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Self-fertile cybrids *Nicotiana tabacum* (+*Hyoscyamus aureus*) with a nucleo-plastome incompatibility

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Abstract Cytoplasmic hybrids (cybrids) in a novel inter-generic combination, *Nicotiana tabacum* (+*Hyoscyamus aureus*), were generated by fusion of protoplasts from a plastome tobacco albino mutant (line R100a1) and γ-irradiated green protoplasts of *H. aureus*. Cybrids possessed a plastome of *H. aureus* and a rearranged mitochondrial DNA. The cybrids displayed a syndrome of nucleo-plastome incompatibility expressed as a partial chlorophyll-deficiency of cotyledonary and true leaves at the early stage of vegetative development of plants grown from seeds in soil. During later development, the plants restored a normal green coloration. This character is phenotypically indistinguishable from the same syndrome in previously generated cybrids *N. tabacum* (+*H. nigrum*). In contrast to the cybrids *N. tabacum* (+*H. nigrum*), cybrids *N. tabacum* (+*H. aureus*) were self-fertile, and did not manifest other features that were interpreted as nucleo-mitochondrial incompatibilities in *N. tabacum* (+*H. nigrum*) plants. Therefore, the cybrids *N. tabacum* (+*H. aureus*) present a self-propagating system of *Nicotiana* (+*Hyoscyamus*) nucleo-plastome incompatibility in its pure form.

Keywords *Nicotiana/Hyoscyamus* · Cybrids · Fertility · Nucleo-cytoplasmic incompatibility · Maternal inheritance · Evolution

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Introduction

Inter-specific sexual hybridization in *Oenothera* (Stubbe and Herrmann 1982; Stubbe 1989), *Pelargonium* (Metzlaff et al. 1982), *Zantedeschia* (Yao and Cohen 2000) and other genera (Kirk and Tilney-Bassett 1978) established that a plastid genome could be functionally incompatible with the nuclear background of certain species. The nuclear-plastome incompatibility in such hybrids prevents the complete differentiation of chloroplasts, that results in chlorophyll-deficiency, virescence or variegation. Sometimes, incompatibility effects are not pronounced phenotypically but are detectable by the decreased functioning of the photosynthetic apparatus (Glick and Sears 1994). The phenomena of the nucleo-plastome incompatibility together with nucleo-mitochondrial incompatibilities, mostly expressed as a different CMS (Hanson 1991; Vedel et al. 1994), have demonstrated the genetic co-operation between nuclear, plastidial and mitochondrial genomes. This, originally genetic concept now actively expands due to molecular and biochemical studies, suggesting new trends in the elucidation of co-operative developmental and metabolic pathways and their signalling (Poyton and McEwen 1996; Leon et al. 1998; Mackenzie and McIntosh 1999).

In the examples described above, the incompatibilities occur between plastomes and nuclear genomes of related species whose nuclear hybridity could be established via sexual crosses. Since most species of the mentioned genera are characterised by biparental inheritance of plastids, their hybrids might contain nuclear genomes and plastomes from both parental species. Depending on the proportion of plastids from each parent, the syndrome of plastome-genome incompatibility could be expressed as different degrees of chlorophyll-deficiency in pure or mosaic phenotypes. Genetic studies revealed a differential contribution of nuclear genomes and plastomes to the expression of incompatible phenotypes (Kutzelnigg and Stubbe 1974; Stubbe 1989), with multiple gene control of incompatibilities (Yao and Cohen 2000). The general limitation of the above systems for multidisciplinary studies is the genetic complexity of hybrids due to biparental inheritance of the cytoplasm and highly heterozygous nuclear genomes.

Alternative models on nucleo-cytoplasmic interactions emerged from alloplasmic cybridization by protoplast fusions (Gleba and Sytnik 1984; Kumar and Cocking 1987; Medgyesy 1994). Cybrids present some advantageous possibilities for studying interactions between genetic compartments. (1) A nuclear genome of only one parent could be combined with only a plastome of another parent (mitochondria might be either of a parental type or recombinant). (2) Parental species for somatic cybridization could be taxonomically very distant and sexually incompatible (for instance, from different genera or tribes). (3) There is a possibility that nucleo-organelle incompatibilities could be overcome by recombination of organelle DNA (Medgyesy 1994). (4) In most systems available for somatic cybridization, inheritance of the cytoplasm is maternal and, therefore, their genetic analysis is quite simple.

