Biparental Plastid Inheritance in *Zantedeschia albomaculata* (Araceae)

F. Santiago Brown, R.C. Snijder and J.M. van Tuyl
Plant Research International, Wageningen University and Research Centre
P.O. Box 16, 6700 AA Wageningen
The Netherlands

Keywords: *Aestivae*, breeding, calla, plastome-genome incompatibility, CAPS

Abstract

Most flowering plants exhibit maternal plastid inheritance. In *Zantedeschia*, however, biparental is known to occur in interspecific hybrids. Moreover, plastome-genome incompatibility exists between the different species. The evolutionary significance of biparental plastid inheritance and plastome-genome incompatibility is unclear. It is possible that the unusual genomic background of interspecific crosses affects plastid inheritance and that plastids inherit maternally in intraspecific hybrids as in most other flowering plants. The mode of plastid inheritance between *Z. albomaculata* subsp. *albomaculata* and *Z. albomaculata* subsp. *macrocarpa* was, therefore, investigated. Subspecies specific CAPS markers were developed and used to demonstrate that biparental plasmid inheritance occurs between these subspecies. This finding suggests that biparental plastid inheritance exists within *Zantedeschia* spp. and is not a result from the unusual genomic background of interspecific crosses.

INTRODUCTION

Zantedeschia is a popular cut-flower and pot-plant. It is mainly grown in The Netherlands, New Zealand and the U.S. The area planted in 2000 was 177 ha (U.S. not included), and the leading producer is The Netherlands with 100 ha in 2002. Production of *Zantedeschia* as a cut flower has increased steadily in recent years. In 2002, 58 million stems were sold through the Dutch auctions, an increase of 16.8% on the previous year (Vereniging van Bloemenveilingen in Nederland, 2003).

The genus *Zantedeschia* consists of eight species distributed over two sections, *Zantedeschia* and *Aestivae*. Section *Zantedeschia* consists of *Z. aethiopica* and *Z. odorata*, and section *Aestivae*, also known as 'colored callas', consists of *Z. albomaculata*, *Z. elliotiana*, *Z. jucunda*, *Z. pentlandii*, *Z. rehmannii* and *Z. valida*. *Z. albomaculata* includes two subspecies *albomaculata* and *macrocarpa* (Singh et al., 1996).

In most flowering plants, plastid inheritance is primarily maternal, although paternal and bipaternal plastid inheritance are reported in genera such as *Oenothera*, *Pelargonium* and *Medicago* (Mogensen, 1996). There are three mechanisms for uniparental plastid inheritance. Paternal plastids degrade in the gamete before or during gametogenesis; they are shed from gametes before fertilization; or they degrade in the zygote after fertilization (Birky, 2001). Biparental inheritance in *Oenothera* has demonstrated different transmission efficiencies of paternal plastids during fertilization (Chiu and Sears, 1993).

Interspecific hybrids within section *Aestivae* may suffer from plastome-genome incompatibility (PGI), due to biparental inheritance. This incompatibility is expressed in seedling leaves as albinism, variegation or virescence. The occurrence of these phenotypes depends on the parental species (New and Paris, 1967). In *Zantedeschia* section *Aethiopica* PGI is a consequence of "miscommunication" between plastids and the nuclei (Yao and Cohen, 2000; Yao et al., 1995). Snijder (2004), in his work on genetics of *Erwinia* resistance, came across PGI in the section *Aestivae*. PGI has been described previously in other interspecific hybrids such as *Azalea* (Ureshino et al., 1999), *Trifolium* (Pandey et al., 1987), *Impatiens* (Arisumi, 1985) and *Pelargonium* (Metzlaff et al., 1982).

To identify plastids and their mode of inheritance between species, differences between plastids had to be identified. To achieve this, phenotypic descriptions of

interspecific seedlings were combined with CAPS markers to show a restriction pattern that discriminated plastids between species. Seedlings from *Aestivae* combinations were analyzed, focusing primarily on *Z. albomaculata* species due to presence of not only interspecific PGI phenotypes, but also intraspecific.

