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Hybrid Variegation in the Genus *Pelargonium*

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Summary. 1) The genetical and cytological analysis of crosses between the wild-type form *'Roseum'* of *Pelargonium zonale (L.) L'Herit. ex Ait.* and the hybrid species *Pelargonium zonale hort. 'Stadt Bern'* revealed the occurrence of hybrid variegation in the genus *Pelargonium.*

2) The plastids in the green-white variegated plants were sorted out in the quick apical way characteristic for *Pelargonium* chimera. Six types of periclinal chimeras could be observed.

3) The DNAs of the two different plastomes can be distinguished by restriction endonuclease analysis with EcoRI and BamHI.

4) The differences in the cleavage patterns allowed a detailed description of the process of sorting out of the two parental plastids types within the variegated hybrids on the level of their plastid DNA. In all cases observed the cleavage pattern of *'Roseum'* plastid DNA was found in the green tissue and the pattern of *'Stadt Bern'* plastid DNA in the white tissue.

5) From these results we draw the conclusion that there is a disharmony between the F_1-hybrid nucleus and the plastome of *,Stadt Bern'*, which blocks the normal differentiation of *'Stadt Bern'* plastids.

Key words: Hybrid variegation – *Pelargonium* – Extranuclear inheritance – Plastid DNA – Restriction patterns

Introduction

The development of photosynthetically active chloroplasts is under a dual genetic control: Both chloroplast and nuclear genes carry the information for chloroplast proteins. Therefore, normal chloroplast differentiation requires a balanced interaction of the gene products of two different genetic system. Crosses between species differing with respect to their genetic information not only in the genome but also in the plastome may lead to a disturbance of this interaction and thus to a defective chloroplast biogenesis. This phenomenon which is phenotypically visible as a bleaching out of the hybrid plants was first described for *Geranium* (Dahlgreen 1923, 1925) and for *Oenothera* (Renner 1924) und is called hybrid chlorophyll deficiency („Bastardbleichheit"). Chloroplast genes are biparentally inherited in *Geranium* as well as in *Oenothera.* Therefore, a hybrid zygote contains two types of plastids in addition to the hybrid nucleus, one of maternal origin the other of paternal origin. If the two types differ in their genetic information, a disharmony may occur between the hybrid nucleus and one of the plastid types at the level of their gene products.

The respective plastid type is unable to undergo a normal differentiation while the other type may develop into normal chloroplasts. The hybrid plant originating from such a zygote has variegated leaves as well as normal green and fully bleached leaves as a result of sorting out of the two plastid types during ontogenesis. This type of variegation is called hybrid variegation. Bleaching is a reversible defect. If the bleached plastids of a hybrid are – as a result of a new cross – again combined with their original nuclear genome, they will develop into normal green chloroplasts.

Hybrid variegation was intensively studied in *Oenothera* (for review see Hagemann 1965; Kirk and Tilney-Bassett 1978). Within the subgenus *Euoenothera* five plastomes und six diploid genotypes (combined from three basic haploid genomes) were distinguished by the occurrence of hybrid variegation in interspecific hybrids (Stubbe 1959). The DNA of these five plastomes has

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been analyzed by means of restriction enzyme digestion. Comparison of the cleavage patterns of the DNAs revealed distinct differences between the plastomes (Herrmann and Possingham 1980; Gordon et al. 1982).

Hybrid variegation has been observed only in a few other genera with biparental chloroplast inheritance (cf. Kirk and Tilney-Bassett 1978). However, these observations were made by chance in most casses and were not followed by a thorough analysis of the phenomenon. Like *Oenothera, Pelargonium* with its biparental plastid inheritance has become a standard object of research on chloroplast genetics. There was only one paper on the occurrence of variegation reporting that the progeny of crosses between green plants of *Pelargnoium denticulatum* and *P. filicifolium* was entirely variegated (Smith 1915 cit. in Chittenden 1927). Recently, extended variations were found between the restriction cleavage patterns of plastid DNA in different *Pelargonium* species and in various cultivars and varieties of the hybrid species *Pelargonium zonale hort.* (Metzlaff et al. 1981). The existence of different plastomes and the possibility to perform interspecific and intervarietal corsses makes the genus *Pelargonium* a potentially interesting subject for studies on hybrid variegation. In this paper we will describe the occurrence of hybrid variegation in the F_1-generation of crosses between *Pelargonium zonale (L.) L'Herit. ex Ait. 'Roseum'* and *Pelargonium zonale hort. 'Stadt Bern'.*