In many cases, the transfer of even distant plastomes results in normally green cybrid plants (Glimelius and Bonnett 1986; Kushnir et al. 1987; Thanh et al. 1988; Babiychuk et al. 1995; Dragoeva et al. 1999). However, deviations in the composition and functioning of their photosynthetic apparatus (Kushnir et al. 1987; Babiychuk et al. 1995; Peter et al. 1999) suggest compartmental incompatibilities at molecular and physiological levels. In some combinations, cybrids could not be generated because of severe nucleus-cytoplasm incompatibilities (Kushnir et al. 1991; Perl et al. 1991; Wolters et al. 1993; Medgyesy 1994).

One of the first examples of phenotypically pronounced nucleo-organelle incompatibility in cybrids became apparent after regeneration of chlorophyll-deficient potato plants combining a nuclear genome of *Solanum tuberosum* with a plastome and chondriome of *Solanum brevidence* (Perl et al. 1990). The well-documented cybrid combination *Atropa belladonna* (+*Nicotiana tabacum*) presents a similar incompatibility system where the tobacco plastome is genetically intact but photosynthetically knocked out into the nuclear background of *A. belladonna* (Kushnir et al. 1991). The white cybrids from both combinations are only able to grow heterotrophically in an aseptic culture. Viable in soil cybrids *N. tabacum* (+*Hyoscyamus niger*) were reported as a system with a distinct complex of nucleo-organelle incompatibilities (Zubko et al. 2001). The transient pigment deficiency of *N. tabacum* (+*Hyoscyamus niger*) cybrids was attributed to nucleo-plastome incompatibility, while different developmental modifications of CMS flowers reflected nucleo-mitochondrial incompatibilities. Here we report on self-fertile cybrids in a similar combination *N. tabacum* (+*Hyoscyamus aureus*) that manifests the transient pigment deficiency but with no other phenotypic characteristics controlled by mitochondria in the cybrids *N. tabacum* (+*H. niger*). Therefore, the plants *N. tabacum* (+*H. aureus*) represent a minimised self-maintaining system on nucleo-plastome incompatibility in an intergeneric combination *Nicotiana* (+*Hyoscyamus*).

Materials and methods

Plant material, protoplast fusion and selection of cytoplasmic hybrids

Wild-type *H. aureus* L. (Marburg, Germany) was used as a donor of cytoplasm in protoplast fusion experiments. The albino plastome mutant of *N. tabacum* L., line R100a1 (Zubko et al. 1991) was used as a recipient. Aseptic plants of both species were propagated on basal Murashige and Skoog medium with 30 g/l of sucrose at a temperature of 25°C and illumination regime 10/14 h of darkness/light.

Isolation and fusion of protoplasts from aseptic leaves were performed according to the previously described procedure (Zubko et al. 1996). Protoplasts of *H. aureus* were γ-irradiated at the dose of 200 Gr prior to fusion. The selection of cytoplasmic hybrids was based on the screening green colonies (Zubko et al. 1996) with tobacco morphology.

Backcrosses and self-pollinations

Several hybrid lines with good growth and rooting capacity in vitro were transferred to soil and studied further. For self-pollination, the stigmas were treated with pollen from the same flowers, and self-pollinated plants were kept separately. For backcrosses, emasculated flowers were pollinated with pollen of *N. tabacum* (cultivars Lehija and Wisconsin 38).

Analysis of cpDNA and mtDNA

Total DNAs isolated from aseptic leaves according to Dellaporta et al. (1983) were digested with the enzymes *Bam*HI, *Hin*dIII and *Pst*I + *Sal*I. DNA digests fractionated by electrophoresis in 0.8% agarose gels were blotted on a Hybond$^+$ membrane and hybridized with ^{32}P-labelled probes at 65°C as described before (Zubko et al. 2001). For cpDNA analysis, a filter containing *Pst*I + *Sal*I digests was hybridized with a 1.75-kb *Eco*RI fragment of the *rbcL* plastid gene from spinach (Zurawski et al. 1981). The plasmid was a gift from Dr. I.K. Komarnitsky, Kiev. For analysis of mtDNA, blots of DNA digested with *Bam*HI and *Hin*dIII were probed with a 1.0 kb *Bam*HI fragment of the *atp*α gene and a 2.2-kb *Eco*RI fragment of the *nad3/rps12* gene cluster from *Arabidopsis* (kindly provided by Dr. W. Schuster, Berlin).

Analysis of nuclear ribosomal DNA

The filter with *Pst*I + *Sal*I DNA digests was re-probed with an 0.5 kb *Pst*I + *Eco*RI fragment of plasmid pUL7 with cloned rDNA of *Citrus lemon* (Kolosha and Fodor 1986). The plasmid was kindly provided by Dr. I.K. Komarnitsky. The conditions for hybridisation of the filter with the ^{32}P-labelled rDNA fragment were the same as described before (Zubko et al. 2001).

