

Male sterility in apiaceae vegetables: advances, mechanisms, and prospects

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Abstract

Apiaceae vegetables, such as carrot, celery, water dropwort, and fennel, typically possess small, bisexual flowers with asynchronous development. These characteristics pose significant challenges to hybrid seed production. The development and application of male sterility are crucial for successful hybrid breeding and seed production in Apiaceae vegetables. To date, male sterile lines have been identified in carrot, celery, fennel, and water dropwort, facilitating the advancement and application of hybrid breeding in this family. This review summarizes the types, genetic mechanisms, and associated genes of male sterility in Apiaceae vegetables and discusses future directions for research and application in this field. It also provides a reference for the utilization of male sterility materials in Apiaceae vegetables in breeding and research.

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Introduction

Male sterility is a genetic phenomenon in which plants do not produce viable or fertile pollen, which is ubiquitous in nature^[1]. The discovery of male sterile materials has played a breakthrough role in the cross-breeding of various crops, and has been widely used in the production of various crops^[2,3]. Until now, only four types of Apiaceae vegetables have been found to create male sterility: carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), water dropwort (*Oenanthe javanica* (Bl.) DC.), and fennel (*Foeniculum vulgare* Mill.). Apiaceae vegetables have small flowers, hermaphroditic, low self-fertility compatibility, which makes their breeding research and other related work have slow progress^[4]. The discovery and application of male sterility are crucial for resolving the bottleneck problem in the breeding of plants of the Apiaceae family^[5].

Classification of Apiaceae

The Apiaceae family is divided into four main subfamilies: Centella, Azorella, Eryngium, and Apioideae, with more than 400 genera and nearly 4,000 species of plants^[6]. The Apiaceae family in China comprises over 90 genera and more than 1,500 species, including over 60 genera and more than 200 species with medicinal value^[7]. It contains *Pleurospermum* Hoffm.^[8], *Hydrocotyle sibthorpioides* Lam.^[9], *Angelica* L.^[10], *Pimpinella* L.^[11], *Chamaesium* H. Wolff^[12], *Bupleurum* L.^[13,14], *Ostericum* Hoffm.^[15], *Heracleum* L.^[16], *Ferula* L.^[17], *Centella* L.^[18,19], *Cryptotaenia* DC.^[20]. Among them, Apioideae encompassed a variety of significant vegetable crops, such as carrot (*D. carota*)^[21], parsley (*Petroselinum crispum* L.)^[22], celery (*A. graveolens*)^[23], coriander (*Coriandrum sativum* L.)^[24], water dropwort (*O. javanica*)^[25,26], fennel (*F. vulgare*)^[27], etc.

Most plants in the Apiaceae family possess dual medicinal and edible properties, which are inseparable from their secondary metabolite components. Some of these important medicinal and aromatic plants, such as *Angelica dahurica* L., *Peucedanum praeruptorum* L., cumin (*Cuminum cyminum* L.), and *Angelica sinensis* L., etc.^[4] have certain effects on regulating human health.

Discovery of male sterility in Apiaceae vegetables

Morphological characteristics of male sterility flowers

The flowers of Apiaceae plants are flat-topped umbel, usually bisexual, small, numerous, with low self-fertilization compatibility, and have inconsistent flowering periods, which include five petals, five sepals, and an enlarged disc combined by two styles (pistil)^[28–31]. Flowers act as one of the important reproductive organs of plants, whose sterility characteristics are primarily manifested in their morphological features^[32], such as the color of flowers, the structure of floral organs, the number of honey glands^[33,34], and so on.

The anthers or filaments of male sterility plants were abnormal (such as small floral organs, short filaments, thin anthers, etc.), which led to pollen sterility or the formation of debility pollen, thereby preventing self-pollination and resulting in sterility. However, their pistil could secrete a large amount of nectar, enabling them to accept foreign pollen for successful pollination, with a relatively high seed-setting rate^[28,35]. The main male sterility types of Apiaceae vegetables are shown in Fig. 1.

