Review

Cytoplasmic male sterility in Brassicaceae crops

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Brassicaceae crops display strong hybrid vigor, and have long been subject to F_1 hybrid breeding. Because the most reliable system of F_1 seed production is based on cytoplasmic male sterility (CMS), various types of CMS have been developed and adopted in practice to breed Brassicaceae oil seed and vegetable crops. CMS is a maternally inherited trait encoded in the mitochondrial genome, and the male sterile phenotype arises as a result of interaction of a mitochondrial CMS gene and a nuclear fertility restoring (*Rf*) gene. Therefore, CMS has been intensively investigated for gaining basic insights into molecular aspects of nuclear-mitochondrial genome interactions and for practical applications in plant breeding. Several CMS genes have been identified by molecular genetic studies, including Ogura CMS from Japanese radish, which is the most extensively studied and most widely used. In this review, we discuss Ogura CMS, and other CMS systems, and the causal mitochondrial genes for CMS. Studies on nuclear *Rf* genes and the cytoplasmic effects of alien cytoplasm on general crop performance are also reviewed. Finally, some of the unresolved questions about CMS are highlighted.

Key Words: Brassicaceae, cytoplasmic male sterility, mitochondrial gene, *Rf* gene, Ogura CMS.

Introduction

Among Brassicaceae crops, six *Brassica* species and *Raphanus sativus* are cultivated worldwide as oil seed and vegetable crops. It has long been known that these Brassicaceae crops show strong heterosis. Oil seed crops such as *B. napus* and *B. juncea* show hybrid vigor in terms of seed yield (up to 200% the parental lines; Fu *et al.* 1990, Jain *et al.* 1994). Vegetables such as cabbage (*B. oleracea*) also exhibit high heterosis (Tanaka and Niikura 2006). Thus, F_1 hybrid breeding is useful for all Brassicaceae crops.

To enable F_1 hybrid breeding, an efficient, reliable and stable method of F_1 seed production without contamination by self-fertilized seeds from each parent is vital. Because of the size and structure of Brassicaceae flowers, it is impossible to implement commercial hybrid seed production based on manual emasculation and pollination, as practiced with Cucurbitaceae crops. The self-incompatibility system, reviewed by Kitashiba and Nasrallah (2014) in this issue, was developed mainly by Japanese breeders and has been effective in F_1 breeding of vegetable Brassicaceae. However, self-incompatibility is not always stable, and may be suppressed by high temperature or drought. Furthermore, amphidiploid species *B. napus* and *B. juncea*, which are the major oil seed crops, do not express self-incompatibility.

Cytoplasmic male sterility (CMS) is another trait applicable to F_1 seed production, which is stable and applicable to all Brassicaceae crops. CMS is a maternally inherited trait encoded by a gene located in the mitochondria. In CMS plants, pollen production is disturbed, whereas the function of the female organ is not generally affected. Expression of a mitochondrial CMS gene can be masked by nuclear fertility restorer (*Rf*) genes, allowing the plant to produce functional pollen. A combination of a nuclear genome that lacks *Rf* genes and a CMS-inducing mitochondrial genome leads to nuclear-cytoplasmic incompatibility and the CMS phenotype. CMS has been reported in a large number of plant species, and is very important both from practical breeding and from the fundamental genetic and evolutionary viewpoints. CMS has been covered in several excellent reviews (Budar and Berthomé 2007, Chase 2007, Delourme and Budar 1999, Gabay-Laughnan and Newton 2012, Prakash *et al.* 2009).

In this article, we focus on CMS in Brassicaceae crops, including the latest information in this field. First, we review the history of studies on Ogura CMS. Among CMS types of Brassicaceae, the CMS discovered by Ogura (1968), which is now popularly refered to by his name, has been studied most extensively and is used worldwide in F_1 breeding of *B. napus*, *B. juncea*, *B. oleracea* and *R. sativus*. Then, we describe other CMS types and their causal genes. Finally, we discuss the studies on *Rf* genes, which are found in the nuclear genome and suppress CMS. We also consider the effects of alien cytoplasm on general crop performance.

History of research on Ogura CMS

Originally described in radish, Ogura CMS (Fig. 1) is also widely used in *B. oleracea*, *B. juncea* and *B. napus*. Ogura CMS is one of a few CMSs that have been studied in detail in terms of both their basic molecular mechanism and their practical use in breeding. The history of research on this CMS system reflects the development of breeding science in the last 50 years (Fig. 2).