Results

Selection of cytoplasmic hybrids *N. tabacum* (+*H. aureus*) after protoplast fusions

Protoplasts of albino plastome tobacco mutant R100a1 never recovered green plants in our previous experiments (Zubko et al. 1991). Since protoplasts of *H. aureus* were inactivated in their divisions by γ-irradiation, all green clones derived after fusing protoplasts of these two partners were supposed to be either asymmetric hybrids or cybrids always containing a plastid genome of *H. aureus*.

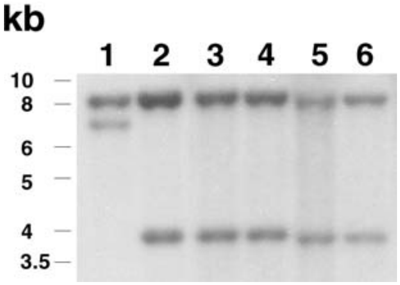

Fig. 1 Inheritance of *H. aureus* plastome in cybrids. Total DNA was isolated from *N. tabacum* Wisconsin 38 (*lane 1*), *H. aureus* (*lane 2*), cybrid lines 6R20-15 (*lane 3*) and 6R20-32 (*lane 4*), and two randomly selected plants from sexual progenies of line 6R20-15 (*lanes 5 and 6*). *Pst*I + *Sal*I digests were separated on agarose gels (1–3 μg of DNA per lane), blotted and hybridised with the *rbcL* probe. The 1-kb DNA ladder was used as a marker

In total, 33 green colonies were selected from protoplast fusions. They differed in the time of appearance and in the mode of regeneration. Some of them regenerated into plants within 2–3 months whereas others did not form actual plants on regeneration media even after 1 year. Twelve lines of plants were recovered with a relatively normal morphology characteristic for tobacco. They all exhibited identical patterns of amylase and aspartate-aminotranspherase isozymes of the tobacco type (Zubko 1992). The plants were able to grow in the soil. Most of them were classified as asymmetric nuclear hybrids on the basis of irregular leaf/flower morphologies, sterility and the presence of characteristic *H. aureus* bands in their esterase isozyme patterns (Zubko 1992). Three lines, 6R20-06, 6R20-15 and 6R20-32, with the most regular morphology and tobacco isozyme patterns, were further analysed as putative cybrids.

Analysis of organelle genomes

The lines 6R20-06, 6R20-15 and 6R20-32 were propagated by cuttings in vitro and grown in soil for backcrossing with wild-type tobacco, in order to maximise the purity of the tobacco nuclear genome within the plants. We found the flowering and fertility of the line 6R20-32 not effective enough to continue the pollination experiments. The lines 6R20-06 and 6R20-15 produced reasonable amounts of seeds after the backcrosses.

Figure 1 shows a blot-hybridisation analysis of the plastome type in the plants. The banding patterns of *Pst*I + *Sal*I DNA digests probed with the *rbcL* plastid gene fragment are clearly different in the parental plants, *N. tabacum* (lane 1) and *H. aureus* (lane 2). Lines 6R20-32, 6R20-15 and their backcross progenies (lanes 3–6) displayed the same patterns as *H. aureus*. There were no differences in the *Hin*dIII restriction patterns of cpDNA from *H. aureus* and the cybrids (data not shown). These results revealed inheritance of the *H. aureus* plastome type within the cybrids.

