Genic male and female sterility in vegetable crops

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Abstract

Vegetable crops are greatly appreciated for their beneficial nutritional and health components. Hybrid seeds are widely used in vegetable crops for advantages such as high yield and improved resistance, which require the participation of male (stamen) and female (pistil) reproductive organs. Male- or female-sterile plants are commonly used for production of hybrid seeds or seedless fruits in vegetables. In this review we will focus on the types of genic male sterility and factors affecting female fertility, summarize typical gene function and research progress related to reproductive organ identity and sporophyte and gametophyte development in vegetable crops [mainly tomato (Solanum lycopersicum) and cucumber (Cucumis sativus)], and discuss the research trends and application perspectives of the sterile trait in vegetable breeding and hybrid production, in order to provide a reference for fertility-related germplasm innovation.

Introduction

Vegetable crops are of essential importance to the human diet, not only because of their enrichment in nutritional substances such as vitamins, polysaccharides, and minerals, but also due to their high content of beneficial health components, including fiber and bioactive compounds [1]. With the rapid growth of global populations and living demands, creating vegetable varieties with high yield and superior quality is one of the urgent tasks to be undertaken by vegetable breeders in the coming decades.

Vegetable crops are generally annual herbaceous plants that predominantly propagate by seeds. Seeds are developed from embryo sacs after double fertilization—the unique mode of sexual reproduction in flowering plants. During this process, one sperm fertilizes the egg and the other sperm fuses with the central cell, which results in the formation of the embryo and endosperm, respectively [2]. Two specialized floral organs are indispensable for double fertilization: the stamen, which produces pollen, and the pistil, which bears ovules. Defects in stamen/pollen or pistil/ovules will attenuate to varying degrees male or female fertility, respectively. In vegetable crops, the morphology of reproductive organs displays great diversification. In tomato (Solanaceae) flowers, anthers join tightly with each other to form an anther cone, which encloses the inner pistil, consisting of a stigma, a long style, and an ovary (Fig. 1a–c) [3]. Cucumber (Cucurbitaceae) cultivars mostly produce unisexual flowers: the male flower produces anthers and the female flower with inferior ovary gives rise to the fruit (Fig. 1d and e) [4]. In lettuce (Asteraceae), which is a typical self-pollinating vegetable, 25–30 flowers cluster to form a capitulate inflorescence that is tightly packed by bracts (Fig. 1f and g). In each flower, anthers are fused to form the tubular androecium, a pistil formed from two carpels is located in innermost whorl, and the elongated style emerges from the tubular androecium to facilitate pollination (Fig. 1h) [5]. Flowers in baby bokchoy (Brassicaceae) are similar to those of Arabidopsis, with an inferior superior ovary surrounded by six stamens (Fig. 1i–k).

Hybrid seeds are widely utilized in the creation of new vegetable varieties with high yield, earliness, and growth vigor, and improved resistance due to heterosis [6]. In most vegetable crops, the global share of hybrid seed production is increasing by 8%–10% per year [7]. However, a large proportion of vegetable crops (except Cucurbitaceae) produce morphologically tiny flowers that have strictly closed pollination (e.g. Brassicaceae, Asteraceae). Creation of male-sterile plants can save the cost of artificial emasculation and thus was commonly applied in hybrid seed production in vegetable crops such as tomato (Solanum lycopersicum) [8], pepper (Capsicum annuum) [9] and Chinese cabbage (Brassica rapa ssp. pekinensis) [10]. Intriguingly, some fruit vegetables, such as tomato (Fig. 1l and m) and cucumber (Cucumis sativus) (Fig. 1n and o), possess parthenocarpic ability (development of a fruit without fertilization) and thus can produce seedless fruits. Seeds generally serve as the source of hormones triggering fruit senescence, and thus the seedless trait is expected to extend the shelf life of the fruit [11]. Studies showed that seedless tomatoes contain more dry matter (except cellulose) and soluble solids than seed cultivars [12]. In cucumber, seedless pickling gherkin is more attractive for consumers for its crunchy, firmer, and fleshier taste than the seeded variety [13]. Taken together, seedless fruit, partially derived from female sterility, is a valuable trait in fruit vegetables, not only for producers, but also for consumers [14].

Therefore, deep understanding of male and female sterility regulation is of significant importance for effective utilization of sterile plants for hybrid seed and seedless fruit production in vegetable crops. This review will focus on genic male and/or female sterility-related mutants and the underlying genes in...
Male sterility

Stamens are composed of filaments and anthers, and the latter contain diploid cells that undergo meiosis to form haploid microspores that subsequently differentiate into the male gametophytes, also known as pollen grains [15]. Male-sterile mutants include plants defective in anther morphology, microsporogenesis, pollen development, and pollen function [16]. Correspondingly, male sterility is classified into structural, sporogenous, and functional types [8].

Structural male sterility

Structural male sterility is caused by defects in stamen morphology. Mutants of this type display deformed stamens with rare pollen production [8]. Genes involved in this process are mostly stamen identity genes, such as B-, C- and E-class floral patterning genes. According to the ABC model in Arabidopsis, the stamen is controlled by the B-class [AP3 (APETALA 3) and PI (PISTILLATA)] and C-class [AG (AGAMOUS)] genes [17, 18]. E-class SEP (SEPALATA) genes act as co-factors with B- and C- homotic genes in specifying stamen primordia [19]. Therefore, mutations in B-, C-, or E-class genes will result in deformed stamens in vegetable crops.

In tomato, TAP3 and TM6 are the homologs of the Arabidopsis B-class AP3 gene. The expression pattern of TAP3, TM6, and other identity genes is indicated in Fig. 2a. Homeotic transformations of petals and stamens upon mutation of TAP3/TM6 led to defective stamens with no pollen [21–23]. Similarly, TM6 RNA interference (RNAi) plants showed carpelloid stamens [22]. In 2019, TM6 was revealed to be the candidate gene for tomato male sterile15(26/47) mutants [24]. As for the other B-class gene PI, there are also two homologs in tomato: SIGLO1/TPIB and SIGLO2/TPI. Stamens of single SIGLO-RNAi lines were weakly affected, while double RNAi mutants displayed complete homeotic conversion of stamens into additional carpel-like organs and thus loss of male fertility [25]. Later, SIGLO2 was demonstrated to be the candidate gene of natural male-sterile mutants sl-2 and 7b-1 [26, 27]. TM5 is one of the E-class SEP genes in tomato, and RNAi of TM5 led to a series of morphologic changes of floral organs, including an altered number of organs per whorl, defective petals and styles, and sterile anthers and carpels [28]. TM29 is another tomato SEP gene, and the RNAi mutants exhibited infertile stamens and ovaries, causing parthenocarpic fruit [29]. In cucumber, three ethylene biosynthesis-related genes, F (Female: CsACS1G) [30, 31], M (Monoecious: CsACS2) [32], and A (Androecious: CsACS11), specify the bisexual floral bud developing into unisexual flowers, and ethylene was shown to promote female flower development [33]. CUM26 (CUCUMBER MADS 26) is the homolog of PI in cucumber (Fig. 2b). The cum26 mutants, also called green petals (gp), displayed two perianth whorls of sepals in both male and female flowers.
Table 1. Summary of gene information mentioned in this article.