Ogura CMS was discovered in Japanese radish (*R. sativus*) of an unknown cultivar (Ogura 1968). Although not well utilized in radish breeding in Japan, this CMS was introduced into European radish. Some European radishes have an *Rf* gene (Bannerot *et al.* 1977, Bonnet 1977), whereas Ogura (1968) observed that Japanese cultivars have no such gene. European scientists introduced Ogura CMS into *B. napus* by intergeneric hybridization and repeated back-crossing (Bannerot *et al.* 1974, Heyn 1976). The resultant alloplasmic lines of *B. napus* showed male sterility, but all of them had chlorotic leaves, yellowing at low temperatures (below 15°C) (Pelletier *et al.* 1983). This chlorophyll deficiency, undesirable for breeders, was suggested to result from functional incompatibility between the *B. napus* nucleus and *R. sativus* chloroplasts.

To overcome chlorosis in Ogura CMS *B. napus*, cells of an alloplasmic male sterile *B. napus* line and a normal *B. napus* variety were fused (Jarl and Bornman 1988, Menczel *et al.* 1987, Pelletier *et al.* 1983), and regenerated plants without chlorophyll deficiency but retaining the male sterility were selected. In these lines, the alloplasmic chloroplasts derived from *R. sativus* were substituted with those from *B. napus*, and the plants grew normally even at low temperature. A CMS line was thus established and used in *B. napus* hybrid breeding. A similar cell fusion approach was also applied in *B. juncea* (Kirti *et al.* 1993, 1995a).

Cell fusion led to recombinations of Ogura CMS and

Fig. 1. Ogura cytoplasmic male sterile (left) and fertile (right) flowers found in a population of Japanese wild radish. Arrows show male sterile (left) and fertile (right) anthers. Scale bar = 1 cm

B. napus mitochondrial genomes, and detailed analysis of the recombined mitochondrial genomes in these male sterile *B. napus* lines identified the causal gene of Ogura CMS (*orf138*) located upstream of *orfB* (Bonhomme *et al.* 1991, 1992, Grelon *et al.* 1994), now known as *atp8* (Heazlewood *et al.* 2003). *Orf138* was co-transcribed with *atp8* (*orfB*) in male sterile plants. At least nine nucleotide sequence variants, including one with a deletion of 39 nucleotides (Kosena type), have been reported for *orf138* (Yamagishi and Terachi 2001). Interestingly, nuclear expression of *orf138* and targeting of ORF138 protein to mitochondria failed to induce male sterility in *Arabidopsis thaliana*, although ORF 138 expression led to an alteration in mitochondria in both plant and yeast cells (Duroc *et al.* 2006). *Orf138* is widely distributed in Japanese wild radishes and another wild species of the same genus, e.g., *R. raphanistrum*, whereas most cultivated radishes have normal cytoplasm (Yamagishi and
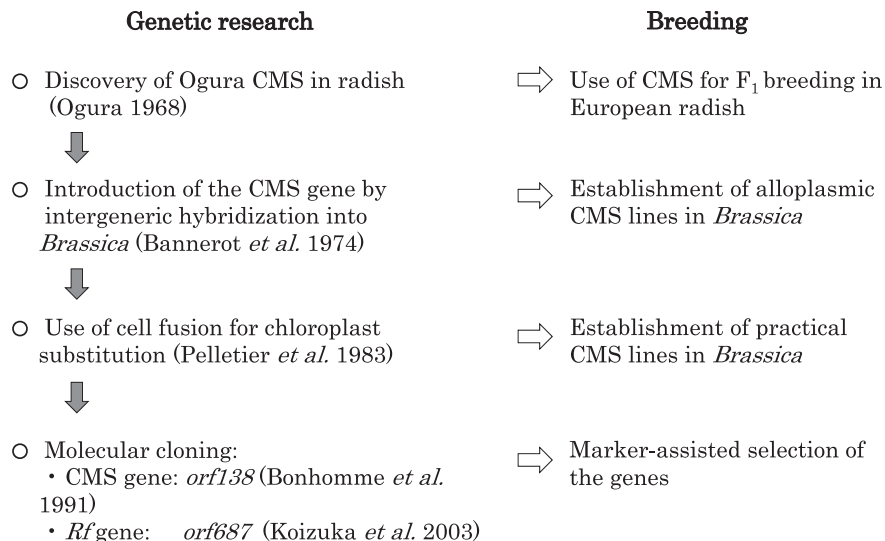
Genetic research

○ Discovery of Ogura CMS in radish (Ogura 1968)