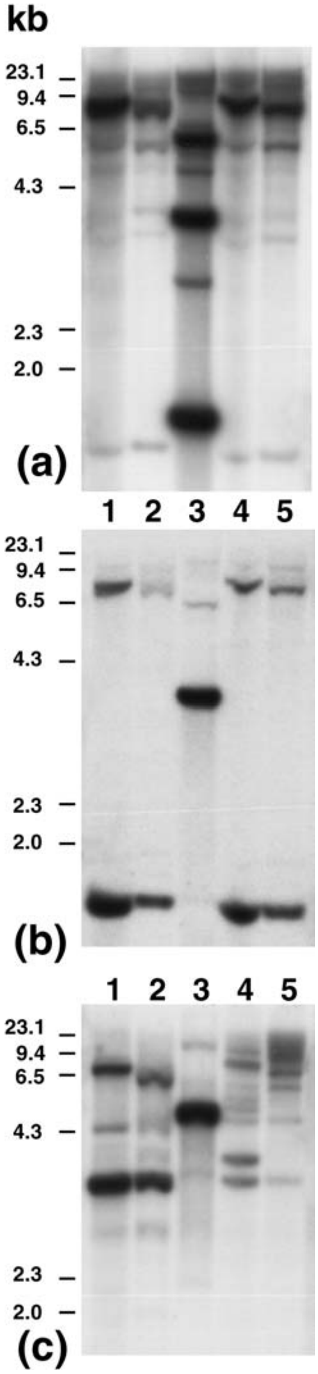

Fig. 2a–c Rearrangement of mtDNA in the cybrids. DNA was isolated from *N. tabacum* albino recipient line R100a1 (*lane 1*), green tobacco Wisconsin 38 (*lane 2*), *H. aureus* (*lane 3*), cybrid lines 6R20-15 (*lane 4*) and 6R20-32 (*lane 5*). (**a**) *Bam*HI DNA digests (hybridisation with the *atp*α probe); (**b**) *Bam*HI DNA digests (hybridisation with the *nad3/rps12* probe); (**c**) *Hin*dIII DNA digests (hybridisation with the *nad3/rps12* probe). *Lanes* contained 3–4 μg of DNA. The 1-kb DNA ladder was used as a marker

The analysis of mitochondrial genomes is shown in Fig. 2. The hybridisation patterns of *Bam*HI-digested DNA from the *N. tabacum* (+*H. aureus*) lines probed with *atp*α and *nad3/rps12* gene fragments (lanes 4, 5) are very similar to those in tobacco (lanes 1, 2).

The DNA digested with *Hin*dIII and probed with the *nad3/rps12* fragment showed different banding patterns

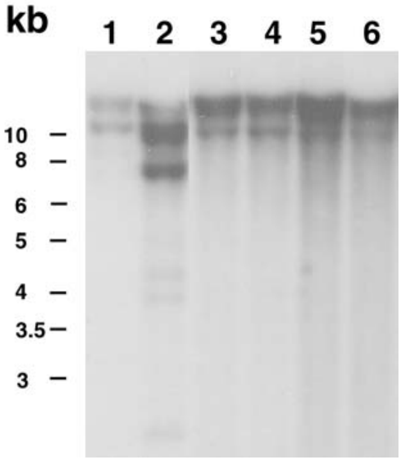

Fig. 3 Southern-blot hybridisation of the cybrid DNA with nuclear rDNA probe. The lanes on the filter contain *Pst*I + *Sal*I digests of total DNA isolated from *N. tabacum* Wisconsin 38 (*lane 1*), *H. aureus* (*lane 2*), cybrid lines 6R20-15 (*lane 3*) and 6R20-32 (*lane 4*), and two plants from sexual progenies of line 6R20-15 (*lanes 5 and 6*)

for the lines 6R20-15 and 6R20-32, which suggests rearrangements in their mtDNA (Fig. 2).

Analysis of nuclear ribosomal DNA

To provide molecular evidence supporting the tobacco-based nuclear constitution of the cybrids, we hybridised *Pst*I + *Sal*I-digested DNA of lines 6R20-32, 6R20-15 and several backcross progenies with the ribosomal DNA repeat of lemon (Fig. 3). All *N. tabacum* (+*H. aureus*) plants manifested the rDNA pattern of tobacco. These data are in agreement with the previous analysis of nuclear-encoded isozymes (Zubko 1992) showing the cybrid nature of the plants analysed.

