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Variations of Chloroplast DNAs in the Genus *Pelargonium* and their Biparental Inheritance

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Summary. The comparison of EcoRI patterns of chloroplast DNAs (ctDNAs) from five species of the genus *Pelargonium* and from 16 cultivars and varieties of *Pelargonium zonale hort.* demonstrates a remarkable inter- and intraspecific ctDNA (plastome) variation. The plastome of the *P. zonale* varieties could be differentiated into groups I, II and III. Reasons for this variation seem to be: occurrence of numerous spontaneous plastome mutations, intense hybridisation by gardeners and breeders, and biparental plastid inheritance.

Crosses of *P. zonale* varieties with different ctDNA types lead to the direct evidence on the molecular level of biparental plastid inheritance and plastid sorting-out in F_1-hybrids.

Key words: *Pelargonium* – Inter- and intra-specific chloroplast DNA variation – Restriction patterns – Biparental plastid inheritance

Introduction

The genus *Pelargonium (Geraniaceae)* exhibits in a wide range of its species a remarkable morphological and physiological variability. The species *P. zonale hort.* is particularly rich in varieties and cultivars due to efforts of gardeners and breeders. In addition to the great variability in flower colours, growth forms, fertility markers etc., numerous spontaneous plastome mutations have been isolated and propagated in the form of a great variety of periclinal chimeras (Hagemann 1964; Kirk and Tilney-Bassett 1978). Such varieties were used as early as 1909 by Erwin Baur. His classical experiments with white-margined forms of *Pelargonium zonale hort.* led him to postulate the theories of plastid inheritance in higher plants, of sorting-out during ontogenesis of genetically different plastids, and of biparental plastid inheritance in *Pelargonium*.

Our investigations were begun with the following objective: is the pronounced morphological and physiological variability in *Pelargonium*, which certainly is nuclear-coded, correlated with a variation in the restriction patterns of the chloroplast DNA of different species and varieties? We analysed the EcoRI patterns of chloroplast DNAs from five species of the genus *Pelargonium* and from 16 varieties of *P. zonale hort.*

Studies on differences in the restriction patterns of ctDNAs of different species were first carried out by Atchison et al. (1976) on several American and Australian *Nicotiana* species. They found differences in the EcoRI patterns of ctDNAs of American and Australian *Nicotiana* species. Further differences were reported for *Nicotiana* – including parasexual hybrids – by Belliard et al. (1978), Frankel et al. (1979) and Aviv et al. (1980). Interspecific plastome variations were also discovered in the genus *Zea* (Timothy et al. 1979; Conde et al. 1979) and in *Oenothera* (Herrmann and Possingham 1980). The first report about persistent intraspecific plastome variations was given by Scowcroft (1979) for the Australian species *Nicotiana debneyi.* By filter hybridisation it was shown that this plastome variation resides in an additional EcoRI restriction site in some populations.

This paper describes a remarkable inter- and intraspecific plastome variation in the genus *Pelargonium*. The distinct EcoRI-patterns of specific plastome allowed us to follow the inheritance of the maternal and paternal plastids on a molecular level, before and after hybrid formation.

Material and Methods

Breeding of Plant Material

The plants were cultivated under greenhouse conditions. The investigations were carried out on outgrown specimens round-the-year. Twenty-four – 48 h before harvesting the plants were put in the dark to reduce the starch content of the plastids. The following species were included in the analysis: *P. zonale* (for varieties see Table 1), *P. radula, P. roseum, P. peltatum* and *P. fragrans.*

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Isolation of Plastids

The plastids were isolated by differential centrifugation according to the method of Herrmann et al. (1975) in a homogenisation medium containing 0.3 M mannitol. For mutant (white) plastids the times of centrifugation were doubled. The described DNase I treatment was omitted because the conditions of restriction enzyme analysis do not permit the cleavage of chromatin from nucleus contaminations.

Restriction Enzyme Analysis

The analysis of chloroplast DNA (ctDNA) was carried out following the method first described by Atchison et al. (1976). In this procedure the isolated plastids are soaked for ten minutes in a buffer containing 200 mM NaCl, 10 mM TRIS-HCl pH 7.8, 20 mM $MgCl_2$. After addition of 30 units of restriction enzyme the digestion of ctDNA was carried out for 4 h at room temperature within the plastids. After lysis by SDS the restriction fragments were isolated by two short CsCl density centrifugation steps and concentrated by ethanol precipitation.