Materials and Methods

Plant Material. In this paper we use the following nomenclature: We will call the wild-type variety of *Pelargonium zonale (L.) L'Herit. ex Ait. 'Roseum'* because the flowers of these plants are rose-coloured. All other *'Zonale'*-hybrids originating from crosses of several *Pelargonium* species including *P. zonale* (cf. Clifford 1958) we will call *Pelargonium zonale hort.* (= *Pelargonium* x *hortorum L. H. Bailey*) since we do not know the actual ancestors of the various cultivars and varieties.

Crosses were made between *P. zonale (L.) L'Herit. ex Ait. 'Roseum'* and the cultivar *'Stadt Bern'* belonging to the group of *'Zonale'*-hybrids. The plants were cultivated under greenhouse conditions.

Light Microscopy. The determination of the periclinal chimera constitution of plants with hybrid variegation was accompanied by both the analysis of the pattern phenotype and anatomical investigations of the leaf. The distribution of green and white tissues in the mesophyll using free hand sections of leaf.

The investigation of plastids in guard cells of leaf epidermisses allowed the differentiation between green and white L_1.

Plastid DNA Analysis. The plastid were isolated from leaf tissues by differential centrifugation according to the method of Herrmann et al. (1975) in a homogenisation medium containing 0.3 M Mannitol. The DNaseI treatment was omitted (cf. Metzlaff et al. 1981). The restriction enzyme analysis of the plastid DNA was carried out following the method first described by Atchison et al. (1976) with some modifications (Metzlaff et al. 1981). The restriction enzymes were isolated and kindly given by Dr. M. Hartmann, Central Institute of Microbiology and Experimental Therapy, Jena, GDR. The digested plastid DNA was fractionated on 1.5% or 1.75% vertical agarose slab gels (Seakem-Agarose, 10 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 as electrophoresis buffer (Loening 1968), 40 V, 40 mA, 18 hours, room temperature. The gels were stained in 5 μg/ml ethidium bromide for 20 minutes, destained in distelled water for one hour and photographed under ultraviolet light on DK 5 (ORWO, Wolfen, GDR).

Results

Phenotype of Hybrids

Three independent reciprocal crosses were made between fully green plants of the wild-type form *'Roseum'* and the variety *'Stadt Bern'*. From all crosses variegated plants were observed in the F_1-generation (Fig. 1). All variegated plants showed the variegation already at the cotyledons. During ontogenesis entirely bleached leaf parts of leaves appeared in several variegated hybrids. In other hybrids variegation disappeared during ontogenesis and they developed into normal green plants. If an extended bleaching occured already at the stage of the cotyledons growth was inhibited in the plantlets and they usually died a few weeks after germination.

An offspring of 57 plants was observed with the three crosses with *'Roseum'* as the female parent. 11 of these 57 plants showed distinct variegation. In the progeny of the reciprocal crosses using *'Stadt Bern'* as the female parent 32 from 89 hybrids showed variegation. It seems likely to obtain more variegated plants, more extended bleaching of the variegated plants and more entirely bleached seedlings in the offspring of the crosses with *'Stadt Bern'* as the female parent.

Cytological Observations

The plastids in the green-white variegated plants of the F_1 were sorted out in the quick apical way characteristic for *Pelargonium* chimera (cf. Hagemann 1964). Six types of periclinal chimeras could be observed with three independent tissue layers and two chimera components as expected for a shoot apex (Fig. 2).

In addition to the chimeras the two homohistonts GGG and WWW were found (the three layers L_1, L_2 and L_3 of the apex and the leaves are green (G) or white (W), i.e. all tissue layers contain the same type (paternal or maternal) of plastids. Only homohistonts were used for the plastid DNA analysis described below. Since it is difficult to distinguish leaves of the chimera GWW with a green epidermis and of the chimera WGG with

Fig. 1. Variegated *Pelargonium* plant which originated after crossing of *Pelargonium zonale (L.) L'Herit. ex Ait.* x *P. zonale hort.* Homohistically white and green buds occur together with periclinal chimera buds, the former being used for plastid isolation

a white epidermis from the white and green homohistonts, respectively, the material for DNA extraction was selected only after a light microscopic analysis.