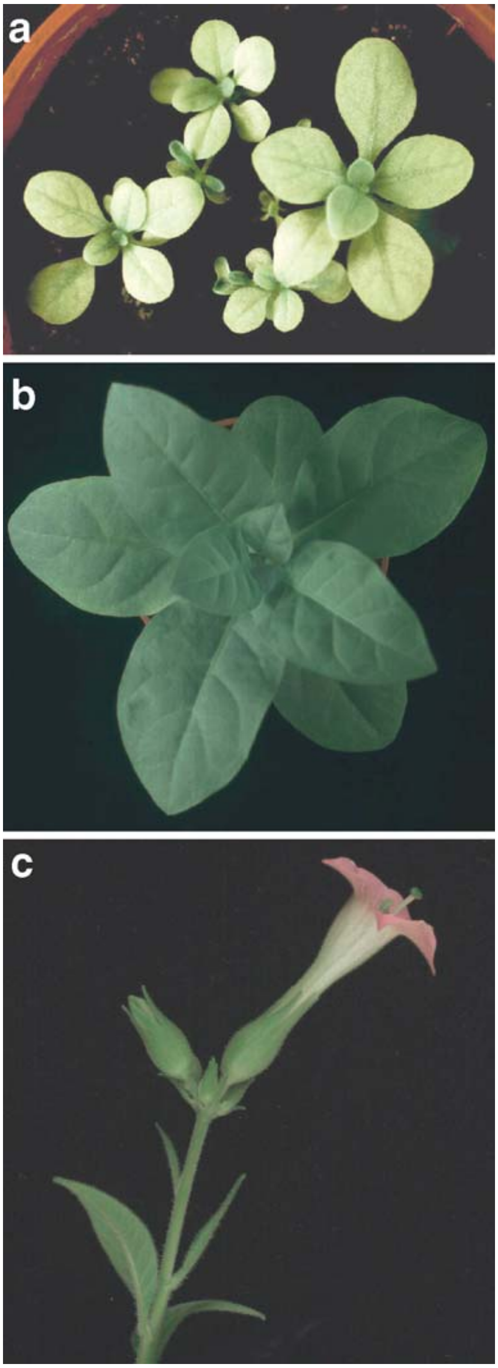

Fig. 4a–c Morphology of cybrid plants *N. tabacum* (+*H. aureus*). **a** Three week-old seedlings of the cybrid line 6R20-15 manifesting partial chlorophyll-deficiency. **b** Eight week-old plant of the line 6R20-15 with a restored green colour of leaves. **c** Flowering plant of the cybrid line 6R20-15

Partial chlorophyll-deficiency and self-fertility of the line 6R20-15

All initial *N. tabacum* (+*H. aureus*) plants recovered after protoplast fusions displayed phenotypes similar to tobacco when growing in vitro. Since most of 6R20-plants manifested different features of asymmetric nuclear hybrids or reduced fertility in the soil, they were excluded from pollination experiments. Two lines, 6R20-6 and 6R20-15, with characteristics of true cybrids were backcrossed with wild-type tobacco after emasculation of their flowers before opening. Seeds collected after backcrossing both lines were germinated in soil (at the light intensity of 180–200 μmol × m^{-2} s^{-1}) and in vitro. The germinating seedlings were yellow coloured from the cotyledonary leaf stage. The first true leaves became greener in seedlings growing in vitro. The true leaves of plantlets in soil remained yellow (Fig. 4a) for about 4 weeks. The morphology of the leaves in the studied plants was of the tobacco type. After 5 weeks of growth in the soil, the leaves gradually became more green, and after 8 weeks their coloration (Fig. 4b) was comparable to the colour of the control tobacco leaves. The growth of the plants during the stage of pigment deficiency was obviously delayed, so their final size was smaller than the size of the wild-type tobacco. When germinating and growing under dim light (10–20 μmol × m^{-2} s^{-1}), the cybrid plants did not manifest the bleaching effect, similar to the cybrids *N. tabacum* (+*H. nigrum*) described previously (Zubko et al. 2001).

The 6R20-6 line was occasionally lost during the vegetative stage of the first backcross. Plants of the line 6R20-15 formed normal flowers of the tobacco type (Fig. 4c). Surprisingly, their anthers produced substantial amounts of pollen grains. The pollen was used for self-pollination of 6R20-15 plants. Self-pollinated flowers produced seeds (500–700 per seed capsule) that were germinated in the soil. The seedlings and young plants manifested the same reduction in pigmentation as the progeny from the backcross. These plants were self-pollinated during flowering, and they were also self-fertile.

The seed progenies after the second round of backcrossing displayed the same phenotypes as the plants from the first backcross and the progenies after self-pollination. These experiments reveal self-fertility of the *N. tabacum* (+*H. aureus*) cybrid as well as maternal inheritance of the pigment-deficiency syndrome.