MATERIALS AND METHODS

Plant Material

Parental lines were obtained from the germplasm collection at Plant Research International (PRI) (Table 1), these genotypes were crossed during 2001 and 2002. Seedlings of these crosses were planted in a controlled greenhouse environment during February 2003 (Table 2).

DNA Extraction

The samples (Fig. 1) were freeze-dried, and ground using tungsten balls, which were placed in QIAGEN collection microtubes on a mixer mill (MM300 Restch) for 90 seconds at 25 shakes per minute. 400 µl of Extraction Mix (0.13 M Tris.HCl, 0.02 M EDTA, 0.9 M NaCl, 0.9% CTAB, 0.15 M sorbitol, 5% $Na_2S_2O_5$, 0.6% sarkosyl) was added to each tube and the tubes were incubated in a water bath at 65°C for 1 hour. After incubation, 400 µl of chlorophorm/isoamylalcohol (24:1) was added. The tubes were shaken, then centrifuged for 20 minutes at 5500 g. The supernatant was transferred to a new tube and 1 volume of isopropanol (-20°C) was added. Inverting the tubes a few times precipitated the DNA. Samples were again centrifuged for 5 minutes at 21000 g and the supernatant was discarded. The pellet was washed gently with 70% ethanol. The DNA samples were centrifuged for 5 minutes at 21000 g and air-dried for 1 minute in a speed vac. The DNA pellet was re-suspended and dissolved in 30 µl of TE (1M Tris, 0.1 M EDTA). After the DNA was suspended 10 µg of RNAseA was added to the mixture, which was incubated at 37°C for 2 hours.

CAPS

Two primers were designed to amplify a 3 kb region in the ptDNA (plastid DNA). This region is between two genes, *trn*D (tRNA-Asp) and *trn*C (tRNA-Cys). The spacer region was amplified by PCR using primer pair DCRon ($DCRon_F$: 5'-AGAGCACCGCCCTGTCAAG-3' and $DCRon_R$: 5'-GCATGGCCRAGYGGTAAGG-3'). The PCR was carried out by adding 24 µl of master mix (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 3.5 mM $MgCl_2$, 0.1% Triton X-100, 1 mM dNTP (Invitrogen), 5 pM DC_F for DC, 10 pM DC_R for DC, 0.5 U SuperTaq (HT Biotechnology) and 0.05 U *Pfu* polymerase (Promega)) to 1 µl (1-2 µg) of template DNA on PCR thin reaction tubes. The samples were placed on a MJ Research PTC-200 thermal cycler. It was programmed to give a 3 min period at 94°C and then 40 cycles of the following sequence: 1 min 94°C, 64.5°C, and 3.5 min at 72°C. The final cycle ended with 15 min at 72°C. DNA was visualized on an agarose gel (0.8% agar in 0.04 M Tris Ac, 2mM EDTA), after staining with ethydium bromide.

To identify polymorphisms, the spacer was digested with several restriction enzymes: *Dpn* II, *Nla* III, *Mse* I, *Sau* 96I, *Mnl* I, *Hpy*CH4 IV, *Alu* I, *Hae* III, *Hinf* I, *Taq* I and *Rsa* I. The amount of restriction enzyme used was 10 U (1 µl) for every 10 µl of PCR reaction volume. After overnight digestion at 37°C (except *Taq* I which was digested at 65°C), the samples were run on a 1.2% agarose gel in Tris-Acetate-EDTA buffer, after staining with ethydium bromide. The gels ran at 60 Volts for 4 hours.