⬇

○ Introduction of the CMS gene by intergeneric hybridization into *Brassica* (Bannerot *et al.* 1974)

⬇

○ Use of cell fusion for chloroplast substitution (Pelletier *et al.* 1983)

⬇

○ Molecular cloning:
  · CMS gene: *orf138* (Bonhomme *et al.* 1991)
  · *Rf* gene: *orf687* (Koizuka *et al.* 2003)

Breeding

⇨ Use of CMS for F₁ breeding in European radish

⇨ Establishment of alloplasmic CMS lines in *Brassica*

⇨ Establishment of practical CMS lines in *Brassica*

⇨ Marker-assisted selection of the genes

Fig. 2. History of genetic and breeding research on Ogura cytoplasmic male sterility.

Terachi 1994, 1996, 1997), indicating that Ogura CMS originated in a wild *Raphanus* species and then spread in Japanese wild radishes. In progenies of spontaneous hybridization between the wild and cultivated radish, the male sterility was expressed and discovered by Ogura. Ogura (1968) described that the male sterile plants were of an unknown variety of Japanese radish which were growing under an escaped condition. This suggests hybridization between a wild radish as a female parent and a cultivated one as a pollen parent.

Some radish lines, including European varieties possess an *Rf* gene for Ogura CMS (Yamagishi 1998), whereas none of *Brassica* species has it. An *Rf* gene is essential for F₁ hybrids of oil seed crops produced through the CMS system to set seeds properly. Therefore, the *Rf* gene (*Rfk1*) was introduced into *Brassica* from *R. sativus* cv. Kosena by protoplast fusion (Sakai *et al.* 1996). *Rfk1* was later cloned independently and almost simultaneously by three groups (Brown *et al.* 2003, Desloire *et al.* 2003, Koizuka *et al.* 2003), whereas Bett and Lydiate (2004) suggested the presence of three *Rf* genes in radish. The cloned *Rf* gene encodes a member of the pentatricopeptide repeat (PPR) family.

Origins of CMS and male sterility-inducing mitochondrial genes

CMS is expressed phenotypically when CMS-inducing mitochondrial genome is combined with a nuclear genome that lacks *Rf* genes. Such combinations can arise *de novo* by spontaneous mutation in the mitochondrial genome, outcrossing within a species, or artificial hybridization. The latter includes sexual interspecific or intergeneric crosses, and somatic cell fusions. Here, Brassicaceae CMS types are described according to their origins; (1) intraspecific variations, (2) alloplasmic origin by interspecific or intergeneric hybridizations, and (3) cell fusion.

(1) CMS caused by intraspecific variations

Polima (*pol*) CMS of *B. napus* is another well-known example of spontaneous male sterility (Fu 1981, Liu *et al.* 1987). As *pol* CMS is temperature-sensitive, its practical use in F₁ hybrid breeding is limited, but the molecular basis of this CMS has been well studied. The mitochondrial genome of *pol* CMS plants contains *orf224*, a chimeric gene located upstream of and co-transcribed with the *atp6* gene. In the presence of *Rf* gene, processing of *orf224*/*atp6* RNA is affected and the transcripts are specifically altered, suggesting that *orf224* is the causal gene of *pol* CMS (L'Homme and Brown 1993, Singh and Brown 1993). Indeed, *orf224* introduced into *B. oleracea* by somatic cell fusion induced male sterility (Wang *et al.* 1995). The structure of the protein encoded by *orf222*, which is responsible for *nap* CMS (another example of a natural mitochondrial variant within B. *napus*), is similar to that of the protein product of *orf224* (L'Homme *et al.* 1997), although *B. napus* varieties in which male sterility is induced and the position of

orf222 in the mitochondrial genome are different from *orf224* (L'Homme *et al.* 1997).

Other spontaneous CMS variants include 681A in *B. napus* originated from cv. Xiangyu (Liu *et al.* 2005) and *hau* in *B. juncea* (Wan *et al.* 2008). Maintainer and restorer lines of 681A CMS are the same as those for *pol*, but 681A lines differ from *pol* lines in their mitochondrial genome organization, suggesting that 681A might be a variant of *pol* CMS. On the other hand, restorers for *hau* cytoplasm have not been identified so far and *pol* or *nap* restorers fail to rescue *hau*-induced male sterility (Wan *et al.* 2008).