Discussion

We describe here self-fertile distant cybrids *N. tabacum* (+*H. aureus*) that manifest a maternally inherited syndrome of developmentally regulated chlorophyll-deficiency co-segregating with the plastome of *H. aureus* and, therefore, could be interpreted as a nucleo-plastome incompatibility. Cybrids from a taxonomically similar parental combination, *N. tabacum* (+*H. nigrum*), possessed the same syndrome of the transient pigment-deficiency associated with the *H. nigrum* plastome as well as a diverse complex of nucleo-mitochondrial incompatibilities causing a morphologically different CMS (including homoeotic phenotypes) and vegetative alterations of the plants (Zubko et al. 2001). However, due to the complexity of the incompatibility syndrome in the *N. tabacum* (+*H. nigrum*) cybrids, a precise consideration of the differential contribution of both organelle genomes to particular components of the incompatibility syndrome was quite difficult, especially because of possible interactions between the plastome and chondriome effects (Zubko et al. 2001). The regular tobacco-type flower and self-fertility of the *N. tabacum* (+*H. aureus*) line implies the emergence of a mitochondriome that is highly compatible with the tobacco nuclear genome on the background of the *H. aureus* plastome. Therefore, due to a minimised nucleo-mitochondrial incompatibility within the *N. tabacum* (+*H. aureus*) line, the pigment-deficiency and the associated retardation in growth are very likely to be pure effects of nucleo-plastome incompatibility in both groups of cybrids, *N. tabacum* (+*H. nigrum*) and *N. tabacum* (+*H. aureus*). Currently, this makes *N. tabacum* (+*H. aureus*) cybrids the simplest genetic system for studying nucleo-plastome incompatibility that is maternally inherited. It overcomes the main limitations for genetic and molecular studies in most of the incompatibility systems: i.e. the chimeric state of tissues, the lethality of fully chlorophyll-deficient plants, genetic heterozygosity and heteroplasmy. Self-maintenance of the *N. tabacum* (+*H. aureus*) genome would allow genetic experiments on complementation of the incompatibility syndrome. On the other hand, self-fertility of these plants indicates a possibility for species-autonomous evolution of nucleo-plastome incompatibility syndromes as components of biological properties in the plant kingdom.

Cybrid plants possessing a nucleus of tobacco and plastids from *Petunia hybrida* (Glimelius and Bonnett 1986; Dragoeva et al. 1999), *Atropa belladonna* (Kushnir et al. 1987) and *Salpiglossis sinuata* (Thanh et al. 1988) were fertile (Glimelius and Bonnett 1986; Kushnir et al. 1987; Thanh et al. 1988) or male-sterile (Thanh et al. 1988; Dragoeva et al. 1999). The emergence of male-sterile and fertile plants within the same alloplasmic combinations has been discussed in the relation to rearrangements of mtDNA. Alterations in the mtDNA of somatic hybrids are often based on recombination events (Hanson 1991; Vedel et al. 1994). Mitochondrial DNA within the self-fertile line 6R20-15 is rearranged in comparison to the mtDNA of tobacco and *H. aureus*, but these alterations did not seem to be very extensive since they were only detectable after hybridisation of *Hin*dIII DNA digests with a *nad3*/*rps12* probe. The probing of two other digests indicated the similarity of the cybrid mtDNA with the tobacco mitochondrion. Non-significant rearrangements of the tobacco-based mtDNA could be a reason for self-fertility of the N. *tabacum* (+*H. aureus*) cybrids. However, it is still not clear whether any distant cybrid lines in the taxonomically similar combination, *N. tabacum* (+*H. nigrum*), could be potentially self-fertile, or whether specific rearrangements of mtDNA are essential for establishing the alloplasmic flower fertility.

Even an inter-specific parasexual cytoplasmic transfer within the *Nicotiana* genus often leads to *de novo* CMS generation due to mtDNA modifications (Aviv et al. 1984; Atanassov et al. 1998; Kumashiro et al. 1988). Moreover, most CMS sources in *Nicotiana* derive from inter-specific sexual backcrosses (Gerstel 1980; Kaul 1988). These facts of sterility in inter-specific cytoplasmic combinations, together with the self-fertility of intergeneric *N. tabacum* (+*H. aureus*) cybrids, indicate that criteria for nucleo-cytoplasmic incompatibility are not strictly associated with the taxonomic distance that occurs for the incongruity of nuclear genomes.

Despite recent progress in the elucidation of plastid gene organisation and expression (Sugita and Sugiura 1996; Bruick and Mayfield 1999), as well as the identification of nuclear genes controlling plastid development (Reiter et al. 1994; Chatterjee et al. 1996; Keddie et al. 1996; Jarvis et al. 1998; Carol and Kuntz 2001; Kroll et al. 2001), the genetic components involved in nucleo-plastome incompatibilities are not yet known. Their number is thought to be significant since many nuclear genes participate in assembling the photosynthetic apparatus (Martin and Hermann 1998). Hypothetically, hybrid nucleo-plastome incompatibilities could be caused by the "mosaic" nature of plastid/nuclear protein complexes due to their encoding by distant and, therefore, functionally non-compatible genes (Babiychuk et al. 1995). In particular, the phylogenetic imbalance might contribute to deviated gene dosages or altered RNA editing in plastids (Schmitz-Linneweber et al. 2001). Further understanding of inter-compartmental interactions might be facilitated due to the availability of new genetically simple models. We believe that *N. tabacum* (+*H. aureus*) cybrids could be a useful system, in which the nucleo-plastome incompatibility is inherited uniparentally, pronounced phenotypically and is not associated with other incompatibilities.