Analysis of Plastid DNA of Variegated Hybrids

Plastids were isolated from leaves of *'Roseum'* and *'Stadt Bern'* as well as from leaves of homohistonts GGG and WWW from variegated hybrids. The plastid DNA was digested in organello by the restriction enzymes EcoRI and BamHI. The plastids from *'Roseum'* and *'Stadt Bern'* can easily be distinguished from each other by the cleavage patterns of their DNA (Metzlaff et al. 1981; Fig. 3).

The differences in the cleavage patterns allowed a detailed description of the process of sorting out of the two parental plastid types within the variegated hybrids on the level of their plastid DNA. In all hybrids analyzed and independent of the direction of the cross (*'Roseum'* or *'Stadt Bern'* as mother) the cleavage pattern of *'Roseum'* plastid DNA was found in the green tissue and the pattern of *'Stadt Bern'* plastid DNA in the white tissue (Fig. 4). Obviously it is the *'Stadt Bern'* plastid which is blocked in its differentiation whereas the *'Roseum'* plastid keeps its ability for normal development within the hybrid cell.

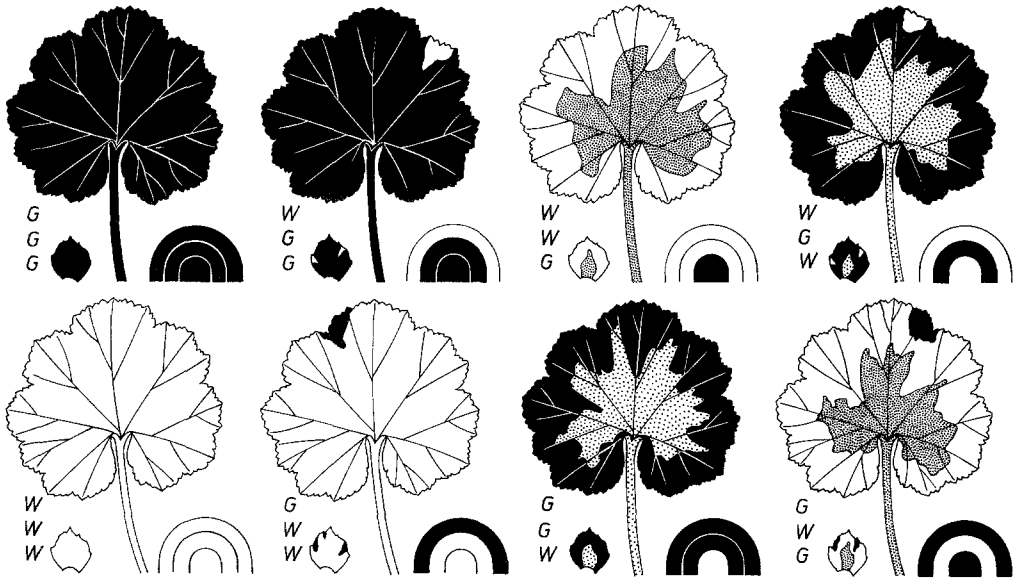

Fig. 2. All periclinal chimera patterns could be observed with *Pelargonium* plants showing hybrid variegation. Tissues from three independent layers in the shoot apex are involved in leaf development, having two different chimera components W (white) and G (green). *Left:* leaf pattern. *Right:* Scheme of the apex constitution