| Gene Ortholog | Function(s) | Phenotype(s) mainly in reproductive organs | Genetic modification |
|---------------|-------------|------------------------------------------|---------------------|
| TAP3/STAMENLESS | MADS-box transcription factor: petal and stamen identity | Carpeloid stamens | Insertion/Pr, SNP/LA0269: chromosomal rearrangement in promoter |
| TM6/SEP15(26/24) | MADS-box transcription factor: stamen identity | Disorganized stamen development | RNAi/SNP(12.7 kb deletion including promoter and first four exons) |
| SlGLO1/TPIB | MADS-box transcription factor: petal and stamen identity | Disorganized stamen development | RNAi |
| SlGLO2/TPI | MADS-box transcription factor: petal and stamen identity | Disorganized stamen development | RNAi |
| CUM26 | MADS-box transcription factor: petal and stamen identity | Homologous conversion and distinct phenotype in male and female flowers | 15 amino acids deletion |
| SpAP3 | MADS-box transcription factor: stamen identity and sex determination | Homeotic transformations of stamens into carpels in male flowers | RNAi |
| SpPI | MADS-box transcription factor: stamen identity and sex determination | Homeotic transformations of stamens into carpels in male flowers | RNAi |
| TAG1 | MADS-box transcription factor: stamen and carpel identity | Homologous conversion and indeterminacy of the floral organs | Antisense RNAi |
| CUM1 | MADS-box transcription factor: stamen and carpel identity | Homologous conversion and distinct phenotype in male and female flowers | Cosuppression by CUM1 ectopic expression |
| SpAG | MADS-box transcription factor: microsporangial development in males and in meristem determination in females | Indeterminate flowers, loss of stamens or carpel | RNAi |
| TM5 | MADS-box transcription factor: floral organ and fruit development | Sterile stamens and ovaries | Antisense RNAi |
| TM29 | MADS-box transcription factor: floral organ and fruit development | Sterile stamens and ovaries | Antisense RNAi |
| CsSEP2 | MADS-box transcription factor: floral organ and fruit development | Sterile stamens and ovaries | Antisense RNAi |
| PsEND1 | A pea anther-specific gene expressed in anther primordium | Male sterility and parthenocarpic fruit | Ectopic expression of barnase driven by promoter PsEND1 |
| SlSES SPL/NZZ | Putative transcription factor: male and female sporogenesis | Male and female sterility | 13-bp deletion |
| CsSPL SPL/NZZ | Putative transcription factor: male and female sporogenesis | Male and female sterility | Antisense RNAi |
| Ms-1 | Microsporogenesis and tapetum development | Male sterility | Insertion in the promoter region and CRISPR/Cas9 |
| Ms32 | Microsporogenesis and tapetum development | Male sterility | SNP |