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References

Atanassov II, Atanassova SA, Dragoeva AI, Atanassov AI (1998) A new source in *Nicotiana* developed via somatic hybridization between *N. tabacum* and *N. alata*. Theor Appl Genet 97:982–985

Aviv D, Bleichman S, Arzee-Gonen P, Galun E (1984) Intersectional cytoplasmic hybrids in *Nicotiana*. Theor Appl Genet 67:499–504

Babiychuk E, Schantz R, Cherep N, Weil J-H, Gleba Yu, Kushnir S (1995) Alterations in chlorophyll a/b binding proteins in Solanaceae cybrids. Mol Gen Genet 249:648–654

Bruick RK, Mayfield SP (1999) Light-activated translation of chloroplast mRNAs. Trends Plant Sci 4:190–195

Carol P, Kuntz M (2001) A plastid terminal oxidase comes to light: implications for carotenoid biosynthesis and chlororespiration. Trends Plant Sci 6:31–36

Chatterjee M, Sparvoli S, Edmunds C, Garosi P, Findlay K, Martin C (1996) *DAG*, a gene required for chloroplast differentiation and palisade development in *Antirrhinum majus*. EMBO J 15:4194–4207

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Dragoeva A, Atanassov I, Atanassov A (1999) CMS due to tapetal failure in cybrids between *Nicotiana tabacum* and *Petunia hybrida*. Plant Cell Tiss Org Cult 55:67–70

Gerstel D (1980) Cytoplasmic male sterility in Nicotiana. Tech Bull NC Agric Res Ser 263:3–31

Gleba Yu, Sytnik K (1984) Protoplast fusion and hybridization of distantly related plant species. In: Schoerman R (ed) Protoplast Fusion, Genetic Engineering in Higher Plants. (Monographs on Theoretical and Applied Genetics 8) Springer, Berlin Heidelberg New York, pp 115–161

Glick RE, Sears BB (1994) Genetically programmed chloroplast dedifferentiation as a consequence of plastome-genome incompatibility in *Oenothera*. Plant Physiol 106:367–373

Glimelius K, Bonnett HT (1986) *Nicotiana* cybrids with *Petunia* chloroplasts. Theor Appl Genet 72:794–798

Hanson MR (1991) Plant mitochondrial mutations and male sterility. Annu Rev Genet 25:461–486

Jarvis P, Chen L-J, Li H, Peto CA, Fankhauser C, Chory J (1998) An *Arabidopsis* mutant defective in the plastid general protein import apparatus. Science 282:100–103

Kaul MLH (1988) Male sterility in higher plants. In: Frankel R et al. (eds) Springer, Berlin Heidelberg New York, pp 775–797

Keddie JS, Carroll B, Jones JDG, Gruissem W (1996) The *DCL* gene of tomato is required for chloroplast development and palisade cell morphogenesis in leaves. EMBO J 15:4208–4217

Kirk JTO, Tilney-Bassett RAE (1978) The Plastids. Elsevier, Amsterdam

Kolosha VO, Fodor II (1986) Cloning of *Citrus lemon* ribosomal repeat. Mol Biol (Moscow) 20:656–659

Kroll D, Meierhoff K, Bechtold N, Kinoshita M, Westphal S, Vothknecht UC (2001) *VIPP1*, a nuclear gene of *Arabidopsis thaliana* essential for thylakoid membrane formation. Proc Natl Acad Sci USA 98:4238–4242

Kumar A, Cocking EC (1987) Protoplast fusion: a novel approach to organelle genetics in higher plants. Am J Bot 74:1289–1303

Kumashiro T, Asahi T, Komari T (1988) A new source of cytoplasmic male sterile tobacco obtained by fusion between *Nicotiana tabacum* and X-irradiated *N. africana* protoplasts. Plant Sci 55:247–254

Kushnir SG, Shlumukov LR, Pogrebnyak NI, Berger S, Gleba Y (1987) Functional cybrid plants possessing a *Nicotiana* genome and *Atropa* plastome. Mol Gen Genet 209:159–163

Kushnir S, Babiychuk E, Bannikova M, Momot V, Komarnitsky I, Cherep N, Gleba Y (1991) Nucleo-cytoplasmic incompatibility in cybrid plants possessing an *Atropa* genome and a *Nicotiana* plastome. Mol Gen Genet 225:225–230

Kutzelnigg H, Stubbe W (1974) Investigations on plastome mutants in *Oenothera*. 1. General considerations. Sub-Cell Biochem 3:73–89

Leon P, Arroyo A, Mackenzie S (1998) Nuclear control of plastid and mitochondrial development in higher plants. Annu Rev Plant Physiol Plant Mol Biol 49:453–480

