Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Physiology and Biochemistry 44 (2006) 1–6
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Research paper

Nuclear-encoded mitochondrial complex I gene expression is restored to normal levels by inhibition of unedited *ATP9* transgene expression in *Arabidopsis thaliana*

María V. Busi [a], Diego F. Gómez-Casati [a,*], Mariano Perales [a], Alejandro Araya [b,c,1], Eduardo Zabaleta [d]

[a] *IIB-INTECH, UNSAM-CONICET, Camino Circunvalación Km6, 7130 Chascomus, Argentina*
[b] *Laboratoire de Réplication et Expression des Génomes Eucaryotes et Rétroviraux (REGER) CNRS, Bordeaux 2, 146, rue Léo-Saignat, Bâtiment 3A, 33076 Bordeaux, France*
[c] *Université Victor Segalen, Bordeaux 2, 146, rue Léo-Saignat, Bâtiment 3A, 33076 Bordeaux, France*
[d] *IIB, UNMdP, Funes 3250 4to nivel, 7600 Mar del Plata, Argentina*

Received 28 July 2005
Available online 06 February 2006

Abstract

Mitochondria play an important role during sporogenesis in plants. The steady state levels of the nuclear-encoded mitochondrial complex I (nCI), *PSST, TYKY* and *NADHBP* transcripts increase in flowers of male-sterile plants with impairment of mitochondrial function generated by the expression of the unedited version of *ATP9* (*u-ATP9*). This suggests a nuclear control of *nCI* genes in response to the mitochondrial flaw. To evaluate this hypothesis, transgenic plants carrying the GUS reporter gene, under the control of the *PSST, TYKY* and *NADHBP* promoters, were constructed. We present evidence that suppression by antisense strategy of the expression of *u-ATP9* restores the normal levels of three nCI transcripts, indicating that the increase in *PSST, TYKY* and *NADHBP* in plants with a mitochondrial flaw occurs at the transcriptional level. The data presented here support the hypothesis that a mitochondrial dysfunction triggers a retrograde signaling which induce some nuclear-encoded mitochondrial genes. Moreover, these results demonstrate that this is a valuable experimental model for studying nucleus–mitochondria cross-talk events.

Keywords: Arabidopsis; Male sterility; Mitochondria; Complex I; Retrograde regulation

1. Introduction

Mitochondria are involved in different cellular processes, such as energy metabolism and respiration [10,21]. Sporogenesis, one of the most important processes in higher plants, requires a proper functioning of mitochondria. In fact, mutations in the mitochondrial genome affect the normal development of spores leading to a male-sterile phenotype [25]. However, little is known about the molecular mechanisms involved in mitochondrial pollen disruption. Previously, it has been reported that a male-sterile phenotype can be induced in tobacco and *Arabidopsis* by expressing a wheat unedited *ATP9* gene (*u-ATP9)*, fused to a gene fragment encoding a transit peptide of yeast *COXIV*. The expression of *u-ATP9* causes a mitochondrial dysfunction characterized by lower rates of respiration [9,13]. The expression of *u-ATP9* in *Arabidopsis* under the control of *CaMV35S* promoter (lines *CaMV35S::u-ATP9)* leads to abnormalities in vegetative development, while its specific floral expression when controlled the by *APETALA3 promoter* (lines *AP3::u-ATP9), specific of* petals and stamens [14] or the *A9* promoter *(lines A9::u-ATP9)* specific of tapetum [20], have little or no bearing on the major phenotypical characters, but a dramatic effect on the male reproductive organs [9]. This observation could be explained by an intense mitochondrial activity occurring in tapetal cells to support sporogenesis where any

* Corresponding author. Tel.: +54 2241 43 0323; fax: +54 2241 42 4048.
E-mail address: diego.gomezcasati@intech.gov.ar (D.F. Gómez-Casati).
[1] To whom reprints should be requested. alexandre.araya@reger.u-bordeaux2.fr (A. Araya).

0981-9428/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.plaphy.2006.01.005

mitochondrial dysfunction disrupts pollen development, and is thus one of the major energy consuming processes in the whole plant [11,27]. Because cells can monitor and respond to changes in the state of their organelles, we postulate that a mitochondrial dysfunction could be sensed by the nuclear genome thus affecting the expression of nuclear-encoded mitochondrial genes. If this idea is correct, inhibition of the deleterious *u-ATP* gene expression should restore the normal sporogenic function in male sterile plants. In the present work we report the specific restoration to fertility of the *AP3::u-ATP9* and *A9::u-ATP9* lines using *ATP9* antisense transcript (*AS-u-ATP9*) under the control of either *AP3* or *A9* promoters, respectively. This experimental model might be useful in developing a better understanding of nucleus–mitochondrial cross-talk, in particular the transcriptional events occurring during flower formation and differentiation, and also in assessing the role of mitochondria during different cellular processes such as sporogenesis. We further analyze the effect of restoring the mitochondrial dysfunction on the expression of three intrinsic subunits of *Arabidopsis thaliana* nuclear-encoded Complex I (nCI), NADH-ubiquinone oxidoreductase genes: the 55 kDa subunit NADH-binding protein (*NADHBP)* (At5g08530), *PSST* of 22 kDa (At5g11770) and *TYKY* of 28 kDa (At1g79010).

