Cytoplasmic male sterility in Brassicaceae crops

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Brassicaceae crops display strong hybrid vigor, and have long been subject to F1 hybrid breeding. Because the most reliable system of F1 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, F1 hybrid breeding is useful for all Brassicaceae crops.

To enable F1 hybrid breeding, an efficient, reliable and stable method of F1 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 F1 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 F1 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 referred to by his name, has been studied most extensively and is used worldwide in F1 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 *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 ORF138 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
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 (Nam 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 coxl 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 (Bangal 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...
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Table 1. Combinations of nucleus and cytoplasm in alloplasmic CMS plants

| Source of the nucleus | Source of the cytoplasm |
|----------------------|-------------------------|
| Brassica rapa        | B. oxyrrhina*, Diplotaxis muralis*, Enarthrocarpus lyra*, Era sativa, Moricandia arvensisab |
| B. oleracea          | D. muralis*, Erucastrum canariense*, M. arvensisab |
| B. juncea            | B. oxyrrhina*, B. tournefortii*, D. berthaultii, D. catholica*, D. erucoides, D. siifolia*, En. lyra*, E. canariense* |
| B. napus             | B. tournefortii**, D. muralis*, D. siifolia*, En. lyra* |
| Raphanus sativus     | B. maurorum |

* Also confers CMS in more than one species of Brassicaceae.
** Also used to produce CMS plants by cell fusion (see Table 2).

Cytoplasm donors 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 lyra, 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. lyra* 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 cytoplasmats (D. berthaultii, 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. lyra* and D. tenuisilique, 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, Kiriti et al. 1993, 1995a, 1995b, 1995c, Mei et al. 2003, Yamagishi et al. 2002, 2008, Yamagishi and Nagakawa 2004). Whereas alloplasmic CMS lines obtained by sexual hybridization inherit the mitochondrial genome of the female parent, somatic hybridization can give rise to novel recombinant 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

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

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

* Also used to produce alloplasmic CMS plants by sexual hybridization (see Table 1 and the main text).
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 *cox1*-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. lyraus* (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 | 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 | coxl | None | R. sativus (DCGMS) | R. sativus |
| orf117 | None | None | A. thaliana | A. thaliana |
| orf72 | atp9 | rps7 | 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 | atp9-2 | B. tournefortii (by cell fusion) | B. napus |
| orf220 | atp8, rps3 | atpA | B. rapa | B. juncea |
| orf288 | nad5 | atp6 | B. rapa | B. juncea |
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(Ashtosh 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. oleracea, 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. ballii-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. berthautilii, 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 Koepp 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 Brassicaceae 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 Brassicaceae crops.

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