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IN VIVO SELF-INCOMPATIBILITY RESPONSE IN THE WILD POTATO *SOLANUM CHACOENSE* BITTER

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ABSTRACT

In tuber-bearing *Solanum* species (potatoes), *S*-locus mediated self-incompatibility is under gametophytic control. In this system, self-fertilization is avoided when the same *S*-allele is expressed in both pistil and pollen. Because the ultrastructural details of the self-incompatibility response in this group of species is unknown, the aim of this study was to identify the cellular events involved in this response by using the diploid species *S. chacoense* Bitter as a model. To this end, pollinations were carried out in two genotypic combinations previously identified as (1) compatible and (2) self-incompatible (self-pollination) for the S-locus. Pollinated pistils were fixed on an hourly basis and observed under a transmission electron microscope to detect ultrastructural changes. In the self-incompatible genotypic combination, pollen grains germinated normally but loss of electron density in the mitochondrial matrix of pollen tubes was observed at 1 hour after pollination (HAP), mitochondrial swelling started at 2 HAP and, finally, mitochondrial content was lost at 7 HAP, with the concomitant cessation of pollen tube growth in the upper third of the style. The gametophytic self-incompatibility response in potato shares similarities with a type of programmed cell death.

Key words: pollen tube mitochondria, programmed cell death, self-incompatibility, tuber-bearing *Solanum*

RESUMEN

En las especies tuberosas de *Solanum* (papas), la auto-incompatibilidad mediada por el locus *S* tiene control gametofítico. En este sistema se evita la autofecundación cuando el mismo alelo *S* se expresa en ambos pistilo y polen. Dado que se desconocen los cambios ultraestructurales de la respuesta auto-incompatible en este grupo de especies, el propósito del presente trabajo fue identificar los eventos celulares involucrados en dicha respuesta usando la especie diploide *S. chacoense* Bitter como modelo. Para tal fin, se realizaron polinizaciones controladas en dos combinaciones genotípicas previamente identificadas, respectivamente, como compatible y auto-incompatible (auto-fecundación) para el locus S. Los pistilos polinizados se fijaron en base horaria y se observaron en microscopio electrónico de transmisión para detectar cambios ultraestructurales. En la combinación auto-incompatible, los granos de polen germinaron normalmente pero se observó pérdida de densidad en la matriz de las mitocondrias de los tubos polínicos a 1 hora después de la polinización (HAP), agrandamiento de las mitocondrias a las 2 HAP y, finalmente, pérdida del contenido de las mitocondrias a las 7 HAP, con el cese concomitante del crecimiento de los tubos polínicos en el tercio superior del estilo. La respuesta de auto-incompatibilidad gametofítica en papa comparte similitudes con un tipo de muerte celular programada.

Palabras clave: mitocondrias del tubo polínico, muerte celular programada, auto-incompatibilidad, *Solanum* tuberosos

INTRODUCTION

In self-incompatible plants, self-fertilization or crossing between closely related individuals is prevented by the action of a self-incompatibility locus (or loci) under either gametophytic or sporophytic control (Frankel and Galun, 1977). This phenomenon is widely present in economically important food, feed and ornamental plants or closely related species of the same families. In sporophytic self-incompatibility, pollen phenotype for the *S*-locus is determined by the somatic genotype of the parental plant and, in incompatible pollinations, pollen tube growth stops on the stigmatic surface (e.g., *Brassicaceae*). In gametophytic self-incompatibility, pollen tube growth can stop either on the stigmatic surface (*Papaveraceae*) or in the upper third of the style (e.g., *Solanaceae*, *Rosaceae*, *Scrophulareaceae*) (de Nettancourt, 1977).

The gametophytic self-incompatibility system of *Solanaceae* and *Rosaceae* has an S-RNase-based control in which a single polymorphic *S*-locus is involved (Wang *et al*., 2003). In this system, pollen grain phenotype is gametophytically determined and fertilization is prevented when the same *S*-allele is expressed in both pollen grain and pistil. Wild and cultivated potatoes (*Solanum* spp.) belong to the *Solanaceae* family, which also includes tomatoes and eggplants. Species in this family share the same gametophytic self-incompatibility system.

There are a few reports on the ultrastructural changes underlying pollen-pistil incompatible reactions. In *Lycopersicum peruvian* Mill. (*Solanaceae*) (de Nettancourt *et al.*, 1973) the self-incompatibility phenomenon involves the destruction of the cell wall and the appearance of vesicles. Alterations in mitochondria have been described for *Papaver* spp. (*Papaveraceae*) (Geitmann *et al*., 2004), *Pyrus pyrifolia* (*Rosaceae*) (Wang *et al*., 2009), *Turnera joelii* and *T. scabra* (*Turneraceae*) (Safavian and Shore, 2010), and *Olea europaea* (*Olaceae*) (Serrano *et al.*, 2010). In these studies, self-incompatibility involved no passive reaction but a reaction closely resembling programmed cell death.

