plants

MDPI

Review

Molecular Regulatory Networks of Plant Male Sterility: Nuclear Regulation of Stamen Development and Mitochondrial–Nuclear Crosstalk

Zihao Li [1,†], Xu Zhou [2,†], Xuemei Jin [2], Chuqi Zhang [1], Yaoyao Wang [1] and Li Cao [1,*]

[1] Agricultural College, Yanbian University, Yanji 133002, China; lizihaoahear@163.com (Z.L.)
[2] Characteristic Industry Development Center of Yanbian, Yanji 133000, China
* Correspondence: lcao@ybu.edu.cn
† These authors contributed equally to this work.

Abstract

Male sterility is the cornerstone of heterosis utilization and hybrid crop breeding. This review provides a comprehensive update on the molecular frameworks of genic male sterility (GMS) and cytoplasmic male sterility (CMS). We highlight how structural sterility is dictated by the ABCDE floral organ identity model, with class B, C, and E genes defining stamen fate. We further detail the SPL/NZZ-triggered DYT1-TDF1-AMS-MYB80-MS1 regulatory cascade that orchestrates sporogenous development encompassing microsporogenesis, tapetum function, programmed cell death (PCD), and pollen wall formation. Additionally, we explore functional sterility, which arises from impaired anther dehiscence and pollen release governed by regulators such as ARF6/8, NST1/2, TCP5, and ICE1. For CMS, we discuss how chimeric mitochondrial ORFs drive sterility via cytotoxicity, energy collapse, aberrant tapetal PCD, and retrograde regulation. The restoration of fertility, primarily achieved through Rf-encoded pentatricopeptide repeat (PPR) proteins, is examined alongside emerging non-PPR restorers acting at DNA, translational, and metabolic levels. The dynamic coevolution of mitochondrial–nuclear interactions is reflected in the rapid expansion of Rf-like gene clusters. We conclude by outlining future perspectives emphasizing single-cell transcriptomics, CRISPR-based precision breeding of sterile lines, and the expanded deployment of male sterility in agriculture.

Keywords: genic male sterility; cytoplasmic male sterility; tapetum; pollen wall formation

Check for updates

Academic Editor: Stefano Del Duca

Received: 5 October 2026
Revised: 29 October 2026
Accepted: 10 November 2026
Published: 16 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Flowers are determinate, specialized, and condensed reproductive shoots that originated through long-term evolutionary adaptation in angiosperms. Species-specific developmental programmes repattern leaf-derived organs into four distinct organ types—sepals, petals, stamens, and carpels. Collectively, these floral organs support the development of the male and female gametophytes and fulfil the requirements for sexual reproduction [1].

The stamen originates from a primordium initiated on the floral meristem and becomes partitioned into two functionally distinct compartments: the pollen-producing anther and the supporting filament. Prior to tapetal breakdown, the anther wall consists of four well-defined somatic cell layers—namely, from the outermost to the innermost, the epidermis, the endothecium, the middle layer, and the tapetum—with the tapetum directly enclosing the sporogenous cells and supplying the metabolites, enzymes, and sporopollenin precursors required for microspore development [2]. The tapetum forms the innermost layer of the

anther wall and lies in direct contact with the developing microspores. This tissue provides metabolites, principally lipids, carbohydrates and proteins, together with the biosynthetic precursors required for pollen wall formation [3–5].

The filament is a slender, stalk-like structure that connects the anther to the receptacle. Together with the pollen-producing anther borne at its apex, it forms a complete stamen. A vascular strand running through the filament continuously delivers water, mineral nutrients and photosynthates to the anther, supplying the energy and structural building blocks required for meiosis in the pollen mother cells, microspore morphogenesis and pollen maturation. Phytohormones, notably auxin, jasmonic acid and gibberellins, are likewise translocated through this vascular tissue, serving as key molecular signals that coordinate anther and pollen developmental programmes.