RESULTS AND DISCUSSION

CAPS-variation among Species

Eight of the restriction enzymes tested showed clear band polymorphisms between species (Table 3). Additionally six showed different band patterns between sub-species

albomaculata and *macrocarpa*. *Z. pentlandii* had the same band pattern as *Z. elliotiana* for all restriction enzymes. *Z. albomaculata* subsp. *macrocarpa* could only be differentiated from *Z. pentlandii* and *Z. elliotiana* by one restriction enzyme, *Alu* I. The differences in band patterns between *Z. albomaculata* subsp. *albomaculata* and *Z. albomaculata* subsp. *macrocarpa*, and the similarity that *Z. albomaculata* subsp. *macrocarpa* has with *Z. elliotiana* and *Z. pentlandii*, suggests that the two subspecies have different origins.

The restriction enzymes *Hae* III and *Alu* I were chosen to differentiate plastids between seedlings (Fig. 2). The DCRon-*Alu* I restriction pattern could differentiate plastomes of *Z. albomaculata* subsp. *albomaculata* and *Z. rehmannii* (Fig. 2a), as well as *Z. albomaculata* subsp. *macrocarpa* and *Z. elliotiana* (Fig. 2b). The size difference between the largest fragments (1.6 kb) from *Z. rehmannii* and *Z. albomaculata* subsp. *albomaculata* was about 50 bp (Fig. 2a.). With the DCRon-*Hae* III restriction pattern, two groups of species could be differentiated within the *Aestivae* section. The first group is *Z. albomaculata* subsp. *albomaculata* and *Z. rehmannii*, which could easily be discriminated from a second group formed by *Z. pentlandii*, *Z. elliotiana* and *Z. albomaculata* subsp. *macrocarpa* (Fig. 2c).

Plastid Inheritance

Seedlings showed three leaf phenotypes: the hybrids were entirely green, had different degrees of variegation or were virescent. The degree of variegation was variable, leaves varied in color intensity and light green area size. CAPS markers combined with visual observations were used to identify the plastome present in dark green leaf phenotypes (Table 4).

A total of 120 pure dark green, light green and virescent leaf sections was analyzed, pure dark green samples had only one plastome type, with the exception of *Z. elliotiana* and *Z. albomaculata* subsp. *macrocarpa* crosses, where dark green and light green samples had both plastome types. The plastome-genome compatibility from both species demonstrates the similarity that exists between these two plastome types, or perhaps the close relationships between their nuclear DNA.

The outcome obtained by this approach helped determine which plastome led to PGI in a particular interspecific crossing direction. Identifying differences between plastomes in parental combinations will help breeders plan crossing combinations to prevent high expression of PGI.

Plastomes from *Z. pentlandii* and *Z. elliotiana* appeared to be closely related and are perhaps identical. Not only did both plastomes show similar PGI behavior, they also demonstrated the same restriction pattern of DCRon spacer after using eight polymorphic restriction enzymes. The numerous differences observed in plastome restriction pattern between subsp. *macrocarpa* and subsp. *albomaculata*, and their crossing incompatibility due to PGI, suggest that subsp. *macrocarpa* should be placed as a different species. Further analysis of genomic sequences, or CAPS digestion on a different plastome section, from all species in section *Aestivae* would help confirm the differences and similarities observed.

Finally, a few other questions are raised for further discussion. Should there be a reclassification of *Zantedeschia* species? How clear is the definition line between species? Does it add any value to science? And considering biparental inheritance was observed in *Zantedeschia* because of the presence of incompatible leaf phenotypes, could it be that all dark green seedlings in intraspecific crosses also inherit plastomes biparentally?

Literature Cited

Arisumi, T. 1985. Rescuing abortive *Impatiens* hybrids through aseptic culture of ovules. J. Amer. Soc. Hort. Sci. 110:273-276.

Birky, C.W.J. 2001. The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. Annual Review Genetics 35:125-148.

Chiu, W. and Sears, B. 1993. Plastome-genome interactions affect plastid transmission in *Oenothera*. Genetics Society of America 133:989-997.