Continued
| Gene       | Ortholog in Arabidopsis | Function(s)                                           | Genetic modification | Phenotype(s) mainly in reproductive organs | Species       |
|------------|-------------------------|-------------------------------------------------------|---------------------|--------------------------------------------|---------------|
| CIATM1     | bHLH10/89/91            | bHLH transcription factor: microsporogenesis and tapetum development | Deletion            | Male sterility                            | C. lanatus    |
| BMTDF1     | TDF1                    | bHLH transcription factor: tapetum development        | Unknown mutation in Wucai accession with abnormal tapetum | Male sterility | B. rapa ssp. chinensis var. rosularis Tsen |
| Msc-2      | MS1                     | Plant Homeo Domain (PHD)-finger transcription factor: microspore and tapetum development | 1 bp deletion       | Male sterility                            | C. annuum     |
| CnMS-5     | AMS                     | bHLH transcription factor: microspore and tapetum development | Deletion and SNP in promoter (possible) | Male sterility | Cucumis melo   |
| CaAMS      | AMS                     | bHLH transcription factor: microspore and tapetum development | VIGS-RNAi           | Male sterility                            | C. annuum     |
| CsWOX1/Mf  | WOX1                    | WUSCHEL-related homeobox (WOX) transcription factor: male and female sporogenesis | 1 bp deletion       | Male and female sterility                  | C. sativus    |
| SPFF       | CEPR1/XIP1              | Leucine-rich repeat receptor-like protein kinase: pleiotropic function including anther development | 2 bp deletion       | Male sterility and other vegetative phenotypes | S. lycopersicum |
| SIMAPK4    | MAPK4                   | Mitogen-activated protein kinase: signal transduction in pollen development of the binucleate stage | RNAi                | Male sterility: pollen abortion           | S. lycopersicum |
| SIMAPK20   | MAPK20                  | Mitogen-activated protein kinase: signal transduction in pollen development at uninnucleate-to-binucleate transition of microgametogenesis | RNAi and CRISPR/Cas9 | Male sterility: pollen abortion           | S. lycopersicum |
| SIFI3      | PIF3                    | bHLH transcription factor: pollen mitotic division    | CRISPR/Cas9         | Male sterility: pollen abortion           | S. lycopersicum |
| SIMYB33    | MYB33                   | R2R3-MYB transcription factor: pollen development at the mature stage | RNAi                | Male sterility: pollen abortion           | S. lycopersicum |
| SGLT1      | GLT1                    | Glutamate synthase: auxin biosynthesis in pollen development | CRISPR/Cas9         | Pollen defect                             | S. lycopersicum |
| SICWIN9    | CWIN9                   | Cell wall invertase: carbon metabolism and sugar signaling in pollen development | CRISPR/Cas9         | Pollen defect                             | S. lycopersicum |
| CsSUT1     | SUT1                    | Sucrose transporter: sucrose support for anther and pollen development | RNAi                | Male sterility                            | C. sativus    |
| CsHT1      | HT1                     | Hexose transporter: metabolic activity of pollen tubes | RNAi                | Reduced pollen germination and pollen tube growth | C. sativus    |
| LeGWD      | GWD                     | α-Glucan, water dikinase: starch phosphorylation in pollens | Transposon Insertion | Male sterility                            | S. lycopersicum |
| SAMDC1/2/3 | SAMDC                   | S-Adenosylmethionine decarboxylase family: reproductive development | RNAi                | Male sterility                            | S. lycopersicum |
| Gene          | Ortholog in Arabidopsis | Function(s)                                                                 | Genetic modification | Phenotype(s) mainly in reproductive organs | Species          |
|--------------|-------------------------|----------------------------------------------------------------------------|---------------------|-------------------------------------------|------------------|
| SlPIN8       | PIN8                    | Auxin transporter: auxin homeostasis in pollens                            | RNAi                | Pollen abortion                           | S. lycopersicum  |
| SIFGL1       | FIGL1                   | AAA-ATPase: male meiosis                                                   | CRISPR/Cas9         | Self-sterility                            | S. lycopersicum  |
| CsMS-3       | MMD1/DUET               | PHD-finger transcription factor: microsporogenesis                         | SNP                 | Male sterility                            | C. sativus       |
| PS-2         | ADPG1                   | Polygalacturonase protein: septum and stromium degeneration, and anther dehiscence | SNP                 | Non-dehiscent anthers                      | S. lycopersicum  |
| SICER6       | CER6                    | 3-Ketoacyl-CoA synthase family: tapetum degradation and microgametogenesis | Insertion           | Non-dehiscent anthers                      | S. lycopersicum  |
| Style2.1     | PRE1/BNQ                | bHLH transcription factor: cell elongation in style                        | Deletion in promoter | Exserted stigma                           | S. lycopersicum  |
| SIDEELLA     | GA1                     | Putative transcription factor: restraining growth in reproductive organs    | Antisense RNAi      | Exserted stigma                           | S. lycopersicum  |
| CsGA20ox     | GA20ox                  | GA biosynthetic enzyme: promoting growth in reproductive organs             | Ectopic expression  | Exserted stigma                           | S. lycopersicum  |
| SlLst/SIETR5 | EIN4                    | Ethylene receptor protein: cell division and differentiation in style       | SNP                 | Exserted stigma                           | S. lycopersicum  |
| SlIAA9       | IAA9                    | Aux/IAA transcription factor: pleiotropic function including inhibition of precocious growth of the ovary | SNP                 | Precocious growth of the ovary            | S. lycopersicum  |
| SlPIN4       | PIN4                    | Auxin transporter: preventing precocious fruit development without pollination | RNAi                | Precocious growth of the ovary            | S. lycopersicum  |
| SmARF8       | ARF8                    | Auxin response factors: negatively regulating fruit initiation             | RNAi                | Precocious growth of the ovary            | S. melongena     |
| SIARF6/8     | ARF6/8                  | Auxin response factors: pleiotropic function including transmitting tract formation | Silencing by Arabidopsis MIR167a | Female sterility: no pollen tube extension in pistils | S. lycopersicum  |
| CsSPT        | SPT                     | bHLH transcription factor: cell differentiation in style transmitting tract | CRISPR/Cas9         | Reduced female fertility                   | C. sativus       |
| CsALC        | ALC                     | bHLH transcription factor: ovular guidance for pollen tubes                | CRISPR/Cas9         | Reduced female fertility                   | C. sativus       |
| CsRALF4/19   | RALF4/19                | Cysteine-rich peptide: pollen tube integrity and ovular guidance           | CRISPR/Cas9         | Male sterility and reduced female fertility | C. sativus       |
| CsPID        | PID                     | Serine/threonine protein kinase: pleiotropic function including ovule initiation | SNP                 | Female sterility                          | C. sativus       |
| Gene | Ortholog in Arabidopsis | Function(s) | Genetic modification | Phenotype(s) mainly in reproductive organs |
|------|------------------------|-------------|---------------------|-------------------------------------------|
| IMA  | MIF2                   | Mini zinc finger (MIF) protein: inhibition of cell proliferation and promotion of cell differentiation in ovules | Antisense RNAi | Female sterility and seedless fruit |
| MIF2 | AG16                   | MADS-box transcription factor ovule formation | Retrotransposon insertion | Abnormal ovule formation and reduced seed set |
| MIF2 | CAM1B/1                | MADS-box transcription factor seed formation | RNAi | Female sterility |
| MIF2 | CMI6                   | MADS-box transcription factor embryo formation | RNAi | Female sterility |
| MIF2 | Pat-k/SlAGL6           | MADS-box transcription factor ovule formation | Retrotransposon insertion | Abnormal ovule formation and reduced seed set |
| MIF2 | SlyAGL11               | MADS-box transcription factor seed coat formation | RNAi | Ovule abortion and seedless fruit |
| MIF2 | SlGAMYB1/2             | R2R3-MYB transcription factor: embryo sac development | Silencing by SlMIR159 | Female sterility |
| MIF2 | PF1/SlHB15A/Pat/Pat-1 | Class III homeodomain leucine-zipper (HD-ZipIII) transcription factor: preventing fruit set in the absence of fertilization | SNP and silencing by miR166 | Aberrant ovule development and parthenocarpic fruit |
| MIF2 | SlCOI1                 | Protein containing Leu-rich repeats and a degenerate F-box motif: embryo sac development | 6.2-kb deletion | JA-sensitive and female sterility |
| MIF2 | SlMYB21                | R2R3-MYB transcription factor embryo sac development | RNAi | Reduced female fertility and parthenocarpic fruit |
| MIF2 | SlCHS                  | Chalcone synthase: flavonoids biosynthesis in reproductive organs | RNAi | Reduced pollen germination and pollen tube growth |
| MIF2 | uORF of SlGGP         | Regulator of ascorbate synthesis: redox homeostasis in plant development including pollen fertility | SNP and CRISPR/Cas9 | Male sterility and virus resistance |
| MIF2 | SlHAK5                 | High-affinity K⁺ transporter: pollen K⁺ uptake and viability | CRISPR/Cas9 | Reduced seed number |
| MIF2 | CHS5                   | Cap binding protein constitutes the eIF4F complex, posttranslational modification of proteins and pollen development | CRISPR/Cas9 (Cas9 driven by PPC2 promoter) | Reduced seed number |
| MIF2 | Ms-cd1                 | EIN3 target gene: pollen development | Higher expression in male-sterile lines | Male sterility |
| MIF2 | Brassica oleracea      | | | |
However, in cum26 male flowers there were indeterminate flower buds or carpels replacing stamens in the third whorl, while in the female flower no obvious phenotypic changes were observed in whorl 3 and whorl 4, suggesting that the function of floral organ identity genes may be sex-dependent in cucumber [34]. In 2016, cucumber AP3 (CsAP3) (Fig. 2b), rather than Arabidopsis AP3, was found to directly bind to the promoter of ethylene receptor ETHYLENE RESPONSE 1 and activate its expression, an essential player for stamen arrest in female flowers, suggesting the novel characteristics of CsAP3 in regulating female flower development in cucumber [35]. In addition to specifying stamen identity, spinach (Spinacia oleracea) SpPI and SpAP3 are required for appropriate organ numbers, whorl development, and sex determination [36]. In lettuce, with a capitate inflorescence, there was no genetic data about stamen or carpel identity genes; however, similar expression patterns of ABC genes in lettuce suggested conserved regulatory mechanisms in floral organ development [5]. Furthermore, there have been studies to generate male sterility using the cytotoxic gene barnase driven by anther-specific promoter FeEND1 (Pisum sativum ENDOTHECIUM 1), and the resulting anther ablation triggered parthenocarpy and seedless fruit in both miniature tomato cultivar ‘Micro-Tom’ and commercial cultivar ‘Moneymaker’, indicating the link of structural male sterility or stamen inhibition with parthenocarpic fruit development in tomato [37, 38].

