TRANSFORMATION OF *BRASSICA NAPUS* WITH THE CMS-ASSOCIATED MITOCHONDRIAL GENE T-*URF*13

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ABSTRACT

Wintertype rapeseed cultivar Ceres and tobacco were transformed with the CMS-associated mitochondrial gene T-*urf*13 of CMS-T maize. For targeting the gene product to the mitochondria T-*urf*13 were linked to a presequence. In tobacco, 50 % of the transformed plants showed modified flower development, 10 % were male sterile. In rapeseed, one transgenic was yet found with modified stamen morphologies ranging from normal to sterile needle-like form.

INTRODUCTION

One of the major factors contibuting to increases in rapeseed productivity over the past few years has been the breeding of hybrid varieties (Brandle and McVetty 1989). There exist three systems for pollination control: self incopatibility alleles for preventing self pollination, nuclear and cytoplasmic male sterilty. The cytoplasmic male sterilty results from nuclear-mitochondria incompatibilities and is a maternally inherited trait. These incompatibilities are caused by interspecific and intergeneric crosses or by mitochondrial rearrangements. In some cases recombination events have been resulted in the generation of new genes specific for the male sterile cytoplasm. New open reading frames often corresponding to chimeric genes has been found in the mt DNA form cms-T maize (T-*urf13*), Petunia cybrids (S-*pcf*), *Phaseolus vulgaris* (*pvs*), *Helianthus annus* (*orf H522*) and *Brassica napus* (*orf224*, *orf138*) (for review: Vedel et al. 1994).

The application of genetic engineering for the development of pollination control systems were shown by Mariani and coworkers (1990, 1992). The authors transformed rapeseed with genes coding for RNases under the control of a tapetum specific promoter. The expression of the RNase genes induced male sterility by destruction of the tapetum cells. Although this engineered male sterilty is a Mendelian inherited trait, male sterile plants can be sorted out by the application of a herbicide. The RNase genes are linked to the *bar* gene which confers resistance to phosphinotricine.

An alternative approach for engineering male sterility might be the transformation with CMS-associated mitochondrial genes. These genes are normally expressed in all plant tissues, but their expression specifically disrupts pollen development. For using this tissue-specific effect we transformed tobacco and rapeseed with the mitochondrial T-*urf*13 gene of CMS-T maize. The T-*urf*13 gene encodes a 13 kD polypeptide (URF13) which is implicated in CMS and sensitivity to the insectizide methomyl and host-specific fungal toxins (for review: Levings and Siedow 1992). Since a direct method for transforming plant mitochondria has not been reported, we fused the T-*urf*13 gene to a presequence for targeting the nuclear gene product to the mitochondria.

EXPERIMENTAL

Transformation of tobacco and rapeseed

For the *Agrobacterium tumefaciens* mediated gene transfer into *Brassica napus* wintertype cultivar Ceres hypocotyl segments were cocultivated following a modified

protocol of De Block et al. (1989). The model plant *Nicotiana tabacum* were transformed according to standard protocols. The T-*urf*13 gene was cloned into a derivate pBI101 (Jefferson et al. 1989) under control of the 1' promoter (Houba-Herin et al. 1990;). For targeting the gene product into the mitochondria the gene were linked to the presequence of the *Nicotiana plumbaginifolia* ATP synthase beta subunit (Chaumont et al. 1993). Control transformation experiments with the *uidA* gene under control of the 35S promoter (Jefferson et al. 1989) or 1' promoter (Houba-Herrin et al. 1990) had shown that the 1' promoter revealed high ß-glucuronidase activity in all stages of microspore development of tobacco and rapeseed. In contrast, the 35S promoter showed high activity in mature pollen, but not in earlier stages of microspore development. The transformation efficiency of the wintertype cultivar Ceres was between 2 and 3%. In transformation experiments with the T-*urf*13 gene the regeneration frequency of tobacco and rapeseed was tenfold decreased. Most rapeseed shoots failed to develop roots.

Flower morphology of tobacco and rapeseed plants transformed with the T-*urf*13 gene

We could find an effect of the T-*urf*13 gene on flower development of transgenic tobacco and rapeseed plants. In tobacco, 10 % of plants transformed with the T-*urf*13 gene linked to the presequence were male sterile. The filaments of these plants were extremely reduced preventing self-pollination. Some viable pollen was formed but incapable of fertilization. Seed set could only be obtained by fertilization with pollen of control plants. The other transgenic tobacco plants were male fertile, but 40 % showed pale flower colour, delayed anther development and shortened filaments. Flower modifications were never observed in plants transformed with the *uidA* gene or with the T-*urf*13 gene without presequence. The modified phenotypes, pale flower colour, delayed anther exertion, shortened filaments were transmitted to the F_1 progenies, whilst the male sterility trait was not inherited in a stable manner.

Rapeseed plants transformed with the presequence-linked T-*urf*13 gene exhibited extreme reduction in growth and root development. So far, flower morphology of two plants could be estimated. Rapeseed flowers possess a tetradynamous arrangement of stamens that consists of four long and two short stamens. In one transgenic rapeseed plant the two shorter stamens varied in morphology. They ranged from normal to a needle-like morphology. In some cases fused stamens could be observed. Most flowers of this transgenic plant developed sterile forms of the two short stamens, but in all flowers the four long stamens exhibited normal morphology and produced fertile pollen.

More transgenic rapeseed plants have to be analyzed for estimating the effect of T-*urf*13 gene expression in rapeseed. It is well known that levels of transgene expression vary between independent transformants. Therefore, many transgenic plants must be screened for analyzing the transgene expression characteristics. The variability is usually explained by differences in transgene copy numbers and/or integration sites (Finnegan and McElroy 1994). Southern blot analysis of tobacco and rapeseed plants transformed with T-*urf*13 revealed the integration of one or two copies. In comparison to transformation experiments with the *uidA* gene, the T-*urf*13 seemed to have a negative effect on transformation efficiency, and on shoot and root growth. Similar observations were reported for tobacco plants transformed with T-*urf*13 linked to the ATPase-ß presequence under control of the 35S promoter (Boutry et al. 1992). The authors did not found a detectable amount of URF13 in mitochondria of the transgenic plants. They suggested that transformants which express T-*urf*13 at normal level were counter-selected, because URF13 has a toxic effect on tobacco when introduced into mitochondria. In contrast, CMS-T maize plants expressing T-*urf*13 are male sterile, but show only very slight differences in other morphological characters compared to plants with normal cytoplasm (Levings and Siedow 1992). The problem of counter-selection in heterologous systems might be overcome by using anther-specific promoters.

Our results demonstrate that male sterilty can be induced by transformation with a CMS-associated mitochondrial gene. Further studies will examine transformation with altered pre-existing mitochondrial genes and restoration of engineered male sterility.

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