For molecular weight determination of ctDNA fragments Lambda phage DNA was cleaved by Eco RI (Thomas et al. 1975) in 100 mM TRIS-HCl pH 7.5, 5 mM $MgCl_2$, 50 mM NaCl and by Hind III (Philippsen et al. 1978) in 10 mM TRIS-HCl pH 7.6, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM mercaptoethanole. All restrictions enzymes were a gift from Dr. M. Hartmann, Central Institute of Microbiology and Experimental Therapy, Jena.

Agarose Gel Electrophoresis and UV-Photography

The DNA fragments were fractionated on 0.7-1.8% vertical agarose slab gel (Seakem-agarose, 20 X 15 X 0.4 cm) under the following electrophoretic conditions: 36 mM TRIS-HCl pH 7.8, 30 mM NaH_2PO_4, 10 mM EDTA (Loening 1968), 40 V, 40 mA, 18 h, room temperature. The gels were stained in 5 ug/ml ethidium bromide for 20 min, destained in distilled water for 1 h and photographed under ultraviolet light on DK 5 (ORWO, Wolfen).

Results

Methodological Aspects

Because only small quantities of plant material were available, it was necessary to use a method which requires only small amounts of leaf material but nevertheless gives clear results. The method of choice was that described by Atchison et al. (1976). With this method the ctDNA is digested by restriction enzymes while it is still within the organelle.

When digestion is carried out in the presence of 0.2 M NaCl, contaminating nuclear DNA is not cleaved to fragments smaller than the size-limiting DNA, but ctDNA is cleaved. Therefore, the presence of contaminating nuclei in the chloroplast preparation does not interfere with the documentation of the ctDNA restriction patterns.

This method cannot be applied to the analysis of ctDNA of all plant species. So we have had some difficulties in obtaining a distinct fragmentation pattern, especially from *Hordeum* ctDNA, as had Atchison et al. (1976). However, for the genus *Pelargonium* this is an excellent method and its practicability was no problem. The method of differential centrifugation could be improved in such a way that no more than 0.5 g of plant material was required for the determination of one EcoRI pattern of ctDNA.

In the buffer system of 200 mM NaCl, 10 mM TRIS-HCl pH 7.8, 20 mM $MgCl_2$ the restriction enzymes BglI, BglII, HindII, SalI, KpnI and PstI could be used successfully in addition to EcoRI. In contrast, the application of HindIII, BamHI and SmaI did not result in suitable restriction patterns. An additional advantage of the method is time economy; within 48 hours the result can be documented.

We are of opinion that the Atchison (1976) technique is an excellent screening method for comparing the ctDNAs of many plant species within a short time.

Restriction Patterns of Chloroplast DNA of Different Pelargonium Taxa

The EcoRI restriction patterns of the ctDNAs of the five *Pelargonium* species *P. roseum, P. radula, P. peltatum, P. fragrans* and *P. zonale hort.* (var. 'Dresdener Rubin') show distinct differences (Fig. 1): a pronounced interspecific variation and remarkable inhomologies of the plastomes. Only the patterns of *P. roseum* and *P. zonale hort.* are comparable to some extent, indicating some closer phylogenetic relationship between these two species. Additional statements about the taxonomy and evolution of the genus *Pelargonium* cannot been derived from these studies because there is no phylogenetic system of the *Pelargonium* taxa and because the selection of the species and varieties studied was random and not done with taxonomic considerations in mind.

The analysis of the ctDNAs of 16 varieties of the (hybrid) species *Pelargonium zonale hort.* reveals the following results: (a) most varieties give an identical EcoRI pattern of their ctDNA; we designated the plastome of these varieties as 'plastome I' (Table 1; Fig. 2, gel b). So far we have found only three varieties with patterns differing from plastome I. (b) The EcoRI patterns of the varieties 'Trautlieb' and 'Mrs. Parker' exhibit only small, trifling differences in comparison with type I. These plastids represent 'plastome II' (Table 1; Fig. 2, gel a). (c) Distinct differences in the restriction pattern are characteristic for the variety 'Madame Salleron' (type BBC; Bergann and Bergann 1959). Its EcoRI ctDNA pattern differs markedly from the other *P. zonale* varieties (Table 1; Fig. 2, gel c).