Another novel CMS gene, *orf288*, co-transcribed with *atp6* was discovered in *hau* CMS (Jing *et al.* 2012). *Orf288* was cytotoxic to *E. coli* and induced male sterility in *A. thaliana* when expressed from the nuclear genome with or without the mitochondrial targeting presequences (Jing *et al.* 2012). It was further shown that ORF288 protein was targeted to the mitochondria even when not fused with mitochondrial targeting presequence. These results indicate that *orf288* might be the causal gene of *hau* CMS.

Recent studies on *R. sativus* discovered types of CMS other than Ogura CMS. One was found in Korean radish collections and named NWB CMS (Nahm *et al.* 2005); the *Rf* genes for this CMS have not yet been found. Another one was found in a radish of Uzbekistan (Kim *et al.* 2007, Lee *et al.* 2008, 2009). A study of the complete mitochondrial genome sequence of this CMS type, designated as DCGMS, identified the causal gene as a novel chimeric gene, *orf463*, consisting of a 128-bp partial sequences of *coxI* and a 1261-bp sequence of unclear origin (Park *et al.* 2013).

Gobron *et al.* (2013) hybridized two distantly related accessions of *A. thaliana* and obtained male sterile strains whose inheritance pattern in reciprocal crosses suggested CMS. Mitochondrial genome analyses of the two accessions revealed that the causal gene of this CMS was *orf117*. The predicted ORF117 peptide is 56% identical and 69% similar to the ORF108 peptide from *Moricandia arvensis* that induces male sterility in *B. juncea* (see next section).

(2) Alloplasmic CMS as a result of interspecific or intergeneric hybridizations

Since the establishment of the CMS line of *B. rapa* with the cytoplasm of *Diplotaxis muralis* (Hinata and Konno 1979), several more male sterile lines have been developed in Brassicaceae (Table 1). In *B. juncea*, the most important oil seed crop in Southern Asia, the cytoplasms of at least eight species induce CMS (Banga *et al.* 2003, Kumar *et al.* 2012, Landgren *et al.* 1996, Malik *et al.* 1999, Pathania *et al.* 2003, Prakash and Chopra 1990, Prakash *et al.* 2001, Rao *et al.* 1994). In addition to *D. muralis*, four other cytoplasms induce CMS in *B. rapa* (Deol *et al.* 2003, Matsuzawa *et al.* 1999, Prakash and Chopra 1988, 1990, Tsutsui *et al.* 2011). Seven *B. napus* CMS lines are known, including the three (Ogura, *pol* and *nap*) mentioned above. The cytoplasm of *D. muralis* induces sterility also in *B. oleracea* (Shinada *et al.* 2006). *Erucastrum canariense* and *M. arvensis*

Table 1. Combinations of nucleus and cytoplasm in alloplasmic CMS plants

Source of the nucleus	Source of the cytoplasm
Brassica rapa	*B. oxyrrhina*[a], *Diplotaxis muralis*[a], *Enarthrocarpus lyratus*[a], *Eruca sativa*, *Moricandia arvensis*[ab]
B. oleracea	*D. muralis*[a], *Erucastrum canariense*[a], *M. arvensis*[ab]
B. juncea	*B. oxyrrhina*[a], *B. tournefortii*[a], *D. berthautii*, *D. catholica*[b], *D. erucoides*, *D. siifolia*[a], *En. lyratus*[a], *E. canariense*[a]
B. napus	*B. tournefortii*[ab], *D. muralis*[a], *D. siifolia*[a], *En. lyratus*[a]
Raphanus sativus	*B. maurorum*

[a] Confers CMS in more than one species of Brassicaceae.
[b] Also used to produce CMS plants by cell fusion (see Table 2).

Table 2. Combinations used to produce CMS plants by cell fusion

Recipient	Cytoplasm donor
Brassica rapa	*Raphanus sativus*[a]
B. oleracea	*Arabidopsis thaliana*
B. juncea	*Diplotaxis catholica*[a], *Moricandia arvensis*[a], *Trachystoma ballii*
B. napus	*A. thaliana*, *B. tournefortii*[a], *Orychophragmus violaceus*, *Sinapis arvensis*

[a] Also used to produce alloplasmic CMS plants by sexual hybridization (see Table 1 and the main text).