**Sporogenous male sterility**

Sporogenous male-sterile mutants exhibit almost normal floral morphology, but are unable to produce functional pollen, or the pollen is defective [8]. In flowering plants, pollen development includes three processes: microsporogenesis, microgametogenesis, and pollen maturation [39]. During microsporogenesis, sporogenous cells differentiate into microspore mother cells after mitosis, and then generate tetrads through meiosis [39]. In premeiotic anthers, the differentiated somatic cells are epidermis, endothecium, middle layer, tapetum, and sporogenous cells from outside to inside (Fig. 3a and b) [40].

The tomato γ-ray mutant sexual sterility (Sises) produced incomplete ovules and wilted anthers devoid of pollen grains, and thus loss of both male and female fertility [41]. SISES encodes Arabidopsis SPL/NZZ (SPOROCYTELESS/NOZZLE) homologous protein and is essential for anther primordium formation in tomato (Fig. 3b) [41]. In Arabidopsis, SPL/NZZ is involved in sporogenesis, and this process is directly activated by C-function AG, which specifies stamen and carpel primordia development [42]. The spl mutant was unable to produce sporogenous cells or tapetal tissue, displaying deformed nucelus and anther wall [43, 44]. Cucumber SPL (CsSPL) is functionally equivalent to tomato and Arabidopsis SPL. Knockdown of CsSPL damaged male fertility and inhibited ovule development [45]. In tomato MS10(35) anthers, pollen mother cells were degenerated and failed to produce tetrads, microspores, and pollen grains, due to dysfunctional meiosis and an abnormal tapetum (Fig. 3b) [46]. MS10(35) is the Arabidopsis DYT1 (DYSPHONY TAPETUM1) homolog in tomato and is essential for chromosome segregation at meiosis anaphase I and programmed cell death of the tapetum during microsporogenesis [46]. MSC-1, the DYT1 homolog in pepper (C. annuum), exerted a similar function in male fertility [47]. Both tomato ms32 mutants and watermelon (Citrus lanatus) Sc18 mutants displayed pollen mother cell and tapetum defects [48, 49]. Their candidates, tomato MS32 (Figure 3b) and watermelon CIATM1 (Abnormal Tapetum 1), were revealed to be the homolog of AthHLH10/89/91, which can form a protein complex with DYT1 to function synergistically in anther differentiation in Arabidopsis [48–50]. DYT1 can upregulate the tapetum-expressed genes AMS (ABORTED MICROSPORES) and MS1 (MALE STERILE 1) in Arabidopsis [51]. In sun and ms1 mutants, tapetal cell abnormalities were obvious, and defective pollen development appeared immediately after microspore release from the tetrads [52, 53]. Another tapetum-specific transcription factor, TDF1 (DESTRUCTIVE IN TAPETAL DEVELOPMENT AND FUNCTION 1) acts downstream of DYT1 and directly promotes AMS expression in Arabidopsis [54, 55]. BrTDF1 of Wucai (B. rapa ssp. chinensis var. rosularis Tsen) was able to complement the tapetum and microspore defect in Arabidopsis tdf1 mutant [56]. In tomato, the expressions of SITDF1, SIAMS, and SIMS1 were all significantly downregulated in ms10(35) and ms32 mutants, combined with the reduced expression of MS32 in ms10(35) mutants and the unchanged MS10(35) transcriptional level in ms32 mutants, indicating a conserved regulatory pathway involving TAG1-SIES-MS10(35)-MS32-TDF1-AMS-MS1 related to sporogenous male fer-

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**Figure 2.** Expression patterns of reported genes involved in floral organ determination in tomato and cucumber. a Longitudinal section of a tomato floral bud at stage 6 [20]. b Longitudinal section of a cucumber floral bud at stage 6 [4]. For each gene, the area between two black lines represents its expression regions. For example, TAG1 is expressed in stamen and carpel primordia in tomato. Car, carpel; Sta, stamen; Pe, petal; Se, sepal. Scale bars = 100 μm.
Sporogenous cell

Anther cell specification

Mature pollen formation

Microsporogenesis

Microgametogenesis

Pollen maturation

Mitosis

Meiosis

Release

Sporogenous cell

Pollen mother cell

Tetrad

Microspore

Pollen

Figure 3. Gene regulatory networks of anther and pollen development in tomato. a Transverse view of a tomato anther at premeiotic stage. b Diagram of anther and pollen developmental stages, and the putative gene regulatory network. Solid frames: genes inferred from functional data with phenotypic effects. Dotted frames: gene function based on indirect data. Solid arrows: genetic relationships. Dotted arrow: presumed genetic relationship. Dotted line: presumed protein interaction.

