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Citation	Plant Genetic Resources, 9(2), 284-287 https://doi.org/10.1017/S1479262111000177
Issue Date	2011-07
Doc URL	http://hdl.handle.net/2115/49479
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Type	article
File Information	PGR9-2_284-287.pdf



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Molecular basis of cytoplasmic male sterility in beets: an overview

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Abstract

Sugarbeet cultivars are almost exclusively hybrids, which are produced using the sole source of cytoplasmic male sterility (CMS), the so-called Owen CMS. Several alternative sources of CMS have been described. One of these, I-12CMS(3), was derived from wild beets collected in Pakistan, and another CMS source, GCMS, has a cytoplasmic origin in wild sea beets from France. During the past decade, male sterility-associated mitochondrial genes have been identified in these three CMS systems. Moreover, the recent development of a variety of DNA markers has permitted the genetic mapping of nuclear restorer-of-fertility genes for both Owen and GCMS. This review focuses on the mechanism of CMS in beets.

Keywords: beets; cytoplasmic male sterility; fertility restoration

Introduction

The identification of cytoplasmic male sterility (CMS) and maintaining genotypes was a major step in the success of hybrid breeding programmes of sugarbeet. The first report of CMS in this crop was made by F. V. Owen, who found male sterile plants in an old cultivar, 'US1' (Owen, 1945). He described that male sterility resulted from the interaction of two recessive nuclear genes (x and z) with the sterilizing cytoplasm and identified the maintainer plants, which had an $xxzz$ genotype and normal fertile cytoplasm. Hybrid seed production in sugarbeet has relied entirely on this single CMS source, the so-called Owen CMS (Bosemark, 2006). Up to now, additional sources of CMS have been discovered in wild beet populations (e.g. Mikami *et al.*, 1985; Halldén *et al.*, 1988; Bosemark, 1998; Touzet *et al.*, 2004) and might offer the opportunity to broaden the genetic base

of the cytoplasm. During the past decade, male sterility-associated mitochondrial genes have been reported in three CMS sources in beets. We present in this study a brief overview of recent advances in our understanding of the mechanism of CMS in beets.

Owen CMS-associated gene

Because of its importance for breeding, the molecular basis of Owen CMS has been extensively investigated. Kubo *et al.* (2000) and Satoh *et al.* (2004) determined the entire nucleotide sequences of the mitochondrial genomes from normal fertile and Owen CMS sugarbeet plants. In-depth sequence comparison of the two mitochondrial genomes, together with a mitochondrial protein assay (Yamamoto *et al.*, 2005), indicated that the 5' leader sequence of *atp6* (designated *preSatp6*) encodes a variant 39 kDa protein that is possibly related to Owen CMS (Table 1). The 39 kDa preSATP6 is closely associated with the mitochondrial membrane and assembles into an Owen CMS-specific protein complex. Interestingly, this characteristic is shared by the sterility-related proteins identified in CMS-T maize and Ogura-CMS radish: URF13 and ORF138 (Grelon *et al.*, 1994; Krishnasamy and Makaroff, 1994; Wise *et al.*, 1999).

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Table 1. CMS sources in beets

Cytoplasm	Origin	CMS phenotype	Causal gene	CMS-associated protein	Rf gene ^a
Owen	Cultivar 'US-1'	Tapetal hypertrophy; microspore degeneration	<i>preSatp6</i>	39 kDa preSATP6 in flower buds and roots	<i>Rf1</i> (III) and <i>Rf2</i> (IV)
I-12CMS(3)	Wild beets collected in Pakistan	Tapetal hypertrophy; microspore degeneration	<i>orf129</i>	12 kDa ORF129 in flower buds and roots	More than two <i>Rf</i> genes
G	Wild beets collected in France	Microspore degeneration	Truncated <i>cox2</i>	31 kDa COX2 in leaves	<i>RfG1</i> (VIII) and <i>RfG2</i>
H	Wild beets collected in France	Microspore degeneration	Not determined	Not determined	<i>RfH</i> (IV)

^aChromosomal location is given in parentheses.

Other CMS sources

A second source of CMS, called I-12CMS(3), was derived from wild beets collected in Pakistan (Mikami *et al.*, 1985). The I-12CMS(3) and Owen cytoplasm show different patterns of male sterility maintenance and male fertility restoration when crossed with the same pollen parents (Mikami *et al.*, 1985). What is worthy of special mention is that preSATP6 is missing in the sterile anthers carrying the I-12CMS(3) cytoplasm, which instead express a CMS-correlated protein of 12 kDa (Yamamoto *et al.*, 2008). This 12 kDa protein proved to be encoded by an unusual mitochondrial gene, designated *orf129* (Table 1). The translation product of *orf129* was found to be primarily present in the matrix and loosely associated with the inner mitochondrial membrane, a feature shared by the PCF protein involved in petunia CMS (Nivison *et al.*, 1994). Further implicating *orf129* with CMS is the observation that ORF129 causes pollen disruption in transgenic tobacco plants when targeted to the mitochondria (Yamamoto *et al.*, 2008).