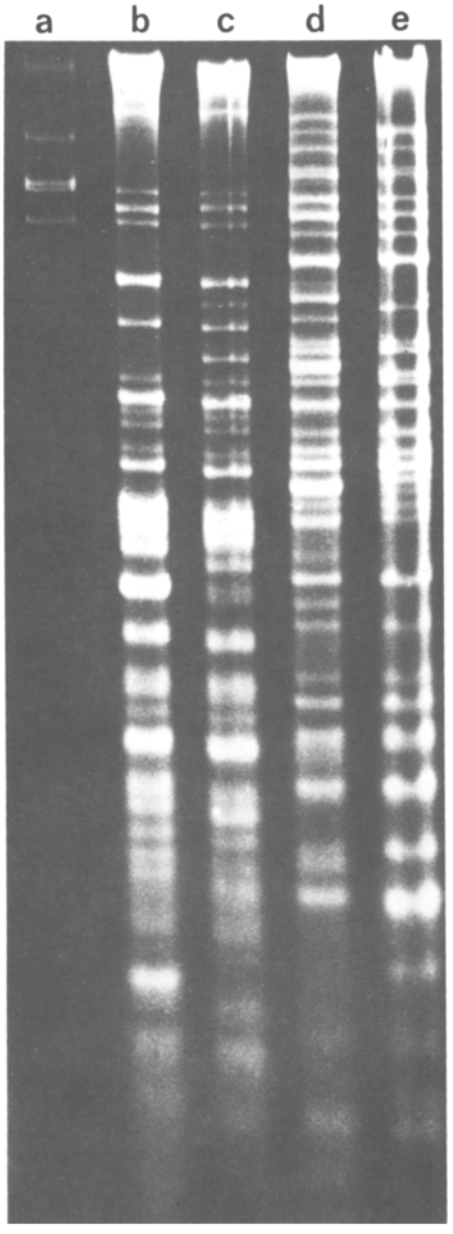

Fig. 3. Agarose slab gel electrophoresis of restriction endonuclease digests of the two DNAs from the parental *Pelargonium* plastomes (1.5% agarose)
Track a: Molecular weight standard: λ-DNA/EcoRI
Track b: *Roseum'* plastid DNA/EcoRI
Track c: *'Stadt Bern'* plastid DNA/EcoRI
Track d: *'Roseum'* plastid DNA/BamHI
Track e: *'Stadt Bern'* plastid DNA/BamHI

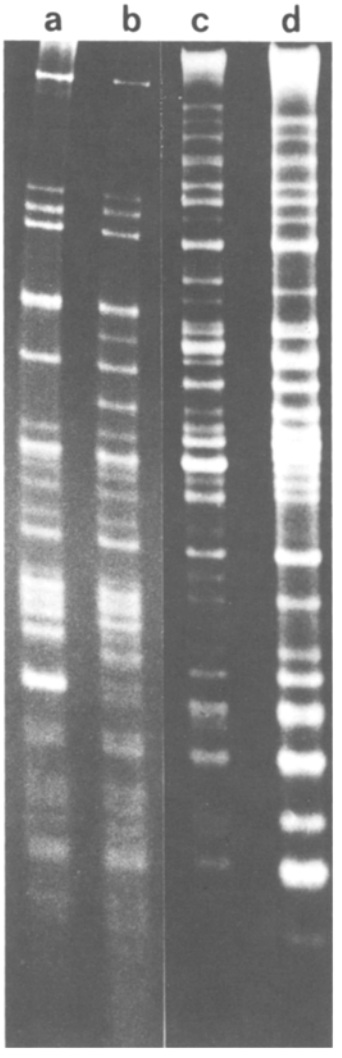

Fig. 4. Agarose slab gelectrophoresis of restriction endonuclease digests of the plastid DNAs from green and white tissue of F_1-hybrid plants (1.5% agarose)
Track a: Plastid DNA from green tissue/EcoRI
Track b: Plastid DNA from white tissue/EcoRI
Track c: Plastid DNA from green tissue/BamHI
Track d: Plastid DNA from white tissue/BamHI
The EcoRI and BamHI patterns of the green tissue are identically with the patterns of *'Roseum'* plastid DNA. The patterns of the white tissue are identically with the patterns of *'Stadt Bern'* plastid DNA

Discussion

Prerequisites to the occurrence of hybrid variegation are biparental chloroplast inheritance and plastome variations among compatible species of a genus or among different varieties of a species. Both biparental inheritance and plastome variation are found in *Pelargonium* (cf. Metzlaff et al. 1981). Therefore we looked for a disharmony between the nucleus and the plastids in hybrids obtained from crosses between the wild-type

form *'Roseum'* and the variety *'Stadt Bern'*. These varieties show distinct differences in the cleavage patterns of their plastid DNAs after restriction enzyme digestion.