The characteristics of the EcoRI pattern of plastome II in comparison to plastome I consist of the lack of two bands

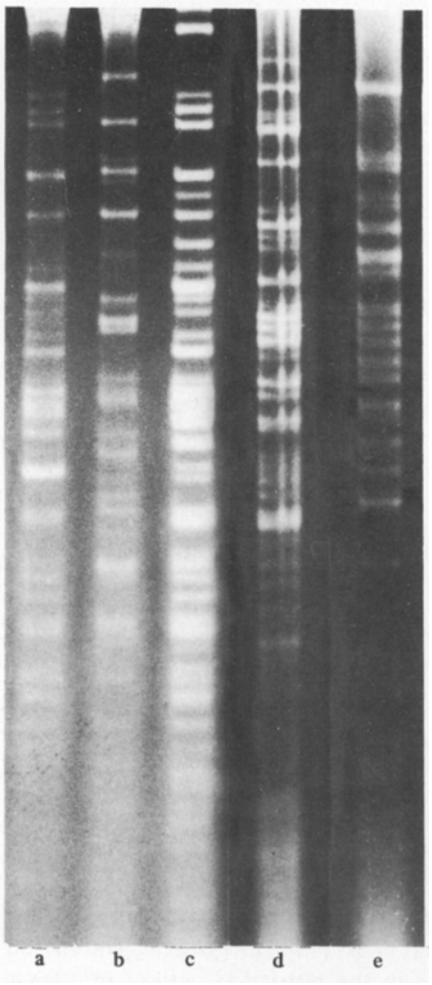

Fig. 1a-e. Interspecific plastome variation in the genus *Pelargonium*. The different EcoRI-patterns of ctDNAs demonstrate the non-homologies of the plastomes. **a** *P. roseum;* **b** *P. radula;* **c** *P. zonale hort.* (var. 'Dresdener Rubin'); **d** *P. peltatum* and **e** *P. fragrans*

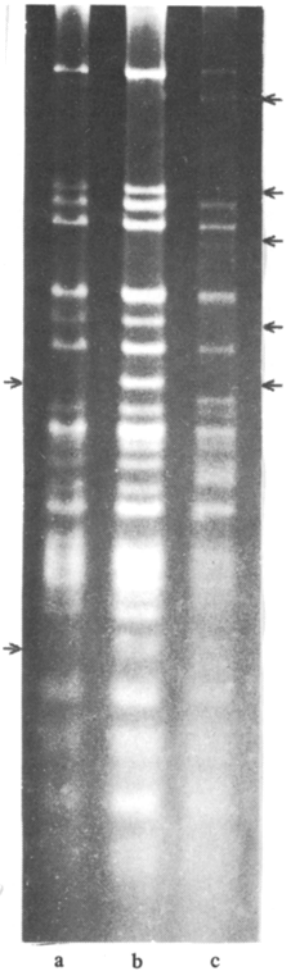

Fig. 2a-c. Intraspecific plastome variation in the hybrid species *Pelargonium zonale hort.* The different EcoRI-patterns of ctDNAs revealed three plastome groups within the species: **a** *P. zonale* – plastome II: **b** *P. zonale* – plastome I; **c** *P. zonale* – plastome III

of 1.9 MD and 0.5 MD respectively in 1.8% agarose gel. additional bands in the range of low molecular weight could not be detected. The plastome III is characterized by numerous changes in the EcoRI-pattern. In comparison with the pattern of plastome I there is an additional band of 5.1 MD molecular weight; two bands of 3.4 MD and 2.2 MD respectively are missing. In the range of low molecular weight bands the conformities prevail. With these results it could be established that there is a distinct intraspecific plastome variation within the species *Pelargonium zonale hort.* which exceeds by far the hitherto known differences within other species.

Inheritance of Plastid DNA Variations

The genetically different types of plastids of the genus *Pelargonium* can be characterized (a) by a particular phenotype of the leaves containing these plastids (green, yellow, yellowish-white, white) and (b) by a typical restriction pattern. The genus *Pelargonium* reveals a biparental mode of plastid inheritance: i.e. the plastids are transmitted both by the egg cells (maternally) and by the sperm cells of the pollen tubes (paternally) (Hagemann 1964, 1979). Due to

Table 1. The plastomes of *Pelargonium zonale hort.* analyzed by EcoRI restriction patterns of ctDNAs

Plastome	Varieties
I	'Stadt Bern', 'Kleiner Liebling', 'Wilhelm Langguth', 'Freak of Nature', 'Mrs. Pollock', 'Flower of Spring', 'Gnom', 'Dresdener Rubin', 'Greifswald', 'Cloth of Gold', 'Happy Thought', 'Dolly Varden','Alex purpurball'
II	'Trautlieb', 'Mrs. Parker'
III	'Madame Salleron'

these characteristics it is possible to pursue the transmission, sorting-out and distribution of maternal and paternal plastids after appropriate crosses, because the biparental plastid inheritance provides the opportunity to combine genetically different plastids in (F_1) hybrid plants.