Mackenzie S, McIntosh L (1999) Higher plant mitochondria. Plant Cell 11:571–585

Martin W, Herrmann RG (1998) Gene transfer from organelles to the nucleus: how much, what happens, and why? Plant Physiol 118:9–17

Medgyesy P (1994) Cybrids – transfer of chloroplast traits trough protoplast fusion between sexually incompatible Solanaceae species. In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry vol 27. (Somatic Hybridization in Crop Improvement I) Springer, Berlin Heidelberg New York, pp 72–85

Metzlaff M, Pohlheim F, Börner T, Hagemann R (1982) Hybrid variegation in the genus *Pelargonium*. Curr Genet 5:245–249

Perl A, Aviv D, Galun E (1990) Protoplast-fusion-derived *Solanum* cybrids: application and phylogenetic limitations. Theor Appl Genet 79:632–640

Perl A, Aviv D, Galun E (1991) Nuclear-organelle interaction in *Solanum*: Interspecific cybridizations and their correlation with a plastome dendrogram. Mol Gen Genet 228:193–200

Peter S, Spang O, Medgyesy P, Schäfer C (1999) Consequences of intergeneric chloroplast transfers on photosynthesis and sensitivity to high light. Aust J Plant Physiol 26:171–177

Poyton RO, McEwen JE (1996) Crosstalk between nuclear and mitochondrial genomes. Annu Rev Biochem 65:563–607

Reiter RS, Coomber SA, Bourett TM, Bartley GE, Scolnik PA (1994) Control of leaf and chloroplast development by the Arabidopsis gene *pale cress*. Plant Cell 6:1253–1264

Schmitz-Linneweber C, Tillich M, Herrmann RG, Maier RM (2001) Heterologous, splicing-dependent RNA editing in chloroplasts: allotetraploidy provides *trans*-factors. EMBO J 20:4874–4883

Stubbe W (1989) *Oenothera* – an ideal system for studying the interactions of genome and plastome. Plant Mol Biol Rep 7:245–257

Stubbe W, Herrmann RG (1982) Selection and maintenance of plastome mutants and interspecific genome/plastome hybrids from *Oenothera*. In: Edelman V, Hallick RB, Chua NH (eds) Methods in Chloroplast Molecular Biology. Elsevier Biomedical Press, Amsterdam New York, pp 149–165

Sugita M, Sugiura M (1996) Regulation of gene expression in chloroplasts of higher plants. Plant Mol Biol 32:315–326

Thanh ND, Páy A, Smith MA, Medgyesy P, Márton L (1988) Intertribal chloroplast transfer by protoplast fusion between *Nicotiana tabacum* and *Salpiglossis sinuata*. Mol Gen Genet 213:186–190

Vedel F, Pla M, Vitart V, Gutierres S, Chétrit P, De Paepe R (1994) Molecular basis of nuclear and cytoplasmic male sterility in higher plants. *Plant Physiol Biochem* 32:601–618

Wolters AMA, Koornneef M, Gilissen LJW (1993) The chloroplast and mitochondrial DNA type are correlated with the nuclear composition of somatic hybrid calli of *Solanum tuberosum* and *Nicotiana plumbaginifolia*. Curr Genet 24:260–267

Yao J-L, Cohen D (2000) Multiple gene control of plastome-genome incompatibility and plastid DNA inheritance in interspecific hybrids of *Zantedeschia*. Theor Appl Genet 101:400–406

Zubko EI (1992) Asymmetric somatic hybrids between distant Solanaceae species. PhD Thesis, Kiev

Zubko MK, Zubko EI, Kapranov PhV (1991) Induction of chlorophyll-deficient tobacco mutants as markers for cell engineering. Biopolymers and Cell 7:72–79

Zubko MK, Zubko EI, Patskovsky YuV, Khvedynich OA, Fisahn J, Gleba YuYu, Schieder O (1996) Novel "homeotic" CMS patterns generated in *Nicotiana* via cybridization with *Hyoscyamus* and *Scopolia*. J Exp Bot 47:1101–1110

Zubko MK, Zubko EI, Ruban AV, Adler K, Mock H-P, Misera S, Gleba YY, Grimm B (2001) Extensive developmental and metabolic alterations in cybrids *Nicotiana tabacum* (+*Hyoscyamus niger*) are caused by complex nucleo-cytoplasmic incompatibility. Plant J 25:627–639

Zurawski G, Perrot B, Bottomley W, Whitefeld PR (1981) The structure of the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase from spinach chloroplast DNA. Nucl Acid Res 9:3251–3270