Genome-wide surveys have suggested that some genes participate in sporogenesis development by regulating the above-mentioned essential genes. For example, the cucumber CsWOX1 (WUSCHEL-RELATED HOMEOBOX 1) transcription factor was shown to interact with CsSPL and regulate sporogenesis through the NZZ/SPL-DYT1-AMS-MS1 pathway (Fig. 5a) [60]. Tomato receptor-like protein kinase XIP1 (XYLEM INTERMIXED WITH PHLOEM 1) plays an essential role in male fertility, and its loss of function mutant spff (small parthenocarpic fruit and flower) showed shrunken anther locules with very few inviable pollen grains [61]. Some genes controlling meiotic progression also affect plant fertility. FIGL1 (FIDGETIN-LIKE 1) was identified in Arabidopsis for its anticrossover role during meiosis, and mutation of FIGL1 resulted in at least a 25% increase in recombination in Arabidopsis hybrid [62], while rice Osfigl1 mutants displayed male sterility, with abnormal chromosome behavior and pollen abortion in the anther [63]. Tomato Slfigl1 mutants were found to be sterile and seedless [64]. Arabidopsis MMD1/DUET (MALE MEIOCYTE DEATH1) is required for male meiotic chromosome organization and progression [65]. Cucumber ms-3, caused by a single-nucleotide polymorphism (SNP) in MMD1 homolog, could not form a tetrad or microspores, thus resulting in pollen absence and male sterility [66].

After release from the tetrad, the microspore undergoes an unequal mitosis to form a large vegetative cell and a small generative cell; subsequently, the latter gives rise to two sperm cells via mitotic division, a process called microgametogenesis [67]. In tomato, SIMAPK4 (MITOGEN-ACTIVATED PROTEIN KINASE 4), SIMAPK20, and SIPIF3 (PHYTOCHROME INTERACTING FACTOR 3) have been demonstrated to play critical roles in microgametogenesis and specifically regulate post-meiotic pollen development through the sugar and auxin metabolism and signaling pathway [68–70]. Knockdown of SIMAPK20 or SIMAPK4 resulted in abnormally degraded cytoplasm at the binucleate stage of pollen development [68, 69]. SIPIF3 mediates the pollen mitosis I process by directly promoting the expression of both SLGLT1 (GLUTAMATE SYNTHASE 1) and SLGLT9 (CELL WALL INVERTASE 9) in the tomato anther [70]. Knockout of SLGLT1/SLGLT9 phenocopied the defective bicellular pollen phenotypes of Slpif3 mutants [70]. Tomato SlMYB33 was shown to function in the pollen maturation process. Pollen grains in SlMYB33-RNAi plants displayed slight irregularity during the binucleate stage and collapse and shrinkage at the mature stage, and thus exhibited aberrant pollen viability and poor male fertility [71]. Plant metabolites, like saccharides and polyamines, can affect male gamete development as well. Cucumber SUT1 (SUCROSE TRANSPORTER 1) regulates anther and pollen development by ensuring carbohydrate supply [72]. CsSUT1-RNAi plants showed shriveled pollens and male sterility [72]. Downregulating cucumber HT1 (HEXOSE TRANSPORTER 1) reduced pollen germination and tube growth [73]. In tomato, mutation of the gene for α-glucan water dikinase (GWD), a key enzyme controlling starch degradation, led to pollen grains accumulating excess starch and male gamete lethality.
with only 0.4% paternal transmission [74]. The reduction of polyamines in tapetal tissue, executed by knocking down the biosynthesis-related gene SAMDC1/2/3 (S-ADENOSYLMETHIONINE DECARBOXYLASE) under the tapetal-specific A9 promoter, led to shrunken and distorted pollen grains and male sterility [75]. In addition, auxin homeostasis is crucial for reproductive development. Knockdown of auxin efflux transport PIN8 (PIN-FORMED 8) resulted in decreased auxin content and ~80% of pollen grains with abnormality and lack of viability [76].

Functional male sterility

In flowering plants, after a successive process of cell degradation in the tapetum, septum, and stomium, mature pollen is released from the anther [77]. In functional male-sterile mutants, the pollen is normal but cannot reach the stigma or cannot germinate on the stigma [8]. To some extent, diclinism in most cucurbit crops (e.g. cucumber) is a kind of natural functional male sterility due to the positional separation of the stamen (in the male flower) and pistil (in the female flower). Manual pollination can restore the fertility of this functional type of male sterility.

In tomato, spontaneous mutants ps (positional sterile) and ps-2 conferred functional male sterility due to unsplit anthers (Fig. 4a–c). A polygalacturonase gene (PG), the homolog of Arabidopsis ADFG (ARABIDOPSIS DEHISCENCE ZONE POLYGALACTURONASE), is responsible for the tomato ps-2 male-sterile phenotype. PGS belong to one of the largest hydrolase families and are associated with a wide range of plant developmental programs, including anther dehiscence [78]. In breeding practice, ps-2 has been utilized for tomato hybrid seed production in Eastern Europe due to its low level of self-pollination and availability of viable pollen by manual breeding [79]. Moreover, mutation in tomato β-ketoacyl-coenzyme A synthase Slcer6 (ECERIFERUM 6), which is required for the biosynthesis of very-long-chain fatty acids, led to defective cuticle formation during anther development. Thus, Slcer6 mutants showed abnormal tapetum development and inhibited pollen dispersal [80]. Slcer6 mutants displayed two times lower seed production than in wild-type self-crossing when used as the pollen donor. Considering the impaired microgametogenesis, Slcer6 mutants exhibited the characteristics of both sporogenous male sterility and functional male sterility [81].

Besides non-dehiscent anthers, an exerted stigma also leads to self-pollination failure because pollen can hardly reach the stigma properly (Fig. 4d and e). Style length was previously found to be associated with allogamy/autogamy (cross-pollination/self-pollination) [82]. Style2.1 accounts for the stigma exertion in wild, allogamous species, and a deletion of 450 bp in the promoter of Style2.1 was responsible for the transition of long style to short style, promoting the evolution of self-pollination in cultivated tomatoes [82]. The phytohormone gibberellin (GA) was found to regulate style length by promoting cell expansion and proliferation. Knockdown of the GA signaling protein gene SIDELLA resulted in excessively elongated style and self-sterility in tomato (Fig. 4d and e) [83]. Similarly, overexpressing citrus GA biosynthetic enzyme GA20OX (GA20-OXIDASE) in tomato caused an elongated style and protruded stigma [84]. Tomato accession T431 exhibited >95% male sterility due to stigma exertion under higher temperature, for which the SiLST (Long Styles) gene encoding an ethylene receptor protein was the key candidate [85]. Further study showed that, unlike the stigma exertion in wild tomato, high temperature-induced stigma exertion was caused by more seriously shortened stamens than pistils in cultivated tomato, and exogenous jasmonate treatment could effectively rescue this type of stigma exertion, suggesting a new function of jasmonic acid (JA) in improving plant abiotic stress tolerance [86].

