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INHERITANCE OF ORGANELLES IN SOMATIC CELL HYBRIDS AND CYBRIDS OF NICOTIANA

The University of Nebraska - Lincoln PH.D. 1987

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INHERITANCE OF ORGANELLES IN SOMATIC CELL HYBRIDS AND CYBRIDS OF *NICOTIANA*

by

Nicola M. Ayres

A DISSERTATION

Presented to the Faculty of

The Graduate College in the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Biological Sciences
(Genetics, Molecular, and Cellular Biology)

Under the Supervision of Professor David W. Galbraith

Lincoln, Nebraska

March, 1987

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TITLE

Inheritance of Organelles in Somatic Cell Hybrids and Cybrids of Nicotiana

BY

Nicola M. Ayres

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INHERITANCE OF ORGANELLES IN SOMATIC CELL HYBRIDS AND CYBRIDS OF *NICOTIANA*

Nicola M. Ayres, Ph.D.

University of Nebraska, 1987

Advisor: David W. Galbraith

In somatic cell hybrids and cybrids of *Nicotiana*, research has indicated the presence of a hierarchy of organelle dominance between various species. This putative hierarchy was based on results using biochemical selection of hybrids. I tested the possibility that biochemical selection pressure biased the results. Protoplasts of various alloplasmic substitutions of *Nicotiana* were fused together; heterologous fusion events were isolated using fluorescence-activated cell sorting. Leaves of plants regenerated from these fusion events were analyzed to identify the types of chloroplast and mitochondrial DNA present.

The results reported here indicate that the hierarchy of organelle dominance appears to be a real phenomenon, even when selective pressures are removed. Chloroplasts from *Nicotiana undulata* strongly outcompeted chloroplasts from *N. tabacum*. Chloroplasts from *N. suaveolens* and *N. glauca* equally competed with chloroplasts from *N. tabacum*. Analysis of mitochondrial DNA revealed that mitochondria segregated independently from

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chloroplasts in the N. undulata - N. tabacum and N. glauca - N. tabacum fusions, but not in the N. suaveolens - N. tabacum fusions. After heterologous fusion events, mitochondrial DNA recombination was detected in some plants, but chloroplast DNA recombination was not observed.

In addition, male fertility was restored in a plant regenerated from a fusion between protoplasts of two cytoplasmic male sterile, alloplasmic substitutions. These alloplasmic substitutions contained the cytoplasm of N. suaveolens or N. plumbaginifolia, each in a N. tabacum nuclear background. Thus both nuclei were identical but each of the two protoplast types had the cytoplasm of either N. suaveolens or N. plumbaginifolia. The fertile plant contained N. plumbaginifolia chloroplast and mitochondrial DNA restriction patterns, and displayed a floral morphology intermediate between the N. plumbaginifolia alloplasmically substituted parent and fertile N. tabacum. The flowers of the fertile plant were only able to set seed when manually pollinated.

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ABBREVIATIONS

CMS, cytoplasmic male sterility; tab, N. tabacum; pbg, N. plumbaginifolia; sua, N. suaveolens; und, N. undulata; gla, N. glauca. The plants are referred to by the nomenclature: nuclear genome/chloroplast genome/mitochondrial genome. Hence tab/und/und refers to the alloplasmic substitution of und in a tab nuclear background. It possesses the chloroplasts and mitochondria of und and the tab. FACS, Fluorescence-activated cell sorting.

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INTRODUCTION

When two different populations of isolated protoplasts are fused, the organelle populations are initially mixed. In regenerated plants, however, only one chloroplast and one mitochondrial type are usually detected. Some interaction between the organelles or between the organelles and the nuclei may govern this segregation. A putative hierarchy of organelle dominance has been implied from the results of protoplast fusion experiments, using Nicotiana protoplasts. In this hierarchy the chloroplasts of N. undulata strongly outcompete, of N. suaveolens slightly outcompete, and of N. glauca equally compete those of N. tabacum. The focus of the studies reported here was to determine whether the hierarchy of organelle dominance actually exists, or was a result of the experimental selective conditions that were employed. A second facet of this work was to study the restoration of male fertility in regenerated plants derived from a fusion of protoplasts of two cytoplasmically male sterile plants.

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LITERATURE REVIEW

Organelle Inheritance

Early events in plant development include the interaction of organelles contributed by each of the parents. When fertilization occurs, the initial zygote is comprised of up to six different genomes: two nuclear, two mitochondrial, and two chloroplast genomes. In sexually produced hybrids, the number of genomes contributed to the zygote is determined by the type of organelle inheritance, either uniparental or biparental. In about sixty higher plants that have been classified for organelle inheritance, approximately two-thirds exhibit maternal inheritance; the remaining third has biparental plastid inheritance (Tilney-Bassett, 1984). Both types of plastid inheritance occur throughout the plant kingdom, indicating that the type of plastid inheritance has little evolutionary significance. The frequency with the change in type of plastid inheritance occurs implies that relatively few genes are responsible for plastid inheritance (Tilney-Bassett, 1984).

