroform — isoamyl alcohol 6 times. The last water phase is overlayed with the same volume of ethanol and the precipitating DNA wound on a glass rod. DNA precipitate is then dissolved in 10times diluted saline-citrate, namely in the volume referring to 1/2—1/1 of DNA solution after the first ethanol precipitation. DNA solution is mixed with 1/10 volume of 3 M sodium acetate, 1 mM EDTA, pH 7.0, and, under continuous stirring, 0.54 volume of isopropanol is added in drops and DNA wound on a glass rod. The DNA precipitate is dissolved in the same volume of saline — citrate as the volume of DNA solution after the second ethanol precipitation of DNA.

DISCUSSION

Spectrophotometric values presented in Table 2 show good quality of DNA preparations Nos. 1—6. But procedures Nos. 1 and 2 gave strongly degraded DNA (Table 3). Molecular masses of DNA preparations Nos. 3, 4 and 6 (No. 5 not estimated) correspond to the normal possibilities of Marmur's method

TABLE 4

Yield of DNA obtained with the help of different isolation procedures

No.	Amount of cells [g] ("wet weight")	Total amount of DNA [mg]	Amount of DNA per 1 g of cells [mg g ⁻¹]
1	50	4.44	0.09
2	28	1.68	0.06
3	30	0.99	0.03
4	50	3.40	0.07
5	40	6.42	0.16
6	40	0.16	0.004

applied to DNA isolation from whole plants or their parts. Comparing the records obtained from analytical centrifuge during molecular mass estimation, however, it was possible to see that only DNA preparation No. 6 contains molecules of very uniform size. Summarizing, the way and conditions of cell homogenization is the crucial step of DNA isolation from plant cell culture because of the risk of DNA degradation in homogenate and the effectiveness of the procedure. The yield of high-molecular DNA from plant cell culture is relatively very low in comparison with other sources. Isopropanol precipitation is very important here, especially because of removing A_{260} absorbing material of lower molecular mass. Pronase action does not influence the quality of the resulting DNA preparations significantly.

REFERENCES

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BOOK REVIEW

COSTES, C. (ed.): *PROTEINES FOLIAIRES ET ALIMENTATION*. — Gauthier—Villars, Paris 1981. 266 pp.

The recently increasing importance of optimal utilization of raw materials and nutritional resources leads to searching new ways of technological use of traditional materials. The book being reviewed, which appeared in the series "Biochimie appliquée", summarizes data enabling the evaluation of the possibilities of efficient processing of plant leaf proteins for alimentation. This subject is treated from many aspects in 10 chapters written by 11 authors.

The editor gives a survey of "static" biochemistry of plant proteins in the first chapter. Analytical methods of determination of proteins and their components are treated by A. Mouranche in the second chapter. R. Douillard deals with the lipids and other lipophilic compounds accompanying the proteins in plant leaves, their structure and properties important from the point of view of preparation and storage of protein concentrates. Potential difficulties in using the protein preparations from plant leaves in nutrition caused by certain specific substances are pointed out by B. Monties. P. Guy deals with the factors affecting the production of plant leaf proteins — selection of plant species and conditions of cultivation and mineral nutrition of plants. The following two chapters are devoted to isolation of plant protein preparations: L. Petit generally outlined the methods of isolation of proteins from plants, while I. Gastineau and O. de Mathan described the methods of industrial preparation of "green proteins" from alfalfa. Technological methods for transformation of the texture of proteins isolated from plants are the subject of the chapter written by J. Culioli. D. Sauvant deals with the use of proteins from plant leaves in alimentation of animals, and A. Chominot with economical aspects of the utilization of proteins of plant leaves in alimentation.

The book, written in French, presents a good survey of appropriate data and some problems related to the use of proteins of plant leaves in alimentation. Individual chapters are provided by a list of references and instructive figures, schemes and tables. The book is supplemented by a useful subject index. The title of the chapter written by B. Monties differs without any reason from that given in the list of contents, where also the name of O. de Mathan, co-worker of I. Gastineau, is omitted.

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