Another cause of functional male sterility is inconsistent maturation of anthers and pistils or preanthesis ovaries, which refers to ovaries that can initiate development without/before pollination stimulation. In tomato, loss of function of the negative regulator of auxin response Aux/IAA9 resulted in seedless fruits due to precocious growth of ovaries, despite the mutant pistils being fertile by hand pollination [87]. Similarly, downregulation of SIPIN4 triggered seedless fruit because of fruit development initiated prior to fertilization [88]. In eggplant (Solanum melongena), the spontaneous dominant mutant 13-3 produced parthenocarpic fruits with reduced expression of auxin response factor SmARF8 (AUXIN RESPONSE FACTOR 8). Silencing SmARF8 by RNAi in eggplant generated seedless fruits, whereas hand-pollination of SmARF8-transgenic plants could produce normal, seeded fruits [89].

Female sterility

In breeding practice, the application of female sterility is much less common than that of male sterility. Related studies are sporadic and unsystematic, mainly focusing on parthenocarpy and seedless fruit. Seeds are considered detrimental to the edible quality of the fruit, e.g. seeds in eggplant can lead to browning and bitterness of the flesh [89]. Therefore, seedless fruit is a desirable trait in many horticultural crops, e.g. triploid seedless watermelon [11], seedless citrus varieties bred from Satsuma mandarin (Citrus unshiu) by somatic hybridizations [90, 91], and seedless grape ‘Corinth’ and ‘Thompson’ cultivars (Vitis vinifera) [11]. Female fertility defects generally result from abnormalities of the ovary or ovule development.

Weakened female fertility caused by ovary defects

The fruit is generally developed from the carpel, and loss of carpel identity genes can cause ovary developmental abnormalities and thus defects or absence of female fertility, such as the C-function genes (tomato TAG1, cucumber CUM1, and spinach SpAG) and E-function genes (tomato TM5 and TM29, and cucumber SEP2) (Fig. 2a and b). In tomato transgenic plants with TAG1 silenced, indeterminacy was detected in the fourth whorl and carpels were replaced with indeterminate floral meristems [28]. Regarding AG homologs in unisexual flowers, they seemed to remain similar in function, although the AG-suppressed plants exhibited unique phenotypic characteristics related to sexual dimorphism in dioecious spinach and monoecious eggplant [34, 36]. In TMS-RNAi tomato, the inner three whorls of the flower were all defective, and the sterile carpel with deformed style was incompletely fused [3]. Knockdown of TM29 in tomato resulted in infertility of the ovary, with ectopic shoots growing from the deformed fruit [29]. In cucumber, mutation of CsSEP2 led to perturbed floral development and infertility of the ovary, with an enlarged stigma and seedless fruit shed precociously from the peduncle [92].

In angiosperms, successful double fertilization begins with pollen grain deposition on the stigma, adhesion, hydration, and germination to produce pollen tubes [93]. Then pollen tubes pass through the reproductive tract tissues [also known as the transmitting tract (TT)] inside the stigma, style, and ovary towards the ovules [94]. Female fertility defects can also result from abnormalities in the stigma, style, or TT that block the pollen
Figure 4. Two types of functional male sterility in tomato. a Schematic drawing of tomato stamen. b In wild-type tomato, dehiscence of mature anthers gives rise to release of pollen grains. c In the ps-2 mutant, indehiscence of mature anthers blocks pollen grain dispersal. d In wild-type tomato, pollen grains adhere to the stigma and germinate, then pollen tubes extend along the transmitting tract towards ovules. e In the Sldella mutant the exserted style prevents pollen grains landing on the stigma, leading to pollination failure.

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tubes targeting the ovules. In Arabidopsis, genes responsible for TT development include SPT (SPATULA) [95], ARF6/8 [96], HECs (HECATEs) [97], NTT (NO TRANSMITTING TRACT) [98], and HAF (HALF FILLED) [99]. Downregulation of tomato ARF6 and ARF8 led to smaller organs and female sterility, as a result of pollen failure to germinate on the stigma or to grow along the TT in transgenic plants [100]. In cucumber, a recent study showed that CsSPT and CsALC (Arabidopsis ALCATRAZ homolog) functioned redundantly in maintaining female fertility. Cspid single mutants showed 60% lower seed set from the defective style TT, while Cspid Csalc double mutants showed complete female sterility due to absence of pollen tube extension channels [101]. In addition, CsSALC participated in pollen tube guidance by positively regulating the expression of CsRALF4 (Rapid ALKalinization Factor 4) and CsRALF19 in cucumber, and mutation of CsSALC led to 95% reduction in female fertility (Cheng et al., unpublished results). Therefore, during the long journey from stigma to ovules, pollen tube extension is mediated by CsSPT at the preovular guidance stage (early stage of pollen tube guidance) and by CsALC to approach ovules at the late stage in cucumber (Fig. 5b).

Damaged female fertility derived from ovule defects

In Arabidopsis, ovules are located within the ovary, attached to the placenta by the funiculus. Mature ovules consist of embryo sacs, inner and outer integuments, and the funiculus. Ovule development is divided into four phases: (i) initiation of ovule primordia from the placenta; (ii) appearance of the megaspore mother cell (MMC) and its differentiation into four haploid megaspores, as well as initiation of the inner and outer integument; (iii) embryo sac formation and integument maturation, in which the most proximal megasporde undergoes three mitotic division cycles to form the seven-celled embryo sac, including two synergids, an egg, a central cell, and three antipodal cells; and (iv) fertilization and embryogenesis [102–104]. Disruption of any of the above-mentioned developmental phases will result in an ovule defect.

The cucumber auxin transport-related gene CsPID (PINOID) plays an essential role in ovule initiation. Cpuid mutants exhibited no ovule formation in the placenta and were female-sterile [105]. CsPID interacted directly with the HD-ZIP III transcription factor CsREV (REVOLUTA), whose homolog in Arabidopsis is required for meristem initiation at lateral positions (Fig. 5a) [106]. In Cspid mutants, two auxin transport-related genes, CsABCB19 (ATP-BINDING CASSETTE B19) and CsABC1B3, as well as the ovule development-related genes CsWox1, CsSPL, and CsWUS (WUSCHEL) were all downregulated, suggesting that they may act in the same pathway in regulating ovule initiation (Fig. 5a).