'Stadt Bern' has the pattern of the *'Pelargonium plastome I'* thus belonging to the larger group of *Pelargonium zonal hort.* varieties and cultivars which, in turn, belong to the group of *'Zonale'*-hybrids (Metzlaff et al. 1981). The pattern of the plastid DNA fragments derived from *'Roseum'* shows a certain degree of homology with plastome I despite the variations thus suggesting a rather close evolutionary relationship between *P. zonale (L.) L'Herit. ex Ait.* and the *P. zonale hort.*-hybrids. Other *Pelargonium* species exhibited completely different plastid DNA cleavage patterns (Metzlaff et al. 1981). Crosses between *'Roseum'* and *'Stadt Bern'* could be performed without any interference by incompatibility.

In our present work we perform crosses of *'Roseum'* and the variety *'Trautlieb'* belonging to the *'Pelargonium plastome II'* of the *'Zonale'*-hybrids (Metzlaff et al. 1981). The first analysis of these crosses revealed the occurrence of hybrid variegation, too.

Two typer of evidence were obtained for the occurrence of hybrid variegation in the progeny of crosses between the two green varieties mentioned:

(1) Variegated plants could be observed in the offspring of all crosses. The percentage of variegated plants was higher when *'Stadt Bern'* as the female parent was used indicating reciprocal differences.

(2) In all cases plastid DNA from the bleached tissue showed the cleavage pattern which is characteristic for *'Stadt Bern'*. Thus, only one of the two plastid types bleached out in the hybrid as is expected in the case of hybrid variegation (cf. Hagemann 1964, 1965; Kirk and Tilney-Bassett 1978).

The results reported in this paper raise several questions: The wild species *P. zonale* is generally assumed to be one of the ancestors of the *'Zonale'*-hybrids (cf. Knuth 1931; Harney and Chow 1971); to what extent did *P. zonale* contribute to the occurrence of plastomes in the hybrid varieties?

Is hybrid variegation a common phenomenon in *Pelargonium*, which could than be used to elucidate the interaction of genome and plastome in chloroplast biogenesis?

Is there a causal connection between the plastome differences observed and the occurrence of hybrid variegation?

Another question was already asked by Chittenden (1927), Ufer (1934) and Renner (1936): Are some of the well-known white or yellow margined *Pelargonium* cultivars due to the hybrid variegation and not to the spontaneous plastome mutations as usually assumed?

Further investigations will be carried out to answer these questions.

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References

Atchison BA, Whitfeld PR, Bottomley W (1976) Mol Gen Genet 148:263–269

Chittenden RJ (1927) Vegetative Segregation. Bibliogr Genet 3:355–442

Dahlgren KVO (1923) Hereditas 4:239–250

Dahlgren KVO (1925) Hereditas 6:237–256

Gordon KHJ, Crouse EJ, Bohnert HJ, Herrmann RG (1982) Theor Appl Genet 61:373–384

Hagemann R (ed) (1964) Plasmatische Vererbung, VEB G. Fischer, Jena

Hagemann R (1965) Advances in the field of plastid inheritance In higher plants. Genetics Today. Proc. XI. Internat. Congr. Genetics, The Hague. Pergamon Press, Oxford, p 613–625

Harney PM, Chow TW (1971) Euphytica 20:286–291

Herrmann RG, Bohnert HJ, Kowallik KV, Schmitt JM (1975) Biochem Biophys Acta 378:305–317

Herrmann RG, Possingham JV (1980) Plastid-DNA – the Plastome. In: Reinert J (ed) Results and Problems in Cell Differentiation: The Chloroplast. Springer, Berlin Heidelberg New York, p 45–96

Kirk JTO, Tilney-Bassett R (eds) (1978) The Plastids, Their chemistry, structure, growth and inheritance. Elsevier, Amsterdam

Loening UE (1968) J Mol Biol 38:355–361

Metzlaff M, Börner T, Hagemann R (1981) TAG 60:37–41

Knuth R (ed) (1931) Geraniaceae. In: Engler, Harms (eds) Die natürlichen Pflanzenfamilien. Engelmann, Leipzig

Renner O (1924) Biol Zbl 44:309

Renner O (1936) Flora 30:218–290

Stubbe W (1959) Z Vererbungsl 90:288–298

Ufer M (1934) Naturwissenschaften 22:271

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