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

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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.

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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...
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-ATP9 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).

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>
<thead>
<tr>
<th>Line</th>
<th>Germinated pollen (%)</th>
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<tbody>
<tr>
<td>Ap3::u-ATP9</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>A9::u-ATP9</td>
<td>82 ± 8</td>
</tr>
<tr>
<td>AP3::u-ATP9 xAP3::AS-u-ATP9</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>A9::u-ATP9 xA9::AS-u-ATP9</td>
<td>&lt;1</td>
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Fig. 1. Structure of the chimeric constructs of u-ATP9 fused to COXIV sequence. The expression of the antisense version of u-ATP9 was driven by CaMV35S, A9 and AP3 promoters (pAGBM 201, 501 and 301, respectively).