Results from Wang *et al*. (2009) in *Pyrus pyrifolia* (*Rosaceae*), using an *in vitro* test, are an important starting point to further explain incompatible reactions under natural conditions, in which not only the S-RNase is present. Nonetheless, it is necessary to confirm this explanation by carrying out assays under natural *in vivo* conditions. Furthermore, no data on the ultrastructural details of the self-incompatible reaction in tuber-bearing *Solanum* species have been published to date. Thus, the aim of the present study was to identify changes at the ultrastructural level in one *S*-locus self-incompatible genotypic combination (selfing) in the diploid wild potato species *S. chacoense* Bitter used as a model.

MATERIALS AND METHODS

Selection of genotypic combinations

Local genotypes of *S. chacoense* were grown in a glasshouse in Balcarce, Buenos Aires province, Argentina (37º45´41˝S; 58º18´41˝W). A full diallel crossing scheme was followed to identify *S*-locus compatible and incompatible genotypic combinations. To this end, flower emasculation and hand pollination were carried out on individual plants (genotypes). Pollinated pistils were removed 48 hs after pollination (HAP), fixed in FAA (40% fomaldehyde: 80º ethanol: glacial acetic acid, 1:8:1 v/v/v) and processed according to Martin (1958). Processed pistils were squashed on a glass slide and examined under an optical microscope with UV light.

Time of occurrence of incompatible reactions during the progamic phase

To determine the time at which self-incompatible pollen tube growth was arrested during the progamic phase, 36 pistils of one plant (genotype) were selfed. Three of these pistils were subsequently fixed per hour, from 1 to 12 HAP, and processed as previously described. Comparison was then made between pollen tube length at each fixation time and at 48 HAP, when fertilization in *S*-locus compatible pollinations already occurred.

Ultrastructural studies

Three pistils from each, one S-locus fully compatible genotypic combination and the selfed male parent of that combination, were prepared for observations with a transmission electron microscope (TEM). To this end, standard procedures with modifications were used (Medina *et al*., 2003). Ultrathin sections (70-90 nm) were obtained with

an ultracut microtome (Reichert, Milton Keynes, UK) using a diamond knife. Sections were then routinely mounted for staining on Formvar© coated 200 mesh copper grids. All sections were analyzed under a Zeiss-910 TEM.

Mitochondrial area was determined in at least 200 mitochondria recorded in three pistils per slide. An Analysis of Variance (ANOVA) was carried out and the Tukey test was used to detect highly significant differences (HSD) between means at the 5% level (R project 2006).

RESULTS AND DISCUSSION

Selection of genotypic combinations

Light microscopy showed differences in pollen tube length among genotypic combinations and selfings at 48 HAP. In some genotypic combinations, pollen tubes were observed to have reached the end of the style at 48 HAP. In contrast, in other combinations and invariably in selfings pollen tube growth was found to be arrested in the upper third of the style. The first group of genotypic combinations was considered to be fully compatible for the *S*-locus whereas the second group was considered to be self-incompatible for this locus because of the characteristic site of reaction. In the incompatible genotypic combinations and selfings, arrest of pollen tube growth occurred at the 7th HAP. One of the compatible genotypic combinations and the male parent of that combination were chosen for the TEM study.

Ultrastructural studies

Since pollen tube arrest in the self-pollination occurred at the 7th HAP, TEM observations were made from the 1st to the 7th HAP. Normal features of active cells were observed in compatible pollen tubes (Fig. 1a-c). In contrast, the following mitochondrial alterations were observed in self-incompatible pollen tubes from the 1st HAP onwards: i) loss of electron density in the mitochondrial matrix at 1 HAP (Fig. 1d), ii) mitochondrial swelling at 2 HAP (Fig. 1e), iii) mitochondrial content degeneration at 7 HAP (Fig. 1f). Average mitochondrial areas per fixation time are shown in Table 1.