cytoplasms were recently reported to confer CMS in *B. oleracea* (Chamola *et al.* 2013a). A CMS line of *R. sativus* with the cytoplasm of *B. maurorum* was produced (Bang *et al.* 2011). As shown in Table 1, five species, *B. oxyrrhina*, *B. tournefortii*, *D. muralis*, *D. siifolia* and *Enarthrocarpus lyratus*, are effective as donors of male sterile cytoplasm for more than one crop species. The cytoplasm of *D. muralis* induces male sterility in *B. rapa*, *B. oleracea* and *B. napus*, whereas *En. lyratus* cytoplasm confers CMS in *B. rapa*, *B. juncea* and *B. napus*. CMS was also obtained in *B. juncea* as a result of interspecific hybridization between *B. rapa* and *B. juncea*; this CMS was later transferred to leaf mustard (*B. juncea* var. *multiceps*) (Yang *et al.* 2005), tuber mustard (*B. juncea* var. *tumida*) (Zhang *et al.* 2003) and stem mustard (*B. juncea*) (Yang *et al.* 2009). Male sterility in *B. juncea* CMS line was found to be associated with a novel gene, *orf220*, which shares 79 homology with *orf222* (*nap*) and 81% with *orf224* (*pol*) of *B. napus* (Yang *et al.* 2005).

Three cytoplasms (*D. berthautii*, *D. catholica* and *D. erucoides*) that confer CMS to *B. juncea* commonly possess *orf108* upstream of *atpA*, and the two genes are co-transcribed. Expression analyses suggested *orf108* as the causal gene in these CMS sources (Kumar *et al.* 2012). *Orf108* was also found in male sterile *B. juncea* obtained by cell fusion between *B. juncea* and *M. arvensis*. The CMS *B. juncea* line derived from the somatic hybridization with *M. arvensis* carried the unaltered mitochondrial genome from *M. arvensis* in which *orf108* is present (Prakash *et al.* 1998). The finding is the first demonstration of a common molecular mechanism underlying CMS in lines of different origin (Ashutosh *et al.* 2008, Kumar *et al.* 2012). Apart from the above species, *orf108* was also found in other wild species including *B. oxyrrhina*, *Sinapis alba*, *En. lyratus* and *D. tenuisilque*, which suggests its wide distribution. However, *orf108* was absent in *D. muralis*, *D. siifolia* and *D. cretasia*. Therefore, it appears that *orf108* is of ancestral origin but is lost or modified in some lineages. Recent

finding in *A. thaliana* of CMS-associated *orf117* with 69% homology to *orf108* (Gobron *et al.* 2013) further supports this view. *Orf263* was inferred to be the causal gene in the CMS lines of *B. napus* and *B. juncea* with the cytoplasm of *B. tournefortii* (Landgren *et al.* 1996). In the mitochondria of *D. muralis*, which induces CMS in three *Brassica* species (Table 1), a novel chimeric gene, *orf72*, located downstream of *rps7* and containing a fragment of *atp9*, was identified. Comparison of gene expression and pollen fertility demonstrated the association of the *orf72* with CMS (Shinada *et al.* 2006).

(3) CMS plants obtained by cell fusion

In contrast to sexual hybridization, cell fusion does not require fertilization, and thus a much wider range of hybridization species can be used. Wild species not used in interspecific and intergeneric sexual hybridization have been used for cell fusion to produce male sterile lines (Table 2). In addition to *B. tournefortii*, *D. catholica*, *M. arvensis* and *R. sativus*, which have been used as cytoplasm donors in sexual hybridization, *A. thaliana*, *Orychophragmus violaceus*, *Sinapis arvensis* and *Trachystoma ballii* cytoplasms were transferred into *B. napus*, *B. juncea* or *B. oleracea* by somatic cell fusion to obtain CMS (Forsberg *et al.* 1994, 1998a, 1998b, Hu *et al.* 2004, Kirti *et al.* 1993, 1995a, 1995b, 1995c, Mei *et al.* 2003, Yamagishi *et al.* 2002, 2008, Yamagishi and Nakagawa 2004). Whereas alloplasmic CMS lines obtained by sexual hybridization inherit the mitochondrial genome of the female parent, somatic hybridization can give rise to novel recombined mitochondrial genomes derived from both parental species, which complicates identification of CMS causal genes. Hitherto, two genes have been identified that demonstrate contrasting features in relation to the CMS genes of alloplasmic lines.

The progeny of somatic hybrid between *B. juncea* and *M. arvensis* showing CMS had chloroplast and mitochondrial genomes identical to those of *M. arvensis* without recombination (Prakash *et al.* 1998). This CMS line had *orf108* upstream of *atpA* as the causal gene, similarly to alloplasmic plants obtained by sexual hybridization. The progeny of a somatic hybrid between *B. napus* and *B. tournefortii* (Stiewe and Röbbelen 1994) had *orf193* upstream of *atp9* (Dieterich *et al.* 2003), whereas in the

alloplasmic lines of *B. napus* with the cytoplasm of *B. tournefortii* (as described in the previous section), *orf263* was the causal CMS gene. Thus, with the same parental combination, cell fusion and sexual hybridization resulted in different CMS genes, *orf193* and *orf263*.