Metzlaff, M., Pohlheim, F., Borner, T. and Hagemann, R. 1982. Hybrid variegation in the genus *Pelargonium*. Current Genetics 5:245-249.
Mogensen, H.L. 1996. The hows and whys of cytoplasmic inheritance in seed plants. Amer. J. Bot. 83:383-404.
New, E. and Paris, C. 1967. Virescence in *Zantedeschia.* Proceedings of the Amer. Soc. Hort. Sci. 92:685-695.
Pandey, K., Grant, J. and Williams, E. 1987. Interspecific hybridisation between *Trifolium repens* and *T. uniflorum*. Austr. J. Bot. 35:171-182.
Singh, Y., Van Wyk, A.E. and Baijnath, H. 1996. Taxonomic notes on the genus *Zantedeschia* Spreng. (Araceae) in southern Africa. S. Afr. J. Bot. 62(6):321-324.
Snijder, R.C. 2004. Genetics of *Erwinia* resistance in *Zantedeschia*. Impact of plastome-genome incompatibility. PhD thesis, Wageningen University. 112p.
Ureshino, D., Miyajima, I., Ozaki, Y., Kobayashi, N., Michischita, A. and Akabane, M. 1999. Appearance of albino seedlings and ptDNA inheritance in interspecific hybrids of azalea. Euphytica 110:61-66.
Vereniging van Bloemenveilingen in Nederland. 2003. http://www.wbn.nl/de/actueel/omzetberichten/2003/kwartaal1/omzetbericht_2002.
Yao, J. and Cohen, D. 2000. Multiple gene control of plastome-genome incompatibility and plastid DNA inheritance in interspecific hybrids of *Zantedeschia.* Theor. Appl. Genetics 101:400-406.
Yao, J., Cohen, D. and Rowland, E. 1995. Interspecific albino and variegated hybrids in the genus *Zantedeschia*. Plant Sci. 109:199-206.

Tables

Table 1. List of parental genotypes used.

PRI No.[1]	Species / Cultivar
00056	*Z. albomaculata* subsp. *albomaculata*
00060	*Z. albomaculata* subsp. *albomaculata*
00075	*Z. albomaculata* subsp. *albomaculata*
00076	*Z. albomaculata* subsp. *albomaculata*
018002	*Z. albomaculata* subsp. *macrocarpa*
018006	*Z. albomaculata* subsp. *macrocarpa*
00069	*Z. pentlandii*
00073	*Z. elliotiana*
00074	*Z. rehmannii*
99022	*Z. rehmannii*

[1]PRI: Plant Research International tracking number.

Table 2. Table of genotype combinations giving seedlings.

PRI No.[2]	Female parent (species)[3]	Male parent (species)[3]	Cross
028112	AA	AM	00075 x 018006
028113	R	AM	00074 x 018002
028114	R	AM	99022 x 018006
028115	R	AM	00074 x 018002
028116	AM	AA	018002 x 00056
028117	AA	AM	00056 x 018006
028118	E	AM	00073 x 018006
028201	AM	AM	018006 x 018006
028202	AM	AM	018006 x 018006
028206	AM	AM	018002 x 018002
028208	AM	AM	018002 x 018002
028213	AA	AA	00075 x 00075
028214	AA	AA	00076 x 00076
018415[1]	P	AA	00069 x 00060
018411[1]	AA	P	00060 x 00069
018408[1]	AA	P	00060 x 00069

[1] These seeds were sown at a different date and location.
[2] PRI: Plant Research International tracking number.
[3] *Z. albomaculata* subsp. *albomaculata*, AM: *Z. albomaculata* subsp. *macrocarpa*, E: *Z. elliotiana*, P: *Z. pentlandii*, R: *Z. rehmannii*.

Table 3. DCRon spacer restriction pattern listing differences and similarities between species when cut with different restriction enzymes.