Changes in self incompatible pollen tubes in *S. lycopersicum* were observed by de Nettancourt *et al*. (1973). In the present study, both rapid alterations in the mitochondrial area and degeneration of mitochondrial contents after pollination were observed in an incompatible genotypic combination (selfing of one genotype) of the tuber-bearing species *S. chacoense*, in comparison with one compatible genotypic combination in which the same genotype was used as the pollen donor. These changes are consistent with those observed in apoptotic programmed cell death (Häcker, 2000). Similar changes in ultrastructure and time of occurrence of the incompatible reaction were previously reported for an S-RNase system in *P. pyrifolia* by Wang *et al*. (2009); however, their study was carried out in a growth medium (*in vitro*). These changes led Wang *et al.* (2009) to conclude that the only factor affecting pollen tube growth was the S-RNase. *In vivo* assays carried out in *Turnera joelii* and *T. scabra* (Safavian and Shore, 2010) and in *Olea europaea* (Serrano *et al*., 2010; Serrano *et al*., 2012), similarly to the observed by Wang *et al.* (2009) and in the present study, showed conspicuous changes in mitochondria. Even more, apparently and independently of the self-incompatibility system, the incompatible reaction leads to a certain type of cellular programmed death. Findings from the present study complement those derived from the above-mentioned studies and provide the first *in vivo* lines of evidence of ultrastructural changes triggered by a self-incompatible reaction in a potato species.

	Pollen-pistil relationships		
	Self-Incompatible	Compatible	
Fixation time (HAP[a])	area[b] mean-sq µm- (SD)	area[b] mean-sq µm- (SD)	P[c]
1	9.19 (1.12)B	9.52 (0.99)A	0.5472
2	12.86 (2.79)A	8.91 (0.91)A	0.0004
7	11.97 (2.20)A	9.56 (0.99)A	0.005

Table 1. Mitochondrial area in self-compatible and self-incompatible genotypic combinations at various fixation times after pollination.

[a]HAP: hours after pollination; [b]over 200 mitochondria measured; [c]significance level. (SD) standard deviation. Means followed by the same letter within a column are not significantly different at 5% level according to Tukey.

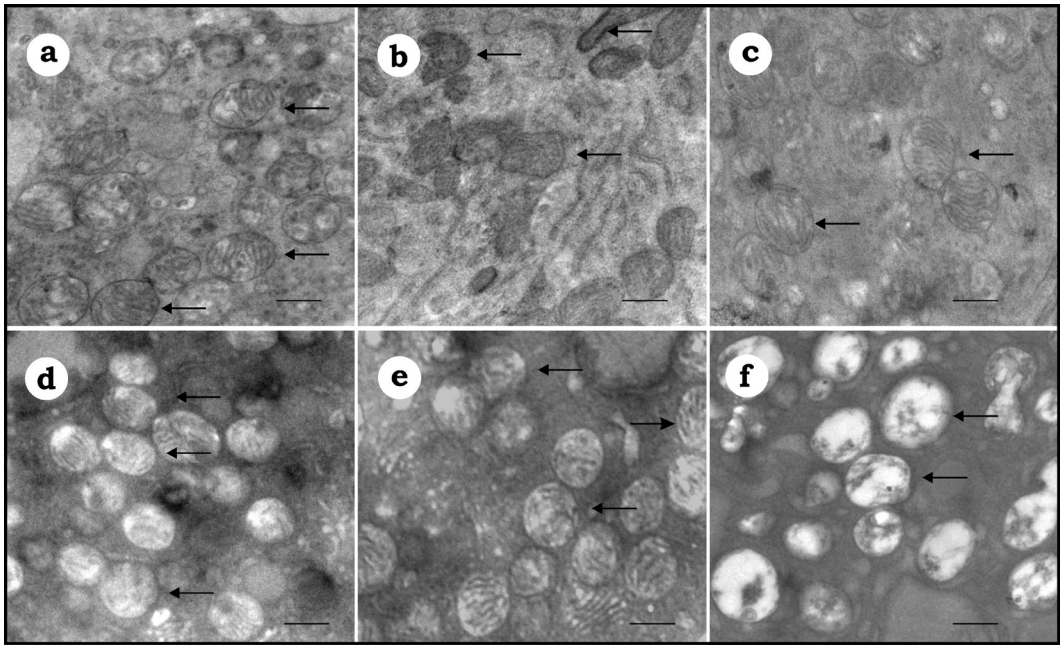

Figure 1. Mitochondrial alterations in gametophytic self-incompatible genotypic combinations (compatible=CC and incompatible= IC). At 1h after pollination (AP): (a) CC and (d) IC, presence of low electron dense mitochondrial content; at 2h AP: (b) CC and (e) IC, swelling and presence of low electron dense mitochondrial content; at 7h AP: (c) CC and (f) IC, mitochondrial content with irregular internal structure. Arrows indicate mitochondria. Bar: 0.28µm.

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