There are four types of plastid inheritance, based on when exclusion of plastids occurs (van Went, 1984). The first type is represented by Lycopersicon; the plastids are absent from the generative cells of the pollen grain. The vegetative cells contain plastids, but only the generative cell contributes to the

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formation of the zygote. In some plants the plastids are present in the generative cells, but are eliminated during subsequent cell development leading to the formation of sperm cells. This type is characteristic of Solanum. In genera such as Triticum, plastids are present in both the generative cell and the sperm cell, but are not transmitted to the zygote. All three of these cases are examples of maternal or uniparental inheritance of plastids. Biparental inheritance, typical of Pelargonium, occurs when generative cell and sperm cell plastids are transmitted to the zygote.

In Pelargonium and Oenothera, the plastids of both parents can be detected in the progeny. In Pelargonium the genetic constitution of the parental plants can affect chloroplast inheritance in the progeny. In studies of crosses between plants containing both green and albino plastids (variegated plants), three groups of progeny are observed. One group of plants contains only green plastids, another only white plastids (lethal), and a third group is variegated. The ratio of plants in these groups is affected by the nuclear genotype of the maternal plant (Birky, 1983). However in Oenothera the ratio of progeny is independent of the maternal genotype. Thus, although the maternal genotype of some plant species can influence the ratio of progeny having parental plastid types, biparental inheritance of chloroplasts may still occur.

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The genes controlling plastid inheritance can be nuclear or extranuclear, or a combination. Genes for many chloroplast polypeptides are encoded in both chloroplast and nuclear genomes and include ribulose-1,5-bisphosphate carboxylase, ATPase subunits, and components of the photosynthetic apparatus. Chlamydomonas displays biparental chloroplast inheritance that is controlled by Mendelian alleles designated mt+ (maternal) and mt- (paternal). Each parental cell contains one large chloroplast. When the cells fuse, the mt- chloroplast is eliminated, most likely through restriction endonuclease activity; the maternal (mt+) chloroplast DNA is protected by methylation (Burton et al, 1979; Sager and Grabowy, 1983). Mutants have been obtained having partial methylation in both maternal and paternal cells (Bolen et al, 1982), however further methylation still occurs during gametogenesis in mt+ but not mt- cells (Sager and Grabowy, 1983). Such a methylation-restriction system is unlikely to be involved in chloroplast inheritance in higher plants, as no methylation has been detected in chloroplast DNA of higher plants.

One nuclear gene that controls chloroplast inheritance is iojap in maize. Wild type or heterozygous plants have fully green leaves and apparently normal plastids. Homozygous recessive plants are either lacking green pigment or are striped white on a green background. If a heterozygote is selfed, three-fourths of the progeny are phenotypically normal. The remaining fourth exhibits the iojap phenotype. This is characteristic of a single recessive nuclear gene. If these homozygous iojap plants

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are crossed to a homozygous wild type female, the progeny are normal. If they are crossed to a homozygous wild type male, the progeny exhibit the iojap phenotype. Thus the trait is inherited maternally even though the nuclear genome encodes the wild type phenotype. The lack of plastid ribosomes is believed to cause this effect, not a mutation in the plastid DNA. Ribosomes are absent in plastids of plants exhibiting the iojap phenotype, but are present when the wild type nuclear genome is present. This indicates that a nuclear-encoded ribosomal protein is involved (Thompson et al, 1983).

Both nuclear and organellar genomes can thus be involved in organelle inheritance. Interactions between these genomes complicates the understanding of the events occurring during plant development. Plastids multiply by division (Butterfass, 1980). Plastid and nuclear DNA synthesis are not coupled, yet the number of chloroplasts per cell has been correlated with ploidy (Heinhorst et al, 1985; Butterfass, 1980). This implies that some coordination must be occurring. Both nuclear and plastid DNA synthesis depend on proteins synthesized in the cytoplasm, not in the plastid (Heinhorst et al, 1985). Thus plastid and nuclear replication are indirectly coordinated. This coordination can be tested by making different organellar combinations through somatic cell fusion.

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Somatic Cell Fusion

As discussed above, most plants exhibit uniparental inheritance of organelles, typically due to exclusion of paternal organelles from the zygote. Somatic cell fusion allows the introduction of multiple genomes (nuclear, plastid, and mitochondrial) within a single cell. This produces novel combinations of genetic information that are excluded by uniparental inheritance or by barriers to sexual fertilization such as morphological or physiological incompatabilities. Somatic cell fusion can be used as a tool to study organelle inheritance in the limited number of plant species in which plant regeneration from protoplasts can be accomplished. Interactions between different organelles can then be studied during the process of regeneration. This process is straightforward in concept; isolated protoplasts are fused together, forming heterokaryons or heteroplasmons, according to whether the nuclear genomes are similar or dissimilar. These fusion products are then regenerated to full plants, which are subsequently examined to determine the relative contributions of the parental organelles to the progeny plant populations.

The genus Nicotiana has been used extensively for somatic cell fusion studies. This genus exhibits exclusively maternal inheritance in sexual hybridization in over sixty species. Most species of Nicotiana respond well in tissue culture and can be regenerated easily and in high frequencies from protoplasts. Thus

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