Cucumber CsWox1 and CsSPL are involved in female organ development by mediating auxin signaling. Cswox1 mutants displayed no ovule initiation and significantly reduced CsSPL transcript accumulation [60]. CsWox1 could interact with CsSPL, and CsSPL could interact with CsTPL (TOPLESS) and CsTCP23 (TEOSINTE BRANCHED1/CYCLOIDEA/PCF family protein 23) (Fig. 5a) [60]. In severe CsSPL-RNAi lines the ovules displayed a finger-like structure without nucellus and integument [45]. Auxin was detected to be greatly decreased in the male and female organ primordia of Cspid, Cswox1, and Cspid mutants [45, 60, 105]. CsSPL stimulated the expression of CsARF3, and was positively regulated by CsARF13 and CsPHB (PHABULOSA) during sporogenesis (Fig. 5a) [45]. CsWox1 may regulate sporogenesis via the CsSPL-mediated pathway and/or by modulating auxin signaling in cucumber (Fig. 5a) [60]. Homologs of SPL in tomato
displayed similar roles in megasporogenesis. There was no nucellus (MMC) or integument development in tomato Sises mutants [41]. Besides auxin, the level of GAs and brassinosteroids (BRs) was reported to regulate ovule numbers in tomato by mediating ovule initiation [107, 108]. GA reduced ovule number and BR promoted ovule initiation by inhibiting GA biosynthesis in tomato [107, 108]. Tomato IMA (INHIBITOR OF MERISTEM ACTIVITY) promoted nucellus differentiation and inhibited cell proliferation by negatively regulating WUS expression [109]. IMA-RNAi plants displayed finger-shaped ovules with overgrowth of integuments or indeterminacy of ovule structure, and no MMC was formed [109]. Loss of function of SLAGL6 (AGAMOUS-LIKE 6) by a retrotransposon insertion resulted in parthenocarpic fruits and defective ovules with abnormal microyle in tomato [110]. AGL11, also known as STK (SEEDSTICK), controls funiculus development and seed detachment in Arabidopsis [111]. Its homolog in tomato, SlyAGL11, was reported to regulate seed coat formation and seed numbers [112]. Suppression of SlyAGL11 led to seedless tomatoes with premature fruit set and faster fruit growth, resembling a strong parthenocarpic phenotype [112].

Even if ovule initiation is normal, embryo sac arrest also can lead to female sterility. JA has been shown to play key roles in ovule late development by analysis of mutants jasmonate-insensitive1-1 (Sjai1-1; mutated in the JA coreceptor CO1) in tomato [113]. SIMYB21 was greatly downregulated in Sjai1-1 mutants, and the ovules of Sjai1-1 and Simyb21 mutants showed similar phenotypes with increased callose deposition and cell vacuolation in the embryo sac, leading to premature elimination of the nucelus before fertilization and thus female sterility [113]. On the other hand, JA promotes SIMYB21 expression, which positively feeds back into JA biosynthesis, and thus promotes ovule late development in tomato [113]. Moreover, the miR159–GAMYB1/2 and miR166–SIHB15A modules are crucial for tomato ovule development and fruit set. SIGAMYB1/2 silencing by SIMR159 overexpression resulted in abnormal growth of the embryo sac and misregulation of pathway genes associated with ovule and female gametophyte development [114]. The mutant pf1 (parthenocarpic fruit 1) produced seedless fruits, and the causal gene was identified as SIHB15A. Knockdown of SIHB15A by miR166 led to aberrant ovules and parthenocarpic fruit [115]. The pat and pat-1 tomato accessions with parthenocarpic fruits were confirmed to be alleles of pf1 [115]. Recently, small peptides have been revealed to play important roles during the reproduction process in Arabidopsis [116], such as RALF4/19 in pollen tube integrity [117], RALF34 in triggering pollen tube burst [118], and LURES in attracting pollen tubes to ovules [119]. Potato (Solanum chacoense) ScrALF3 participates in both male and female gametophyte development. Scralf3 mutants produced pollen grains with reduced viability and fewer seeds, primarily due to abnormal nuclear distribution and asynchronous nuclear divisions in the embryo sac [120, 121].

Besides the above, in traditional breeding, gamete sterility derived from chromosome behavior disorder during meiosis is a key application in seedless fruit production, e.g. the self-infertility of triploid watermelon and banana from gametic chromosome imbalance [122]. Chromosome translocation is another way to generate seedless fruits in watermelon and banana [123, 124]. Recently, a homozygous translocation occurring on watermelon chromosome 6 led to meiotic defects at metaphase I and thus the less seed phenotype of the F1 hybrid, providing new reference...
for the application of reciprocal translocation in less seed fruit breeding at diploid level [122].

**Perspectives**

Fertility genes and related mechanisms are intensively explored in model plants, including Arabidopsis and grain crops. Due to the importance of hybrid seed production, more and more male-sterile mutants and related genes have been characterized in vegetable crops. However, the underlying mechanisms and regulatory networks remain unclear and fragmented. Besides the above-mentioned genes directly affecting fertility, more regulators have been found to participate in reproductive development in an indirect manner. Several studies have shown the close relationship of secondary metabolites and plant fertility. Silencing of tomato chaconone synthase (CHS), the first gene in the flavonoid pathway, caused diminished pistil fertility and parthenocarpic fruits [125]. Besides, overproduction of ascorbic acid, caused by mutation of the ascorbate synthesis regulator GGP (GDP-L-GALACTOSE PHOSPHORYLASE), resulted in disrupted anther and pollen development and male sterility in tomato [126]. There are other examples of indirect regulators. Knockout mutants of the high-affinity transporters SlHAK5 in tomato exhibited impaired pollen germination and pollen tube growth, and resultant reduced seed set, indicating the importance of K+ uptake during reproduction [127]. In melon, a recent study showed that mutation of eIF4E, encoding a cap-binding protein functioning in mRNA circularization and cap-dependent translation, led to melons with increased virus resistance and male sterility. Mutants of eIF4E displayed postmeiotic defects in both microsporocytes and tapetum [128].

In the past decades, mining of male/female-sterile genes has relied mainly on natural or artificial mutants. With advances in plant genetic transformation and genome manipulation technology such as the CRISPR/Cas9 system, it is much easier, faster, and more precise to create mutant materials and perform functional analysis. For example, male-sterile tomato plants without exogenous vector were obtained by targeting SIMS10 gene via CRISPR/Cas9-mediated genome editing [129]. By targeting a stamen-specific gene, the putative strictosidine synthase gene (SISTR1), a novel male-sterile tomato line with abnormal pollen grains was generated [130]. Meanwhile, a transgenic maintainer was created in the background of a male-sterile line transformed with SISTR1 and a seedling-color marker gene (R2R3 MYB transcription factor gene ANTI) [130]. Offspring of crosses between a homozygous male-sterile plant and hemizygous maintainer will be half male-sterile plants and half fertile maintainer plants that can be easily identified by seedling color [130]. This biotechnology-based platform has great practical potential for hybrid seed production in diverse vegetable crops [130]. Meanwhile, the fluorescence marker gene (mCherry, GFP) and the visualized marker gene (Ruby) could also be adopted as a proxy for isolation of transgene or transgene-free vegetable crops on a large scale, which will greatly save time and workload [131]. Notably, TKC (Transgene Killer CRISPR system) technology could achieve self-elimination of the CRISPR construct and produce all-transgene-free progenies [132, 133]. In addition, ITRAQ (isobaric tags for relative and absolute quantification)-based proteomics was applied in the exploration of the protein regulation network of the male-sterile mutant ms7 (35), and fatty acid metabolism was speculated to be the cause of male sterility [134]. ITRAQ and PRM-based proteomics analysis showed that metabolism pathways, including sugar, lipid, and fatty acid pathways, played important roles in pollen abortion in the tomato ms7 mutant [135].