Another interesting case is GCMS, which has a cytoplasmic origin in wild sea beets from France. Ducos *et al.* (2001) reported that GCMS is associated with a mitochondrial *cox2* gene lacking eight highly conserved, C-terminal amino acids, and that cytochrome oxidase activity is decreased by 50% in vegetative tissues (Table 1). The GCMS can be classified as a loss-of-function mutant, in contrast to Owen CMS and I-12CMS(3), which are considered to be gain-of-function mutants. Beets thus appear to maintain distinct CMS cytoplasm, each capable of conferring male sterility by an apparently different mechanism.

Microsporogenesis breakdown in Owen CMS

In Owen CMS plants, male meiosis proceeds normally until the tetrad stage, after which the microspores degenerate either during tetrad formation or immediately after microspore liberation from the tetrads. Concurrently, the anther tapetum shows marked symptoms of abnormality. The most common irregularity is extensive vacuolation and enlargement (hypertrophy) of the tapetal cells, accompanied by mitochondrial disorganization (Kaul, 1988; Majewska-Sawka *et al.*, 1993). At this stage of development, demand for energy or particular mitochondrial biosynthetic products may be markedly high, so the impairment of mitochondrial function is devastating (Budar and Berthomé, 2007). Consistent with this hypothesis is the observation that plants expressing an antisense mitochondrial pyruvate dehydrogenase subunit gene in tapetal cells are male sterile (Yui *et al.*, 2003). Male sterile transgenic plants, in common with Owen

CMS plants, exhibited poor development of tapetal mitochondria and aberrant formation of microspore walls. These results led us to speculate that the synthesis of preSATP6 protein in the tapetum of Owen CMS plants results in dysfunctional mitochondria, which in turn could cause pollen abortion.

Restorer-of-fertility (*Rf*) genes

Hagihara *et al.* (2005a) found that male fertility restoration in Owen CMS by a Japanese breeding line is controlled by a single dominant gene (designated *Rf1*). They constructed a regional map encompassing the *Rf1* locus (Hagihara *et al.*, 2005b). The mapping data also provided a clear indication of the location of the *Rf1* locus on chromosome III, and the locus most likely corresponds to the *X* gene described previously by Owen (Pillen *et al.*, 1993; Table 1). The *Rf1* locus could be further narrowed to the 250 kb region, which was delimited by two DNA markers (Hagihara *et al.*, 2005b). A second *Rf* locus (*Rf2*, *Z*) of Owen CMS has been located on chromosome IV (Schondelmaier and Jung, 1997; Hjerdin-Panagopoulos *et al.*, 2002; Table 1). In GCMS, at least two genes were reported to restore fertility, and one (*RfG1*) was mapped on chromosome VIII and was not co-located with either *Rf1* or *Rf2* (Touzet *et al.*, 2004; Table 1). This is consistent with the conclusion that the causal mechanism of male sterility in Owen CMS is different from that in GCMS.

An additional source of CMS (designated HCMS) from wild beets has been described by Boudry *et al.* (1993), but its sterilizing gene remains unclear. Genetic analysis of the fertility restoration of HCMS led to the conclusion that a single dominant gene (*RIH*) is involved (Laporte *et al.*, 1998). Intriguingly, *RIH* is located on chromosome IV and is linked to the gene for monogerm seed character (Table 1). Several authors have reported weak linkage between the monogerm locus and the *Rf2* gene associated with Owen CMS (Hogaboam, 1957; Roundy and Theurer, 1974). The question inevitably arises whether the same mitochondrial gene (*preSatp6*) is responsible for both Owen CMS and HCMS or whether these two types have a different CMS mechanism but share a common *Rf* gene. This is worthy of further investigation.

Future prospects

How does the accumulation of CMS gene products, such as preSATP6 and ORF129, in anther tissue lead to failed pollen development? This issue has hardly been addressed. As mentioned earlier, it has been shown that sexual reproduction, and pollen production in particular,

is highly sensitive to mitochondrial dysfunction. It is therefore interesting to define a range of anther-specific genes that may act downstream of *preSatp6* or *orf129* and that are responsible for aberrant biochemical and physiological processes leading to defective microsporogenesis, in response to mitochondrial dysfunction (Matsuhira *et al.*, 2007). Another important clue to the CMS mechanism will certainly come from identification of the *Rf* gene products. This research is underway in our laboratory.

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