Among them, the research on the flowers of male sterility in carrot was more, earlier, and deeper^[36,37]. There are two types of

male sterility in carrot: petaloid-type (anthers and filaments transform into sepals or petals or succulent, pt) cytoplasmic male sterile (pt-CMS, Fig. 1a–c) and brown-anther male sterility (Fig. 1d). (1) pt-CMS refers to a condition where stamens transform into petal-like structures (also called 'ps-CMS'), resulting in sterility^[38,39]. According to the degree of petalization, it can be divided into four types: filamentous, spoon, incomplete petalization, and complete petalization. In addition, there are other types, such as 'snakelike', 'liguliform', 'carpellate', polystyle of twining, stylotwining/twin style, loose closure of nectary hole, and nectary atrophy, and so on^[40–42]. The petalized stamens are green, white, light green, and so on^[43,44]. (2) Brown-anther male sterility refers to a condition where pollen development is initially normal, but the anthers later turn brown and become deformed (with filaments essentially degenerated), leading to premature pollen degeneration and sterility. However, the structure of its nectaries, pistil, and petals is consistent with that of normal flowers^[45,46].

The cause of male sterility

In higher plants, male sterility is mainly caused by abnormal self-condition and changes of environmental conditions^[47–51]. The male sterility of Apiaceae plants was mainly caused by their own factors. The details are as follows: (1) Alteration or degeneration of their stamens, as seen in carrot^[41,43], celery^[52], fennel^[53], etc. (2) Abnormal development of microspores and the tapetum cells, exemplified by carrot (brown-anther male sterility)^[54], celery^[35,55,56], ferula^[57], etc. (3) Abnormal activity of ATPase, disorder of active oxygen metabolism *in vivo*, accumulation of free proline and malondialdehyde, etc.; the representative plant was the carrot^[58,59], celery^[60], and so on.

Environmental conditions, especially temperature and light, could enable the conversion of male sterility to fertility within specific ranges^[61], such as carrot (brown-anther male sterility)^[45,62]. So it is

not suitable or applicable for breeding research due to the instability of its infertility, which led to a scarcity of research in crops^[63].

Identification of male sterile materials in Apiaceae vegetables

Carrot

In the middle of the 20th century, many researchers gradually discovered the male sterility materials of carrot^[36]. However, research on male sterility in carrots commenced relatively late in China^[45]. The pt-CMS carrot material (named 'Wuye-BY') was obtained from the wild carrot materials in Wuhan, whose chromosome number was $2n = 18$, and had no obviously enlarged fleshy root^[64]. In the hybridization offspring, the pt-CMS with hairless seeds of carrot (named 'Wuye-MG') was obtained^[65].

Celery

In the late 20th century, male sterility materials of celery were discovered abroad. In China, a total of four cases of male sterility celery had been found, namely '01-3A'^[66,67], 'MS1'^[55], 'W99A'^[68], and 'QCBU-001'^[52] (Fig. 1e).

Water dropwort

Research on the male sterility of water dropwort has also been relatively late. So far, only three cases of male sterility materials have been found, of which two cases were *Oenanthe thomsonii* C. B. Clarke (named 'OtBY001'^[50] and 'OtBY002'^[69]) and one case was *Oenanthe javanica* (Blume) DC. (named 'OJB001')^[51]. The male sterility type of water dropwort includes no-pollen (Fig. 1f), succulent (Fig. 1g), and pollen abnormality (Fig. 1h). The characteristics of fertile water dropwort were filament elongation and anther exsertion (Fig. 1i), while the characteristics of male sterility water dropwort styles (pistil) were elongation (Fig. 1j).

