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Biparental plastid inheritance in *Zantedeschia albomaculata* (*Araceae*)

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Introduction

Section *Aestivae* of the genus *Zantedeschia*, "colored callas", consists of six species, *Z. albomaculata*, *Z. elliotiana*, *Z. jucunda*, *Z. pentlandii*, *Z. rehmannii* and *Z. valida*. *Z. albomaculata* includes two subspecies *albomaculata* and *macrocarpa*. Interspecific hybrids within section *Aestivae* may suffer from plastome-genome incompatibility (PGI), due to biparental plastid inheritance. This incompatibility is expressed in seedling leaves as albinism, variegation or virescence (see Fig. 1). In section *Aestivae* PGI is a consequence of "miscommunication" between plastids and the nuclei.

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.

Material and Methods

Plant Material:

Parental species used to produce interspecific seedlings were *Z. albomaculata* subsp. *albomaculata* (AA), *Z. albomaculata* subsp. *macrocarpa* (AM), *Z. elliotiana* (E), *Z. pentlandii* (P) and *Z. rehmannii* (R). Seedlings from the following parental combinations were analyzed: AAxAM, AMxAA, RxAM, ExAM, AAxAA, AMxAM, PxAA and AAxP. All parental accessions were obtained from the Plant Research International collection.

CAPS Markers:

Two primers were designed to amplify a 3kb 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'). 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.

Results

CAPS-variation among species:

Eight of the restriction enzymes tested showed clear band polymorphisms between species. Between sub-species *albomaculata* and *macrocarpa* six restriction enzymes showed different band patterns. *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 restriction enzymes *Hae* III and *Alu* I were chosen to variegation or virescencent (see Fig. 1). 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 (see Table 1).

Table 1. Association of plastome with dark green leaf phenotype.

Cross combination	Plastome type in dark green leaves
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*.

Plastome type was analysed in 120 dark green, light green or virescent leaf sections. Pure dark green, light green or virescent leaf sections only had one plastome type, with the exception of *Z. elliotiana* and *Z. albomaculata* subsp. *macrocarpa* seedlings, where nine out of twenty dark green samples from different seedlings had two plastome types. The other dark green samples had either the maternal or paternal plastome.

a. *b.* . *c.*

Figure 1. Illustrations of the range of leaf colors expressed in different phenotypes: a. Dark green, b.Light green [Sampled section], e.Virescent.

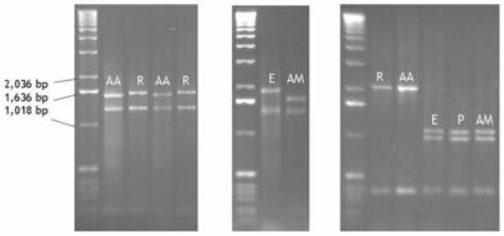

Figure 2. Restriction pattern of DCRon spacer using Alu I (a. and b.) and Hae III c.) (AA: Z. albomaculata *subsp.* albomaculata, *AM: Z.* albomaculata *subsp.* macrocarpa, *E:* Z. elliotiana, *P: Z.* pentlandii, *R: Z.* rehmannii.

Conclusions

- 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.
- Plastomes from *Z. pentlandii* and *Z. elliotiana* appeared to be closely related and are perhaps identical.

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