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MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF POTATO PLANTS EDITED IN THE MITOCHONDRIAL GENOME BY MITOTALEN AND MITOTALECD APPROACHES

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The lack of efficient conventional and biotechnological tools to study the direct and indirect role of mitochondrial genes involved in nuclear-cytoplasmic interactions makes the development of novel editing methods for chondriomes of higher plants highly desirable. We exploited two TALE-based approaches to produce several potato plants edited in the region of the mitochondrial genome containing *orf125*, putatively involved in male sterility of *Solanum commersonii* (+) *S. tuberosum* interspecific somatic hybrids.

A male sterile somatic hybrid was stably transformed by *Agrobacterium*-mediated transformation to constitutively express two constructs with the *Fok*I nuclease (mitoTALEN), targeting two regions of *orf125*, and two with the DddA cytidine deaminase (mitoTALECD), inducing base editing in the same gene regions. In all cases, the expressed nuclear constructs were guided into the mitochondria by using the N-terminal presequence of the *Arabidopsis* mitochondrial ATPase delta-prime subunit.

Relatively short deletions (236 – 1066 bp) were generally induced in the target region by mitoTALEN, due to the repair of the induced

double strand break (DSB) through recombination of short direct repeats (11 – 12 bp). In one case, induced DSB and subsequent repair resulted in the amplification of a substoichiometric molecule showing a 4288 bp deletion spanning the target sequence. With the mitoTALECD approach, base substitutions induced both missense and nonsense mutations, the latter leading to premature stop codons. Deletions and single nucleotide mutations were either homoplasmic or heteroplasmic, but only the former were generally retained in vegetative offspring.

Plants with physical or functional knock-out of *orf125* did not show any obvious alteration in plant growth and other vegetative traits. On the other hand, they displayed reversion to male fertility, strongly suggesting a role of *orf125* in nuclear-cytoplasmic interactions leading to male sterility in some potato interspecific hybrids. The fertility phenotype was stably maintained through vegetative tuber propagation. The involvement of *orf125* in the induction of male sterility was also confirmed by over-expression studies in which it was stably inserted into transgenic plants of a male fertile hybrid and its product expressed in the mitochondria.