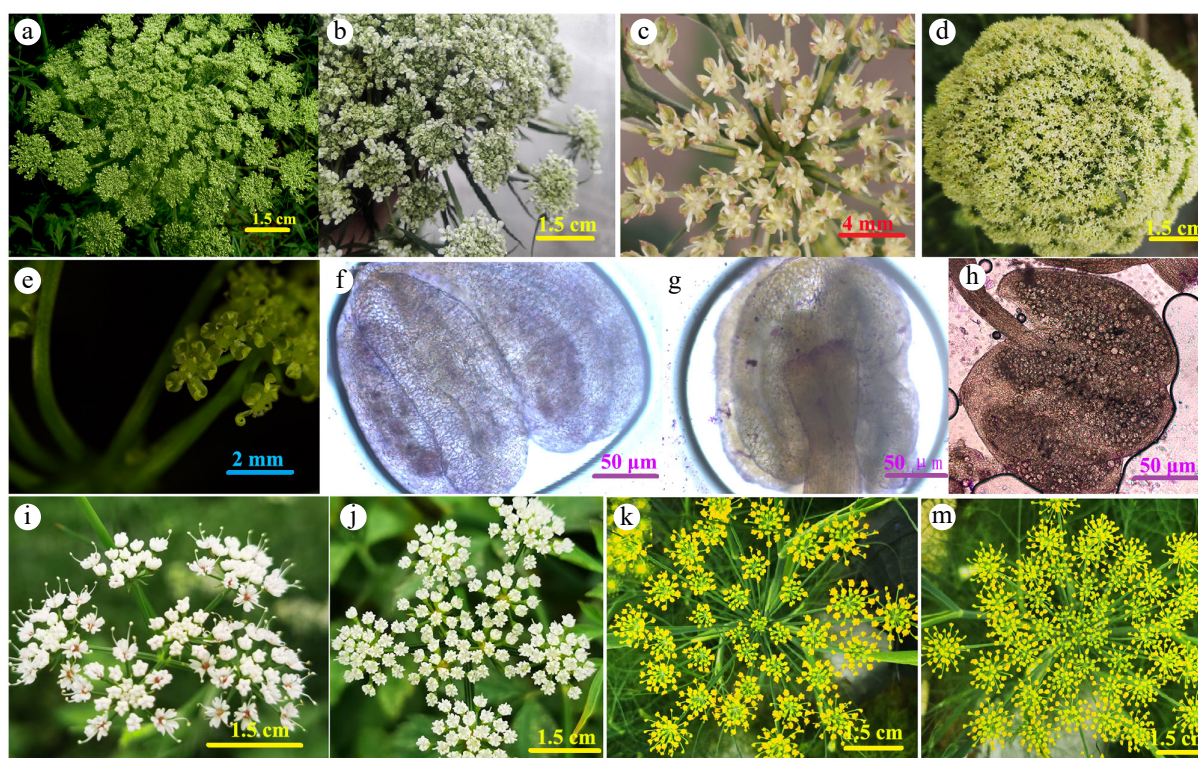


Fig. 1 The type of male sterility in Apiaceae vegetables. (a) Carrot, sepaloidy. (b) Carrot, petaloidy. (c) Carrot, succulent. (d) Carrot, brown-anther. (e) Celery, succulent (no-pollen type has been found). (f) Water dropwort, no-pollen. (g) Water dropwort, succulent. (h) Water dropwort, pollen abnormality. (i) The flower characteristics of fertile water dropwort. (j) The flower characteristics of male sterility water dropwort. (k) The flower characteristics of fertile fennel. (m) The flower characteristics of male sterility fennel.

Fennel

Sterile materials of bulbous fennel have been discovered by Palumbo et al.^[70]. The research group used fertile seed fennel (Fig. 1k) to successfully create male-sterile materials (named 'FVGZKY001', Fig. 1m), whose filaments did not extend significantly and had no pollen grains in anthers^[53].

The utilization of male sterility in Apiaceae vegetables

According to the differences of genes causing male sterility, male sterility was divided into two types, including genetic male sterility (GMS) and cytoplasmic male sterility (CMS). CMS has been increasingly reported and utilized in plants for its maternal inheritance sterility, genetic stability, such as food crops^[71,72], vegetable crops^[5,73–75], and so on. The discovery of these sterility materials could eliminate the need for artificial emasculation, save manpower, reduce the cost of seed production, and ensure the purity of the seeds. This is crucial for the cultivation and creation of a large number of high-quality hybrid varieties, and it plays a significant role in hybrid breeding and production^[1,48]. The discovery of male sterility materials in the Apiaceae family provides an effective solution for creating novel hybrid breeding materials in small-flowered crops, whose cross-utilization has significantly accelerated the development of hybrid breeding programs.