2. Results

2.1. Phenotypic analysis of plants carrying AS-u-ATP9 constructs

Previously, we reported that the expression of *u-ATP9* gene induces a mitochondrial dysfunction in *Arabidopsis* leading to a male-sterile phenotype [9]. To determine whether this phenotype was indeed the effect of *u-ATP9* expression, we constructed different *Arabidopsis* lines by transformation with recombinant pZP212 plasmids, containing the antisense version of the *u-ATP9* transgene driven by three different promoters, *CaMV35S, A9* and *AP3* (Fig. 1). Three transgenic lines, *CAMV35S::AS-u-ATP9, AP3::ASu-ATP9* and *A9::AS-u-ATP9* plants were generated after transformation with *A. tumefaciens*. The presence of the transgene in transformed plants was verified by PCR analyses and the expression of the antisense gene was determined by RT-PCR. All *AS-u-ATP9* expressing plants were fertile and the vegetative and reproductive organs were normal (size, number and shape). Pollen grains were compared to wild type plants and no significant differences were observed. *AS-u-ATP9* expression levels were determined by RT-PCR. Then, only plants showing higher levels of expression were used in crosses with male-sterile *u-ATP9* plants. The progeny of plants carrying both sense and antisense constructs (*AP3::u-ATP9 xAP3::AS-u-ATP9* and *A9::u-ATP9 xA9::AS-u-ATP9*) were identified by PCR using the primers TNOS, u-atp9 and u-atp9r (see Section 4). In hybrid plants, the *u-ATP9* transcripts were not detected after RT-PCR (36 cycles), indicating that antisense specific inhibition of *u-ATP9* expression was successful (data not shown).

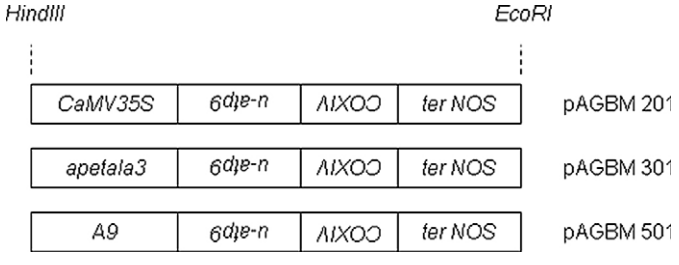

Fig. 1. Structure of the chimeric constructs of *u-ATP9* fused to *COXIV* presequence. The expression of the antisense version of *u-ATP9* was driven by *CaMV35S, A9* and *AP3* promoters (pAGBM 201, 501 and 301, respectively).

2.2. Expression of AS-u-ATP9 gene restores pollen morphology and fertility

Pollen grains from *u-ATP9* plants showed an abnormal morphology, with differences in color, shape and size, and were deficient in germination with less than 1% of viable pollen (Table 1); while *AS-u-ATP9* expressing plants showed normal levels of pollen viability. Pollen grains of *AS-u-ATP9* plants under the control of either *AP3* or *A9* promoters were used to pollinate *u-ATP9* male-sterile plants. To maximize the chance of an antisense effect, the lines used in crosses expressed the sense and antisense transgenes under control of the same promoter. To evaluate the effects of the expression of *AS-u-ATP9* transgene in *u-ATP9* plants, the morphology, and the ability of pollen grains to germinate as well as the plants' capacity for self-pollination were analyzed (Fig. 2 and Table 1). Pollen grains from hybrid lines (*AP3::u-ATP9 xAP3::AS-u-ATP9* and

Table 1
Restoration to fertility after crossing of *u-ATP9* with *AS-u-ATP9* lines

Line	Germinated pollen (%)
Wt	92 ± 4
AP3::u-ATP9	< 1
A9::u-ATP9	< 1
AP3:: u-ATP9 xAP3:: AS-u-ATP9	86 ± 5
A9::u-ATP9 xA9::AS-u-ATP9	82 ± 8

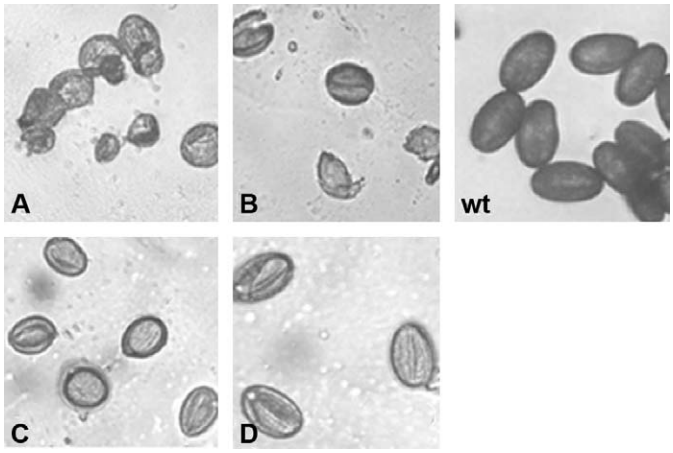

Fig. 2. Pollen grains from transgenic plants expressing *u-ATP9* under the control of *AP3*, and *A9* promoters (A and B, respectively) compared to wt. Figures C and D corresponds to pollen grains extracted from anthers of crossed lines (*AP3::u-ATP9 xAP3*::*AS-u-ATP9* and *A9::u-ATP9 xA9::AS-u-ATP9*, respectively).