We analysed the F_1-hybrids of crosses between the varieties 'Flower of Spring' (white or white-margined shoots, with white plastids of plastome type I) and 'Trautlieb' (green shoots with wild-type chloroplasts of plastome type II). The green-white variegated hybrids grew into a chimerical structure. Leaves or leaf parts containing green or white plastids were investigated separately by restriction analysis. The ctDNA from white leaves or leaf parts showed unambiguously the EcoRI pattern of plastome type I, i.e. their plastids are from 'Flower of Spring'. In contrast, the ctDNA from green leaf parts revealed the EcoRI pattern of plastome type II, i.e. their plastids are from 'Trautlieb' (Fig. 3).

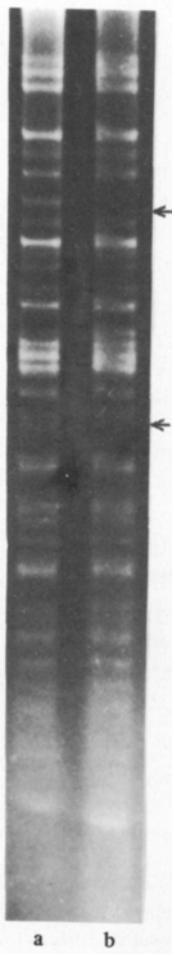

Fig. 3a and b. The biparental mode of plastid inheritance in *Pelargonium*. The EcoRI-analysis of the ctDNAs of the F_1-hybrids 'Flower of Spring' X 'Trautlieb' showed a maternal restriction pattern for the white plastids (a = plastome I) and a paternal restriction pattern for the green plastids (b = plastome II)

These investigations demonstrate that genetic analysis of plastid inheritance, sorting-out and distribution is no longer confined to the use of phenotypic plastid differences, but can also be performed using different characteristic ctDNA restriction patterns as markers.

Discussion

The restriction analysis of several species of the genus *Pelargonium* and of many varieties of the (hybrid) species *P. zonale* has revealed a remarkable plastid DNA (ctDNA) variation. During the past years ctDNA variation has also been reported for other genera, e.g. *Nicotiana*, *Zea* and *Oenothera*, as described in the Introduction.

On the other hand, different species were often found to have the same ctDNA restriction pattern. The three American *Nicotiana* species, *N. langsdorfii*, *N. bonaviensis* and *N. tabacum*, do not differ from each other in their EcoRI pattern; neither do the Australian species, *N. excelsior*, *N. guaveoleus* and *N. gossei* (Atchison 1976). Krahnert et al. (1978) found identical BamHI patterns in three *Lycopercison* species (*L. esculentum*, *L. hirsutum* and *L. peruvianum*).

Differences in the restriction patterns of ctDNA within a species have – apart from *Pelargonium zonale* – only been described (Scowcroft 1979) for the species *Nicotiana debneyi*.

At present we cannot trace the differences in the ctDNA restriction pattern between several *P. zonale* varieties to particular differences in the physiology or morphology of these varieties. But further analysis may lead us to such differences. Atchison et al. (1976) suggested a correlation between differences in the restriction pattern of ctDNA and incompatibility reactions after interspecific crosses. However, this certainly does not apply to *Pelargonium*: its species can easily and successfully be crossed even though they belong to different plastome groups. The intraspecific plastome variation within *Pelargonium zonale* is remarkable. The following facts seem to have favoured this variation: *Pelargonium* has a biparental mode of plastid transmission. If a spontaneous non-lethal plastome mutation affecting the ctDNA variation had occurred, it could be distributed much faster than would be the case for uniparental maternal plastid inheritance.

Moreover, *P. zonale* has been a cultivated plant for a long time. Gardeners and breeders have transformed it into a compound hybrid species. Crosses made to combine numerous spontaneous nuclear mutations have also unintentionally combined plastome differences, which subsequently segregated by the sorting-out of plastids. Chlorophyll deficiencies were valuable traits for gardeners, because they could be kept in the form of periclinal chimeras and led to well-known ornamental varieties which were easily kept by cuttings.

The analysis of ctDNA of F_1-hybrids provided evidence for the biparental mode of inheritance and sorting-out of

plastids directly on the DNA level. The maternal and paternal plastids remain genetically unchanged in the hybrid. Up to now we could not find any indication for a recombination between maternal and paternal ctDNA molecules as has been described for *Chlamydomonas* by Sager (1976) and Boynton et al. (1976). No changed or mixed ctDNA restriction patterns could be detected after hybrid formation.

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