Cross-utilization

Carrot

The research on the utilization of crossbreeding for male sterility in carrots has been conducted for a long time^[76–79]. Over 95% of the commercial carrot varieties in foreign countries are hybrid varieties bred through male sterility techniques^[80,81].

Ghemeray et al. conducted a hybridization combination using ten pt-CMS (petaloid-type cytoplasmic male sterile) lines and ten self-fertilizing lines rich in antioxidants for generating 100 testcross progenies, finding that four sterility lines ('KT-28A', 'Kt-62A', 'KT-80A', and 'KT-95A') had a good binding ability to antioxidants, and 'KT-98A × KS-50' had been identified as the best heterotic combiner for CUPRAC (cupric ion reducing antioxidant capacity) and FRAP (ferric reducing ability of plasma) content^[82]. Janani et al. had developed 60 F₁ hybrids by crossing 15 testers and four petaloid CMS lines, and evaluated their vegetative and economic traits. The results showed that most of the yield-related traits (as root weight, root length, and the pith part) were mainly influenced by non-additive gene action, and had the highest heterosis percentage (33%)^[83]. Huang et al. developed a new red carrot F₁ hybrid 'Jingyu 189' by crossing inbred line 'P666F' as male parent and CMS line 'P633A' as female parent, which was suitable for most regions in China in summer-autumn and the North of China in spring^[84]. Meanwhile, a new purple carrot F₁ hybrid 'Ziyu 199' was developed by crossing inbred line '738F' as male parent, and CMS line 'P27A' as female parent, which was suitable for most regions in China and was resistant to immature bolting^[85]. Li et al. developed a new carrot F₁ hybrid 'Wanshen No. 8' by crossing the inbred line 'P133C' as male parent and the CMS line 'P34A' as female parent, which was suitable for field cultivation in the northern of Anhui Province and the adjacent region^[86].

Celery

The researchers had bred CMS line '0863A' and GMS line '01-3AB' from CMS '01-3A' in celery, and developed new hybrid varieties 'Jinqi 1', 'Jinqi 2' and 'Jinrui 1' by '01-3AB', which had entered the initial adoption phase in commercial production^[35,66,67,87,88]. Liu et al. carried out hybridization using 35 male sterile, maintainer, and 16 inbred lines of celery, and created 158 single cross hybrids and 29

three cross hybrids. The results showed that three cross hybrids had a positive advantage rate (41.4%) and a super standards advantage (86.2%) with single plant weight positively exceeding their parents^[89]. He et al. developed a new celery hybrid 'Jinrui 76' by CMS line 'Y811A' as female parent and inbred line 'Y1002' as male parent, which showed moderate resistance to celery early blight and was suitable for cultivation all over the country^[90].

Fennel

Scariolo et al. used three parental lines, including one CMS line, one maintainer line, and one paternal line for breeding, and obtained F₁ hybrid progenies with high levels of heterozygosity and hybrid vigor. This evaluated the genetic structure of breeding populations of fennel by codominant molecular markers, and they also observed that a trend in hybrid heterozygosity was increased when the genetic similarity decreased between the respective parental lines^[91].

Cultivation of heterozygous sterile lines

Plant protoplast fusion can produce cell hybrids and obtain new varieties. However, protoplast asymmetric fusion reduces the occurrence frequency of incompatibility in fusion hybrids due to the introduction of fewer exogenous genes, making it more likely to obtain the target traits and thus being the most widely applied in crops.

Among the Apiaceae vegetables, carrots were commonly used as the acceptor^[92–94], while celery was a minority^[95], both of them have successfully integrated the carrot pt-CMS gene, and then developed new germplasm of CMS. Bruznican et al. used celeriac as the acceptor and carrot as the donor for protoplast asymmetric fusion experiments, and detected the presence of the carrot specific *atp1* marker in plants after fusion events, opening the path for the isolation of novel CMS lines of celeriac^[96].

Genes of male sterility in Apiaceae vegetables

The male sterility of different crops is controlled by different genes, including mitochondrial genes, nuclear genes, or genes involved in the interaction between the nucleus and the cytoplasm. The differences related to CMS are mainly reflected in the types of open reading frames (ORF) of the genes^[97,98]. At present, four species (carrots, celery, watercress, and fennel) of male sterility materials have been discovered and identified in Apiaceae vegetables, whose sterility genes also have been discovered (Table 1).