An improvement of a male-sterile line in *B. juncea* due to mitochondrial recombination has been reported. Plants with Ogura cytoplasm and *B. juncea* nucleus had flowers with petaloid anthers and poor female fertility. A fully female-fertile, anther-sterile CMS line was obtained through somatic hybridization (Kirti *et al.* 1995a).

A variation in the mitochondrial *coxI-2* gene was suggested to cause CMS in a cell fusion between *B. juncea* and *D. catholica* (Pathania *et al.* 2007), although this has not been definitely proven. A recombined mitochondrial genome was found in *B. napus* CMS lines derived from somatic hybridization with *A. thaliana* (Carlsson *et al.* 2007, Leino *et al.* 2003, 2004, Teixeira *et al.* 2005). The association of fertility restoration with markers on chromosome 3 of *A. thaliana* suggested that the male sterility genes are also derived from *A. thaliana* (Leino *et al.* 2004).

CMS lines are characterized by comparing the mitochondrial genome organization and gene expression in male sterile, maintainer and fertility restored lines. The association of a mitochondrial gene with CMS is inferred when the gene is expressed only in the male sterile plant but is suppressed in the fertility restored plant carrying a nuclear *Rf* gene. Table 3 lists the CMS causal genes in Brassicaceae crops identified so far. Twelve genes have been cloned from ten species of original cytoplasm donors. Among the genes, eight have a chimeric structure consisting fragments of known mitochondrial gene and sequences of unknown origin.

Rf genes for the CMS

When CMS system is applied for F_1 hybrid breeding of oil seed crops (*B. juncea* and *B. napus*), the F_1 hybrid should be male fertile to ensure proper seed set. Therefore, the discovery of *Rf* genes capable of conferring male fertility to CMS plants is critical for exploiting CMS. *Rf* genes for

intraspecific cytoplasmic variants are usually found among the germplasm lines of that species. For alloplasmic CMS lines derived from sexual or somatic hybridization, *Rf* genes are not generally found in the recipient species and need to be introgressed from species used as cytoplasm donors. *Rf* genes have been successfully transferred from other species into cultivated *Brassica* species for the CMS-inducing cytoplasms such as Ogura (Heyn 1976), *T. ballii* (Kirti *et al.* 1997), *M. arvensis* (Kirti *et al.* 1998), *Er. canariense* (Prakash *et al.* 2001), *En. lyratus* (Banga *et al.* 2003) and *B. tournefortii* (Janeja *et al.* 2003). Surprisingly, an *Rf* gene of *M. arvensis* restores fertility of three other cytoplasms, namely, *D. catholica*, *D. erucoides* and *D. berthautii* (Bhat *et al.* 2005, 2006, 2008). Fertility restoration in most *Brassica* CMS lines is sporophytic (i.e. F_1 hybrid plants are 100% pollen-fertile). However, in CMS lines of *M. arvensis*, *D. erucoides* and *D. berthautii*, fertility restoration is gametophytic (Bhat *et al.* 2005), i.e., only *Rf* gene-carrying pollen is functional and thus F_1 hybrid plants are 50% pollen-fertile (Fig. 3). Hence, the self progeny of gametophytically restored plants do not segregate for male sterility and fertility.

The relationships between mitochondrial CMS genes and their counterpart *Rf* genes are interesting from the point of view of studying co-evolution of mitochondrial and nuclear genomes. Such studies have been conducted for *pol* and *nap* CMS of *B. napus*, alloplasmic CMS lines of *B. juncea* and the Ogura CMS. As mentioned above, the proteins encoded by *pol* CMS and *nap* CMS genes have similar structure. The *Rf* genes for *nap* (*Rfn*) and *pol* (*Rfp*) are allelic (Li *et al.* 1998), and both affect mRNA processing of the CMS genes by inducing the removal of sequences from the 5′-ends of the transcripts (Brown 1999, Geddy *et al.* 2005, Menassa *et al.* 1999). *Rf* genes of different origin have a common function in alloplasmic *B. juncea*. As stated above, in *B. juncea*, four CMS lines have a common CMS gene (*orf108*, in Table 3), and share a common restorer gene. In the presence of the restorer, the bi-cistronic *orf108-atpA* transcript is cleaved just downstream of the start codon of *orf108*, leading to the production of monocistronic *atpA* transcript

