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Detection of Two Different Nuclear Genomes in Parasexual Hybrids by Ribosomal RNA Gene Analysis

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Summary. Restriction endonucleases discriminated between the rDNAs contained in callus tissues derived from *Nicotiana glauca, N. langsdorffii,* and their somatic hybrids produced by protoplast fusion. With XbaI, a single repeat fragment of 7.5×10^6 daltons was produced from *N. glauca* rDNA compared to a single repeat fragment of 4.2×10^6 daltons produced from *N. langsdorffii* rDNA. Both kinds of XbaI fragments were found in somatic hybrids.

Key words: *Nicotiana* – Somatic hybrids – Ribosomal RNA genes – Restriction endonucleases

Introduction

Attempts to create parasexual hybrids between plant species would benefit from a method to discriminate hybrids from parental species. In some cases, identification of somatic hybrids is achieved by chromosome morphology, isoenzymes and biochemical complementation (Schieder and Vasil 1980).

Here we report that nuclear ribosomal RNA genes can be used to identify somatic hybrid cell lines of *Nicotiana glauca* and *N. langsdorffii*.

Materials and Methods

DNA Preparation

One to five gram callus tissues of *N. glauca, N. langsdorffii* and their hybrids were used for the preparation of nuclear DNA by the method reported elsewhere (Oono and Sugiura 1980). Average yield of DNA was 7 μg DNA/g callus.

Endonuclease Digestion, Electrophoresis and Hybridization

Endonucleases tested in this investigation were: AccI, BamHI, EcoRI, HindIII, HpaII, KpnI, SalI, SmaI and XhoI (Takara Shuzo, Kyoto, Japan); AvaI, Sau961 and XbaI (Bethesda Res. Lab., USA); ClaI (Boehringer Mannheim, Germany); SacI (New England BioLabs, USA). In most cases, 2.5 μg DNA was digested with 5 UT enzyme in the presence of appropriate buffers specified by suppliers at 37 °C for 3 – 5 h. Following digestion of DNA, EDTA (30 mM final concentration) was added to stop the enzyme reaction. The DNA solution was applied to a gel containing 0.7% agarose, 40 mM Tris · HCl (pH 7.8), 20 mM Na-acetate and 2 mM EDTA. After electrophoresis at 25 V for 15 h, DNA in a gel slab was stained with ethidium bromide and photographed. Then, the same gel was alkaline-treated, and denatured DNA was transferred to a nitrocellulose filter (Southern 1975). A filter was baked at 80 °C for 3 h, and hybridized with ^{32}P-labelled-rRNA (a mixture of 25S and 17S rRNA of rice) prepared by the method reported elsewhere (Oono and Sugiura 1980). Finally, an autoradiographic image of probe hybridization was obtained.

Isolation of Protoplasts and Fusion Treatments

Protoplasts were prepared from suspension cultured cells of either *N. glauca* or *N. langsdorffii* according to the modified method reported previously (Uchimiya and Murashige 1974). Enzyme solution for protoplast isolation consisted of 0.2% Macerozyme R-10, 1% Cellulase "ONOZUKA" R-10, 0.01% Pectolyase Y-23 and 0.5 M mannitol. Protoplasts of two tobacco species were mixed together, and fused with the aid of polyethylene glycol following the method described previously (Uchimiya 1982).

Selection of Hybrid Cell Lines and Plant Regeneration

Protoplast mixture treated with polyethylene glycol was cultured in "Medium A" containing Murashige and Skoog (1962) salts and the following (mg/l): sucrose (30,000), glucose (54,000), inositol (100), thiamine · HCl (10), pyridoxine · HCl (10), biotin (0.05), nicotinic acid (5), folic acid (0.5), glycine (2), 2,4-D (1) and kinetin (0.1). pH of the medium was adjusted at 5.7 with 1 N NaOH before Millipore sterilization. After 2 weeks of culture, cell clusters consisting of dividing cells were collected by centrifugation at 350 × g for 2 min, and washed with "Medium B" having the same composition as Medium A but lacking phytohormones. Only somatic hybrid callus can grow in this type of medium (Carlson et al. 1972). Washed cell clusters were embedded in 0.6% agar containing Medium B. After 1 month culture, hybrid cell colonies which developed were transferred to fresh Medium B (with 0.6% agar) lacking glucose where they were maintained and used for DNA preparation.

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Hybrid calli or regenerated shoots were grafted on stems of *N. glauca* as root stocks so as to obtain hybrid plants.

Fraction I Protein

Polypeptide composition of Fraction I protein contained in leaves of regenerated plants was determined by the method reported elsewhere (Uchimiya et al. 1979). Somatic hybrid plants regenerated from hybrid cell colonies showed the presence of small subunits of both parents, confirming that hybrids contained nuclear information of both parents (data not presented).

Results and Discussion

The present investigation was carried out to find out appropriate restriction enzyme(s) which yield(s) distinctive differences in rDNA fragments between *N. glauca* and *N. langsdorffii*.

AvaI, ClaI, HpaII, SalI and SmaI did not completely digest the DNA preparation of the two *Nicotiana* species under investigation. This might be due to the sequence specific methylation of tobacco rDNA (Uchimiya et al. 1982). AccI and EcoRI digestion resulted in the production of identical rDNA fragments from both *Nicotiana* DNAs. BamHI, KpnI, HindIII, Sau961, SacI, XbaI and XhoI yielded at least one rDNA fragment which was different in the two tobacco species. Among these, clear-cut results were obtained with XbaI. As seen in Fig. 1, *N. glauca* contained a 7.5×10^6 dalton rDNA fragment, while *N. langsdorffii* yielded a 4.2×10^6 dalton fragment. A mixture of rDNA from the two species contained both rDNA fragments. Analysis of rDNA of three different somatic hybrids showed these hybrid cell clones to contain rDNA characteristic of both parents.

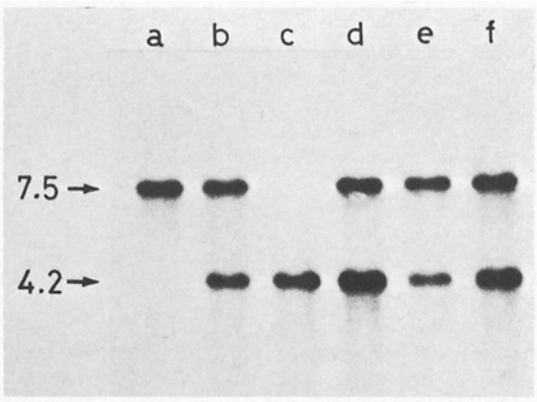

Fig. 1. Autoradiography of Southern image hybridization of ^{32}P-labelled-25S + 17S rRNA to XbaI digests of nuclear DNA from callus tissues of *N. glauca* (*a*), *N. langsdorffii* (*c*), and somatic hybrids (*d–f*). The track (*b*) is an equal mixture of (*a*) + (*c*). A reaction mixture contained 2.5 μg DNA, 5 UT XbaI, 50 mM Tris · HCl (pH 7.5), 35 mM $MgCl_2$, and 500 mM NaCl. Numerals indicate molecular weight; $\times 10^6$ daltons

Theoretically, as little as 0.1 μg DNA (equivalent to 15 mg tissues) would be enough to identify rDNA by currently available hybridization technique. Therefore, restriction endonuclease analysis of rDNA may serve as a sensitive method to characterize somatic hybrids.

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