Carrot

The research suggested that the brown pollen male sterility and CMS of carrots were associated with its unique patterns of mitochondrial gene rearrangements, transcripts, and proteins^[99–101]. The pt-CMS carrot is controlled by specific nuclear genes^[42,102]. The plants exhibited normal flower morphology by carrying normal N-cytoplasm, while causing transformation of stamens into petal-like (Sp) organs by carrying S_p-cytoplasm^[103]. Some studies have also shown that the petal differentiation of pt-CMS carrot was determined by the interaction between cytoplasm and nuclear factors^[38].

The sterility trait of carrots is mainly controlled by mitochondrial genes^[104,105], such as *orfB*^[106,107], *atp6*^[108–110], *atp8*^[111,112], *atp9*^[41,103,113,114], *cox1*^[46], *MADS*^[39], and so on. Szklarczyk et al. had found that a 130/172 bp sequence repeat existed in the *atp9* loci, whose ORFs were C to U edited in four nucleotide positions in the S_p-cytoplasm carrot. Meanwhile, the level of the respective mRNAs and content of their protein was approximately three times higher than in N-cytoplasmic (exhibiting normal flower morphology)^[103]. Petaloid abnormalities intensified with increasing distance from the *atp9*. Notably, *atp9* mRNA expression in Sp-cytoplasm was threefold

Table 1. The main male sterile materials of Apiaceae vegetables.

Species	Name	Discovery time	The type of male sterile	Sterility gene	Research institutions	Ref.
Carrot	Tendersweet	1945	Brown-anther type	Unknown	U. S. Department of Agriculture, Charleston, Smth Carolina, etc.	[36]
	Imperator	1962	Brown-anther type	Unknown	Chinese Academy of Agricultural Sciences	[45]
	Swoden	1966	Petaloid type	Unknown	United States Department of Agriculture and the Idaho Agricultural Experiment Station	[46]
	Wisconsin	1970	Petaloid type	Unknown	Department of Horticulture and Forestry, University of Arkansas, Fayetteville	[46,99]
	A8901, A8902, A8903, A8904	1996	Petaloid type	Unknown	Vegetable Institute of Inner Mongolia Academy of Agricultural Sciences	[46,100]
	Slendero	1987	Petaloid type	Unknown	DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland	[100]
	New kuroda	1997	Brown-anther type	Unknown	Jiangnan University	[46]
	Wuye-BY	2016	Petaloid type	<i>atp6</i>	College of Horticulture, Nanjing Agricultural University	[64]
Celery	MS1	1986	No pollen in anthers	Nuclear recessive gene	University of California	[55]
	01-3A	2002	No pollen in anthers	Nuclear recessive gene	Tianjin Agriculture Academy	[66]
	W99A	2005	Succulence of filaments and anthers	<i>orf768a</i>	College of Horticulture, China Agricultural University	[67]
	QCBU-001	2018	Succulence of filaments and anthers	<i>cox 1</i>	Institute of Horticulture, Guizhou Academy of Agricultural Sciences	[52]
Fennel	CMS of <i>F. vulgare</i>	2020	No pollen in anthers (Bulb fennel)	<i>atp6</i>	University of Padova	[70]
	FvGZBY001	2019	No pollen in anthers (Seed fennel)	Unknown	Institute of Horticulture, Guizhou Academy of Agricultural Sciences	[53]
Water dropwort	OJBY001	2019	No pollen in anthers	Unknown	Institute of Horticulture, Guizhou Academy of Agricultural Sciences	[51]
	OtBY001	2018	Lack of pollen vitality	Unknown	Institute of Horticulture, Guizhou Academy of Agricultural Sciences	[50]
	OtBY002	2021	Succulence of filaments and anthers	<i>ccmFN</i>	Institute of Horticulture, Guizhou Academy of Agricultural Sciences	[69]

higher than in N-cytoplasm, while ATP content increased in Sp-cytoplasm but decreased in N-cytoplasm^[103].