Table 3. CMS genes identified in the mitochondria of Brassicaceae crops

Gene	Normal gene compounding *orf*	Adjacent gene	Origin	Recipient
orf138	None	*atp8*	*R. sativus* (Ogura)	*R. sativus*, *Brassica* species
orf125	None	*atp8*	*R. sativus* (Kosena)	*R. sativus, Brassica* species
orf224	*atp8, rps3*	*atp6*	*B. napus* (*pol*)	*B. napus*
orf222	*atp8*	*nad5c, orf139*	*B. napus* (*nap*)	*B. napus*
orf463	*coxI*	None	*R. sativus* (DCGMS)	*R. sativus*
orf117	None	None	*A. thaliana*	*A. thaliana*
orf72	*atp9*	*rsp7*	*D. muralis*	*B. oleracea*
orf108	None	*atpA*	*D. erucoides*, *D. berthautii*, *D. catholica*, *M. arvensis* (by cell fusion)	*B. juncea*
orf263	*nad5*	*atp6*	*B. tournefortii*	*B. napus, B. juncea*
orf193	*atp6*	*apt9 -2*	*B. tournefortii* (by cell fusion)	*B. napus*
orf220	*atp8, rps3*	*atpA*	*B. rapa*	*B. juncea*
orf288	*nad5*	*atp6*	*B. rapa*	*B. juncea*

○ ; Fertile pollen
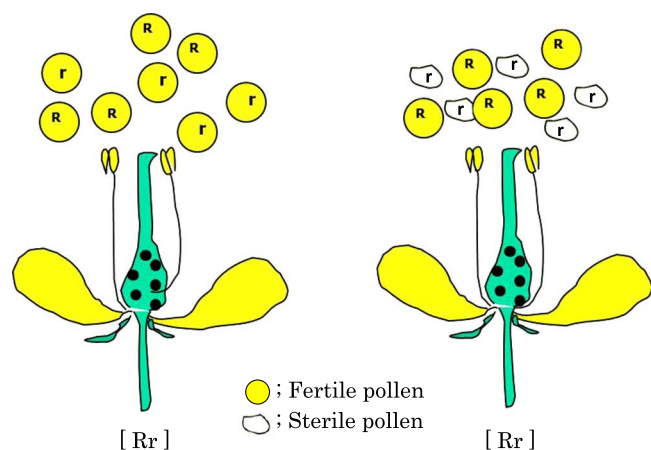◌ ; Sterile pollen

[Rr]   [Rr]

Fig. 3. Sporophytic (left) and gametophytic (right) modes of pollen fertility restoration. R; Functional restorer allele, r; Non-functional restorer allele.

(Ashutosh *et al.* 2008, Kumar *et al.* 2012). These findings are noteworthy, as they indicate that the common genetic mechanisms of CMS and fertility restoration have evolved and are shared by different species and genera.

For Ogura CMS, the *Rf* gene, *orf687* (Brown *et al.* 2003, Desloire *et al.* 2003, Koizuka *et al.* 2003), does not affect transcription of the *orf138*, but regulates *orf138* mRNA translation. In Japanese wild radishes, where *Rf* genes for Ogura CMS are common, the frequency of plants with *orf687* is small (Yasumoto *et al.* 2008). Another *Rf* gene (*Rft*), found in most Japanese wild radishes, regulates *orf138* mRNA processing (Yasumoto *et al.* 2009). This finding indicates that *Rf* genes with different molecular mechanisms have evolved in radish to suppress the expression of *orf138*, the causal gene of Ogura CMS.

Cytoplasmic effects, retrograde regulation and polymorphic floral phenotypes

Availability of alien CMS systems in Brassicaceae crops raises the question of adverse effects associated with alien cytoplasm, as deployment of CMS for commercial hybrid development would require absence of any such effects, which could be due to plastid or mitochondrial genomes. The negative effect of incompatible plastids causing leaf chlorosis in CMS *B. rapa*, *B. juncea* or *B. napus* lines with Ogura, *B. oxyrrhina*, *M. arvensis* cytoplasm, and chloroplast substitution as an approach to overcome this problem, have already been discussed above (Kirti *et al.* 1993, 1995a). CMS lines of *B. juncea* carrying Ogura, *D. catholica* or *T. ballii* cytoplasm show poor female fertility (Chamola *et al.* 2013b, Pathania *et al.* 2003). In most cases, only those mitochondrial genes that are directly associated with male sterility have been studied. Although the expression patterns of many other mitochondrial genes are also altered in the CMS lines, the effects of such changes and the role of *Rf* genes in correcting other defects have rarely been investi-