Despite sterile mutants having advantages of decreasing cost and increasing seed purity during hybrid seed production, complete sterility (referring to both male and female sterility) is undesirable due to the difficulty in germplasm retention. An ideal male/female sterile line should be deficient in fertility, but with normal vegetative growth or fruit development, such as the widely used functional male-sterile mutant ps-2 in tomato breeding practice. For genes with pleiotropic functions, disruption in targeted reproductive organs can be achieved via knockout driven by reproductive organ (pollen or ovule)-specific promoters. For example, knockdown of SlIAA9 in tomato fruits using two flower-specific promoters, Soly03g007780 (Psiol80) and Soly02g067760 (Psiol60), resulted in parthenocarpic fruits without other defects in vegetative tissues [136]. Similarly, fruit-specific gene editing of tomato SIEZ2, a SET-domain-containing polycomb protein, through the Cas9 system driven by a phosphoenolpyruvate carboxylase 2 (PPC2) gene promoter enabled observation of fruit phenotypes lacking additional developmental perturbations [137]. Tissue-specific gene editing will have more applications in vegetable crops to obtain male-/female-sterile mutants by targeting essential genes under reproductive organ-specific promoters.

In agricultural practice, in addition to male-sterile lines, sustainable seed production requires restorer lines and maintainer lines, which entail huge costs. The dominant genic male-sterility (DGMS) system enables an efficient cultivation mode with a 1:1 ratio of male-sterile to male-fertile plants in F1 hybrid plants without seed sorting and separation processes, and eliminates the risk of genetically modified pollen entering the ecosystem, and thus it is highly appreciated in cross-pollinated crops like maize and oilseed [138]. One of the rare examples of DGMS application in vegetable crops is the spontaneous mutant 79-399-3 in cabbage (Brassica oleracea), which is controlled by single dominant gene, Ms-cd1, and has served as the donor for the creation of dominant male-sterile lines in multiple Brassicaceae crops, e.g. cabbage, broccoli, kohlrabi, and Chinese kale [139, 140]. Map-based cloning showed that the Arabidopsis homolog gene SIED1 (SALT-INDUCED AND EIN3/EIL1-DEPENDENT 1) was the Ms-cd1 gene and a KASP marker was developed for rapid identification of Ms-cd1 loci [140, 141]. Recently in watermelon, a new DGMS gene, HSP70 (HEAT SHOCK PROTEIN 70), was identified as the candidate of Cms1 mutant, providing a new gene resource for the DGMS system [142]. In addition, the fusion of cytotoxic genes to tapetum-specific promoters led to genetic male-tissue cell ablation and could be an effective biotechnological tool to generate dominant male sterility [38]. As mentioned above (see section Structural male sterility), the chimeric construct of the ribonuclease gene (barnase) driven by the anther-specific promoter PsEND1 gave rise efficiently to dominant male sterility in Brassicaceae and Solanaceae [38, 143]. Some other promoters from sporogenesis-related genes, e.g. TDF1, AMS, and MS1, can be applied to trigger the expression of cytotoxic genes in specific male tissue to cause DGMS.

Compared with DGMS, an inducible male-sterility system is more labor-saving due to its elimination of the dependence on maintainer lines and restorer genes during hybrid seed production [144]. Chemicals such as herbicides act as common inducers, e.g. the glyphosate-mediated male sterility system developed in maize, which exploited the differential expression of the glyphosate-resistance gene in male tissue and the rest of the plant [145]. Other chemical-inducible male sterility systems are established on the basis of conversion of a non-toxic to a toxic chemical catalyzed by an anther-localized conversion gene, e.g. the L-ornithinase (arg6) gene converted the non-toxic compound N-acetyl-phosphinothricin (N-ac-PPT) into the herbicide
phosphinothricin (PPT) in tobacco and rice [146, 147], and the TAP1-DMR1DAOO gene converted d-glucosinate into l-glucosinate in tobacco [148]. In addition to chemical agents, environmental signals can also induce male sterility, such as photoperiod/thermosensitive genetic systems [also called environmentally sensitive genic male sterility (EGMS)] in rice [149, 150]. The above induction systems can be optimized and utilized in vegetable crops.

In addition to genic male sterility (CMS), cytoplasmic male sterility (CMS), caused by incompatibility of the nuclear and mitochondrial genomes, has been broadly used in hybrid breeding and genetic improvement of vegetable crops, e.g. the utilization of Ogura CMS, Polina CMS, and nap CMS in Chinese cabbage (B. rapa ssp. pekinensis) germplasm innovation [151, 152]. Nuclear genes that regulate mitochondrial genes relevant to CMS were called fertility restorer genes [152]. So far, many mitochondria-located CMS genes have been identified in vegetable crops, e.g. ORF138 for Ogura CMS in radish (Raphanus sativus) [153, 154], ORF220 in mustard (Brassica juncea) [155, 156], and orf137 in tomato [157], but not all corresponding restorer genes have been identified. More information about the mechanism of CMS and its application can be found in a recent review [151].

As elite seeds derived from hybrid breeding are the core competitiveness of the vegetable industry, dissecting fertility-related genes and mechanisms can not only further our understanding of sexual reproduction, but also create more sterile plants to facilitate crossbreeding or obtain seedless fruit. In addition to traditional breeding goals (yield, disease resistance), specific traits such as nutritional or bioactive component enrichment [158], tolerance of extreme environments (e.g. high temperature, salinity), and adaptability to different cultivation modes (e.g. dense planting and soilless culture) will attract more attention during vegetable breeding in the future.

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Author contributions

C.Z. and S.W. wrote the original draft and prepared the figures; Z.X. supervised the work and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declared no conflict of interest.

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