Celery

Studies had shown that the sterility of celery variety '01-3A' was controlled by a pair of recessive genes in the cell nucleus^[66], which was consistent with the previous research results^[55], while celery variety 'W99A'^[68] and 'QCBU-001'^[52] were controlled by the mitochondrial *cox1* gene. Furthermore, the mitochondrial *atp6* gene may be related to the development of celery flowers^[115].

Water dropwort

Studies had shown that the sterility of water dropwort 'OJBY001' might be maternally inherited, while 'OtBY001' might be a recessive trait controlled by a single gene^[50]. The sterility of water dropwort 'OtBY002' was caused by a mutation in the mitochondrial *ccmFN* gene^[69].

Fennel

Research on male sterility is relatively scarce in fennel. Palumbo et al. found that male sterility in fennel was associated with the deletion of a 300 bp fragment of the mitochondrial *atp6* gene^[70]. Based on previous studies, there are many genes that cause crop sterility, such as *cox2*, *cox3*, *cob*, *atpA*, *atp1*, *MS4/MS5*, *BY1949/2562*, *nad6*, *nad4*, *nad3*^[1,2,5,48]. In other Apiaceae vegetables, which genes control their male sterility still require further study and determination (Fig. 2).

Research of restorer fertility in Apiaceae vegetables

The nuclear-cytoplasmic interaction sterility type belongs to CMS, whose sterility is controlled by the interaction between cytoplas-

mic genes and nuclear genes, and only when these two types of genes coexist and interact, can male sterility be manifested^[115,116]. Currently, the majority of genes of the restoration of fertility (*Rf*) identified in crops belong to the genes of the *PPR* (pentatricopeptide repeat) family, such as rice^[117,118], *Brassica napus*^[119], and soybean^[120]. It is generally believed that the expression of CMS is jointly regulated by the mitochondrial gene *atp6* and its corresponding *Rf* gene, and this has been confirmed in relevant crops, such as rice^[121], *B. napus*^[122], maize^[123], and so on. The *PPR* protein is a large protein family in plants and has been distributed in over 3,000 species^[124]. Most *PPR* proteins are located in mitochondria or chloroplasts, while a small number are located in the cell nucleus. They regulated the growth and development of plants as well as their defense responses to adverse environmental stresses^[125], and also regulated pollen development^[116]. However, there is limited research on *PPR* and fertility restoration in Apiaceae vegetables at present. The research found that the brown pollen grains CMS and restorer in carrots were both controlled by two pairs of dominant complementary nuclear genes for the restoration of fertility^[99]. The carrot pt-CMS line originated from wild materials^[126], whose sterility was caused by the result of nuclear and cytoplasmic interaction^[127], and was controlled by two genes, and existed one or two fertility-restoring loci^[33]. Then a restorer line was also discovered from the pt-CMS materials, where the restorer gene was controlled by a nuclear gene^[33,128]. Nothnagel et al. demonstrated that the male sterility of different pt-CMS carrots was caused by the result of nuclear-polymer interaction through genetic analysis^[127]. Alessandro et al. found that the carrot pt-CMS was controlled by the *Vrn1* gene located on chromosome 2 with flanking marker at a range of 0.70–0.46 cm, and its *Rf1* was located on chromosome 9 with the flanking marker at a range of 4.38–1.12 cm^[129].

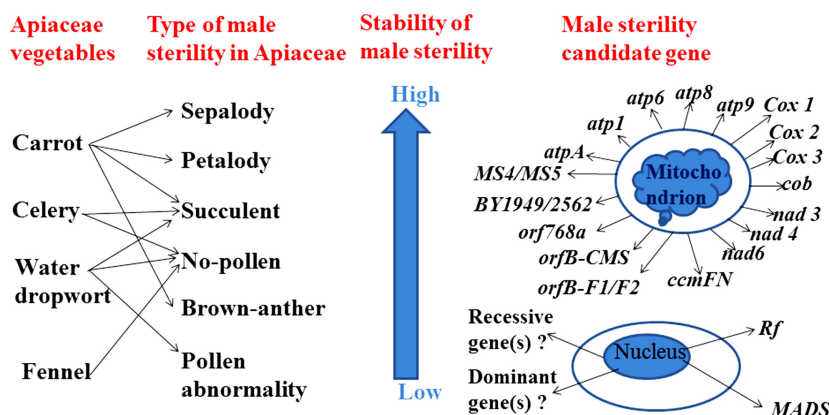


Fig. 2 Summary of research progress on Apiaceae vegetables.