gated. Recently, Chamola *et al.* (2013b) compared in detail the effects of alien cytoplasms in *B. juncea*. In the *T. balli*-based CMS system, a restorer gene could completely reverse the negative effect of cytoplasm on seed yield and yield-contributing traits, whereas in *D. catholica*-based CMS, a restorer gene was only partially effective. Another effect that could come into play in alien CMS systems is the linkage drag associated with the introgressed *Rf* gene. Introgression of a restorer gene for Ogura CMS from *R. sativus* into *B. napus* brought along an unwanted gene responsible for seed glucosinolate synthesis, and it took considerable effort and time to break this linkage (Delourme *et al.* 1995, Primard-Brisset *et al.* 2005).

Although CMS has been attributed to specific mitochondrial genes, diverse floral phenotypes induced by the same cytoplasm in different species have not been fully investigated. For example, although *orf108* causes CMS in *B. juncea* lines carrying *D. catholica*, *D. berthautii*, *D. erucoides* or *M. arvensis* mitochondria, and all these CMS lines are restored by a common restorer (Bhat *et al.* 2005, 2006, 2008), *D. catholica* cytoplasm leads to a petaloid anther, multilocular silique, and poor female fertility whereas others result only in gametophytic pollen sterility. Ogura cytoplasm leads to petaloid anthers in *B. juncea* but sterile anthers in *B. napus*. The fact that the same fertility restorer gene from *R. sativus* restores male fertility in CMS lines of *B. napus* and *B. juncea* carrying Ogura cytoplasm indicates that *orf138* causes male sterility in both the cases. Therefore, how *orf138* expression in *B. juncea* leads to petaloid anther remains unresolved. Yang *et al.* (2008) have shown that CMS phenotypes could be partially mimicked by treating plants with chemical inhibitors of mitochondrial electron transport. Therefore, polymorphic phenotypes are likely to result from differences in the energy status of cells due to expression of CMS-inducing mitochondrial genes in different nuclear backgrounds. For instance, *B. napus* CMS lines carrying a recombined *A. thaliana* mitochondrial genome exhibited altered sugar metabolism (Teixeira *et al.* 2005) and showed retrograde regulation of floral homeotic genes *AP3* and *PI* (Carlsson *et al.* 2007). Similar retrograde regulation of *AP3*, *PI* and *AG* was also reported in CMS lines of stem mustard displaying petaloid anthers (Yang *et al.* 2008). Retrograde regulation of *BjRCE1* gene in CMS *B. juncea* led to altered auxin response (Yang *et al.* 2012) which would in turn affect multiple traits. Differences in miRNA expression between CMS and maintainer lines of *B. juncea* also indicate that retrograde regulation leads to various changes (Yang *et al.* 2013). Recently, Dong *et al.* (2013) compared the floral transcriptomes of Ogura CMS Chinese cabbage (*B. rapa* ssp. *pekinensis*) and the maintainer line, and found more than 100 differentially expressed genes. Thus, traits besides male sterility could be affected in CMS lines, and future studies should focus on global transcriptome changes in male sterile and fertility restored lines to assess the effects of CMS on agronomic performance.

Although novel mitochondrial *orfs* causing male sterility

have been discovered, their precise mode of evolution and significance beyond male sterility has not been critically examined. For example, in corn, the Texas CMS also makes plants susceptible to Southern corn leaf blight pathogen *Cochliobolus heterostrophus* (Miller and Koeppe 1971) and this adverse effect is not rectified by the restorer gene. Likewise, molecular details of processing of CMS-associated transcripts or proteins by the nuclear *Rf* genes remain to be elucidated in the Brasicaceae crops.

Studies discussed above suggest that CMS in lines of different origin may have common molecular mechanisms. A similar resemblance may also be found among *Rf* genes. On the other hand, multiple ways of fertility restoration through evolution of different *Rf* genes (as in the case of Ogura CMS) are also evident. Further detailed and integrated studies are expected to clarify the evolutionary relationships of CMS and *Rf* genes. Such studies would also contribute to establishing efficient F$_1$ hybrid breeding systems in Brasicaceae crops.

Acknowledgement

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