Restriction Enzyme	Aestivae section
Dpn II	R/ AA , E/ P/ AM
Mse I	R , AA , E / P / AM
Hinf I	R , AA , E / P / AM
Nla III	R , AA , E / P / AM
Taq I	AA , R / E / P (AM not available)
Rsa I	R/ AA , E / P (AM not available)
Hae III	R/ AA , E / P / AM
Alu I	R , AA , E / P , AM

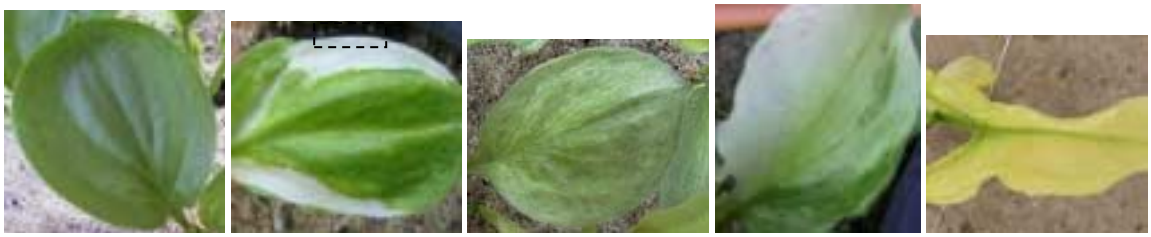
AA: *Z. albomaculata* subsp. *albomaculata*, AM: *Z. albomaculata* subsp. *macrocarpa*, E: *Z. elliotiana*, P: *Z. pentlandii*, R: *Z. rehmannii*.
(,) = difference, (/) = similarity

Table 4. Association of plastome with dark green leaf phenotype.

Cross combination	Plastome associated with dark green phenotypes
AA x AM	AA
AM x AA	AA
E x AM	E and AM
R x AM	R
P x AA	AA
AA x P	AA

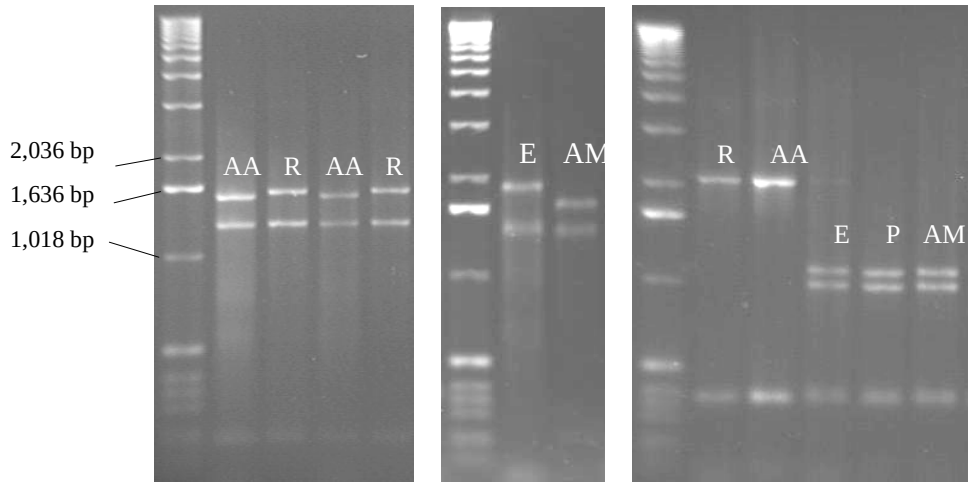
AA: *Z. albomaculata* subsp. *albomaculata*, AM: *Z. albomaculata* subsp. *macrocarpa*, E: *Z. elliotiana*, P: *Z. pentlandii*, R: *Z. rehmannii*.

Figures



a. *b.* *c.* *d.* *e.*

Fig. 1. Illustrations of the range of leaf colors expressed in different phenotypes: a. Dark green, blight green [Sampled section], c. Variegated: Mixed dark, d. Variegated: Mixed light, e. Virescent.



a. *b.* *c.*

Fig. 2. Restriction pattern of DCRon spacer using *Alu* I (a. and b.) and *Hae* III (c.) (AA: *Z. albomaculata* subsp. *albomaculata*, AM: *Z. albomaculata* subsp. *macrocarpa*, E: *Z. elliotiana*, P: *Z. pentlandii*, R: *Z. rehmannii*).