At present, only two nuclear genes (*Rf* and *MADS*) have been identified to be related to male sterility^[48,63,116], while other recessive gene(s) and dominant gene(s) in other Apiaceae vegetables and other crops are still not clear (Fig. 2).

Genomics research on male sterility in Apiaceae vegetables

In recent years, omics technologies such as proteomics, metabolomics and transcriptomics have been increasingly applied to the research on male sterility in various crops^[130], such as wheat^[131], cabbage^[132], onion^[133], rice^[134], pepper^[135], pea^[136] and so on. Moreover, its gradual application in Apiaceae crops has played a highly significant role in mining male sterility genes and characterizing their functions.

Genomics

By analyzing the genetic diversity of the mitochondrial, chloroplast genome, and nuclear genomes of carrots, it is speculated that the CMS lines of carrots mainly originated from their wild species^[137,138], and their genomes had different evolutionary paths and unique fragment structure^[98,139].

Tan et al. conducted a mitochondrial genome analysis on the male sterile material of celery 'QCBU-001', and the results showed that sequencing of its mitochondrial genome was 260,872 bp, containing 21 tRNAs, seven rRNAs, and 52 mRNAs^[52]. Cheng et al. conducted mitochondrial genome analysis of a cytoplasmic male sterile (CMS) line and its maintainer line in celery, and the research identified 21 unique regions containing 15 open reading frames (ORFs) specifically in the CMS line. Among these, only *orf768a* was characterized as a chimeric gene. This gene consisted of a full-length 1,497 bp *cox1* sequence fused to an 810 bp novel sequence located within a unique region. Computational prediction indicated that the *orf768a* protein product possessed 11 transmembrane domains^[67].

Tan et al. analyzed mitochondrial genome sequencing of the water dropwort sterile line 'OtBY002' and the fertile line 'OtKY002', and the results showed that 'OtBY002' and 'OtKY002' both contained a large loop mitochondrial genome (with 336,668 and 336,731 bp, respectively) and a small loop mitochondrial genome (with 48,051 bp), and contained 75 genes. The length of the *ccmFN* gene sequence was significantly different between 'OtBY002' and 'OtKY002', with 1,050 and 1,740 bp, respectively. The mutation of the *ccmFN* gene at 921 bp was the cause of infertility in 'OtBY002'^[68]. Palumbo et al. had assembled the mitochondrial genomes (mtDNA) both fertile and sterile genotypes of fennel, the lengths of mtDNA are 166,124 and 296,483 bp, respectively; and identified two *atp6* sequences (*atp6⁻* and *atp6⁺*), *atp6⁻* being found only in the mtDNA

of CMS with a 300 bp truncation at the 5'-end while *atp6⁺* been detected only in male fertile sample mtDNA^[69].

Transcriptomics

Ou et al. conducted a DGEs (differentially expressed genes) analysis on the flowering process of carrots, identifying a total of 45 genes related to flowering, which were mainly in the aspects of photoperiod, vernalization, autonomy, gibberellin pathways, and flower integration^[140]. Liu et al. conducted transcriptome analysis between its maintainer line (P2M) and the pt-CMS line (P2S) at four flower developmental stages (T1–T4), finding that a total of 2,838 genes were differentially expressed, among which 1,343 and 1,459 genes were significantly upregulated and downregulated in the CMS line, respectively. Functional analysis showed that most of the DEGs were involved in biosynthesis, protein processing in the endoplasmic reticulum, and plant hormone signal transduction. The low expression of *DcAG* and *DcPI* genes associated with oxidative phosphorylation might inhibit the expression of *MADS-box* genes at stage T2 in P2S, while the upregulated expression of *MADS-box* genes and *HSP* (heat shock protein) genes might make some fertile revertants at stages T3 and T4 in P2S^[141].

Wang et al. identified a total of 1,255 DGEs and 89 DEPs (differentially expressed proteins) between the CMS line W99A and its maintainer line in celery by proteomic and transcriptomic analyses, of which 25 genes were differentially expressed at both the protein and transcript levels; they also identified ten DGEs involved in the outer pollen wall and fleece layer development by GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses, most of which were downregulated in the CMS line W99A^[142].

Discussion and outlook

Three-line hybrid breeding in Apiaceae vegetables

Improving the efficiency of three-line (restorer, sterile, and maintainer lines) hybrid breeding is an effective approach to resolving the genetic basis of fertility restoration for CMS, which is particularly evident in carrot breeding^[100]. The CMS-restorer system is a useful tool to exploit heterosis in crops. Song et al. had compared transcripts of CMS-D2 and CMS-D8 systems, which were linked with fertility restorer genes *Rf₁* or *Rf₂* in upland cotton, supposing that *Ghir_D05G032450* and *Ghir_D05G035690* could be the candidate genes related to restorer gene *Rf₁* and *Rf₂*^[143]. Wang et al. had cloned the gene of *Rf-m*, the major restorer gene for the M-type CMS, which consisted of seven PPR candidate genes and contained eight to 14 PPR motifs in soybean. The phylogenetic analysis of seven GmPPR proteins showed that proteins in the same subfamilies cluster^[119]. Wang et al. had identified a new form of CMS (*hau*

CMS) associated with the mitochondrial transcript *orf288* in *B. napus*, and found that a candidate gene encoding a mitochondria-localized PPR protein successfully restored the fertility when it was identified and transferred into the *hau* CMS line^[120]. Zhao et al. had found that *MSH1* (*MutS homolog 1*) mediated fertility reversion via the CMS-associated mitochondrial *ORF220*, which had been a response to physiological signals, and suggested that this nuclear-mitochondrial interplay influences fertility reversion in CMS plants in *Brassica juncea*^[144]. However, the genetic determinants of male fertility restoration in Apiaceae vegetables are still largely unknown, and there are still no materials available, including in carrot and celery, which seriously restricts hybrid breeding in Apiaceae vegetables.

Intensifying the cultivation and research of male sterile materials in other Apiaceae vegetables

As discussed previously, male sterile materials have been discovered in carrots, celery, fennel, and water dropwort, and have been gradually being widely utilized in breeding in the world. However, there have been no research reports on male sterile materials for other Apiaceae vegetables, such as parsley (*P. crispum*), coriander (*C. sativum*), dill (*Anethum graveolens* L.), cumin (*C. cyminum*), etc., which seriously restricts the hybrid breeding and variety protection of these Apiaceae vegetables. At present, some gene technologies have been applied and studied in Apiaceae vegetables, such as gene assembly technology in carrot^[145], transgenic and subcellular localization technology in celery^[146,147], and protein interaction technology in *C. japonica*^[148]. Next, gene editing technology, mutagenesis technology, cell biotechnology, and other methods will be used to screen and obtain the required male sterile materials to meet the market demand for excellent vegetable seeds in the Apiaceae family. Meanwhile, this study will be combined with the existing multi-omics measurement results, and further studies on the selected candidate genes will be conducted using relevant molecular biology techniques, further enriching research on male sterility in Apiaceae vegetables.

Conclusions

The Apiaceae family contains many global vegetables, and the creation, cultivation, and utilization of male sterility are key to achieving hybrid breeding. This paper systematically summarizes the current research status of Apiaceae vegetables (Fig. 2). Various methods will be used to create male sterile materials and apply them to breeding and promoting the development of hybrid breeding for Apiaceae vegetables in the future.

Author contributions

The authors confirm contributions to the paper as follows: data collection: Tan GF, Zhong XL, Zhu SH, Li MY, Xiong AS, Ma J, Zhang J, Luo Q; draft manuscript preparation: Tan GF, Luo Q; final manuscript approval: Tan GF, Zhang J, Li MY, Luo Q, Zhang J, Xiong AS. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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