In crops with perfect flowers, hybrids have traditionally been produced by hand emasculation and pollination, but this is labour-intensive and time-consuming [6,7]. Pollination-control mechanisms such as sporophytic self-incompatibility [8,9], sex forms [10] and male sterility [7] have therefore enabled cost-effective hybrid breeding. Male sterility is the failure of a plant to produce functional, viable male gametes (pollen), or to release them, while female fertility remains intact; it may manifest as stamenless flowers, indehiscent anthers or failed pollen formation [7]. As male-sterile plants cannot self-pollinate, this circumvents tedious emasculation and further ensures hybrid purity [11]. First observed by the German botanist Joseph Gottlieb Kölreuter in 1763 and now reported in hundreds of plant species, it is classified as either cytoplasmic male sterility (CMS) or genic male sterility (GMS) [12,13].

2. Classification of Plant Male Sterility

2.1. Genic Male Sterility (GMS)

Genic Male Sterility (GMS) is controlled exclusively by nuclear genes, and its progeny follow Mendelian inheritance patterns. Among currently reported GMS genes, most exhibit monogenic recessive inheritance [13]. According to phenotypic features and abortive stages that trigger male sterility, GMS can be further classified into structural, sporogenous, and functional groups. This classification system is applicable to most flowering plant species [13,14].

2.1.1. Structural Male Sterility

Structural male sterility arises from mutations in genes related to floral organ development. Homeotic conversion of stamens, or their complete absence or malformation, prevents the assembly of normal anther structure and thereby leads to male sterility [3,15,16]. Most of these defects arise from mutations in genes of the ABCDE floral organ identity network, either lesions that abolish gene function or regulatory changes that perturb their precisely controlled spatiotemporal expression. These genes are core transcription factors governing cell-fate determination and morphogenesis of the four whorls of floral organs including sepals, petals, stamens and carpels [1,17]. They mainly comprise class-A genes AP1 (APETALA1) and AP2, class-B genes AP3 and PI (PISTILLATA), class-C gene AG (AGAMOUS), and functionally redundant class-E genes SEP1-4 (SEPALATA1-4). Except for AP2, most core ABCDE model genes controlling floral-organ development belong to the MADS-box transcription-factor family [17,18].

The specification of third-whorl stamen identity requires the coordinated action of class-B, class-C and class-E genes [19,20] (Figure 1). This central regulatory module has been well validated by genetic and molecular experiments. Class-B genes deliver key signals for stamen-identity specification, class-C genes define reproductive-organ development, and class-E genes act as central co-regulators. Class-E proteins form heterotetrameric complexes with class-B and class-C proteins. These complexes specifically bind to CArG-

box motifs in the promoters of downstream target genes and activate transcription of genes responsible for stamen and carpel development [21,22]. Accordingly, aberrant expression of any of these genes directly causes structural male sterility characterized by defective stamen morphology [3,15,16]. In the solanaceous plant tomato, the TAP3 and TM6 are the B-class genes. Mutation of the *TAP3* and silencing of *TM6* both cause homeotic transformation of stamens and petals, resulting in malformed stamens that fail to produce pollen grains [23,24]. The *sus2* male-sterile mutant lacks functional *TM6* regulation, and most stamens exhibit carpel-like characteristics [25]. In Arabidopsis, AG (AGAMOUS) is initially expressed in stamen and carpel primordia and later shows specific expression in particular cell types of stamens and carpels [19]. AG activates the expression of the sporogenesis-related master gene SPOROCYTELESS/NOZZLE (SPL/NZZ) to initiate microspore development [26,27]. AG also cooperates with class-B genes PI and AP3 to regulate stamen-identity specification and subsequent organ development. Furthermore, AG upregulates DAD1 (DEFECTIVE IN ANTHER DEHISCENCE 1). This gene encodes a key enzyme in the jasmonic acid (JA) biosynthetic pathway [28–30]. The Arabidopsis dad1 mutant displays male-sterile phenotypes including non-dehiscent anthers, impaired pollen maturation and insufficient filament elongation [28].

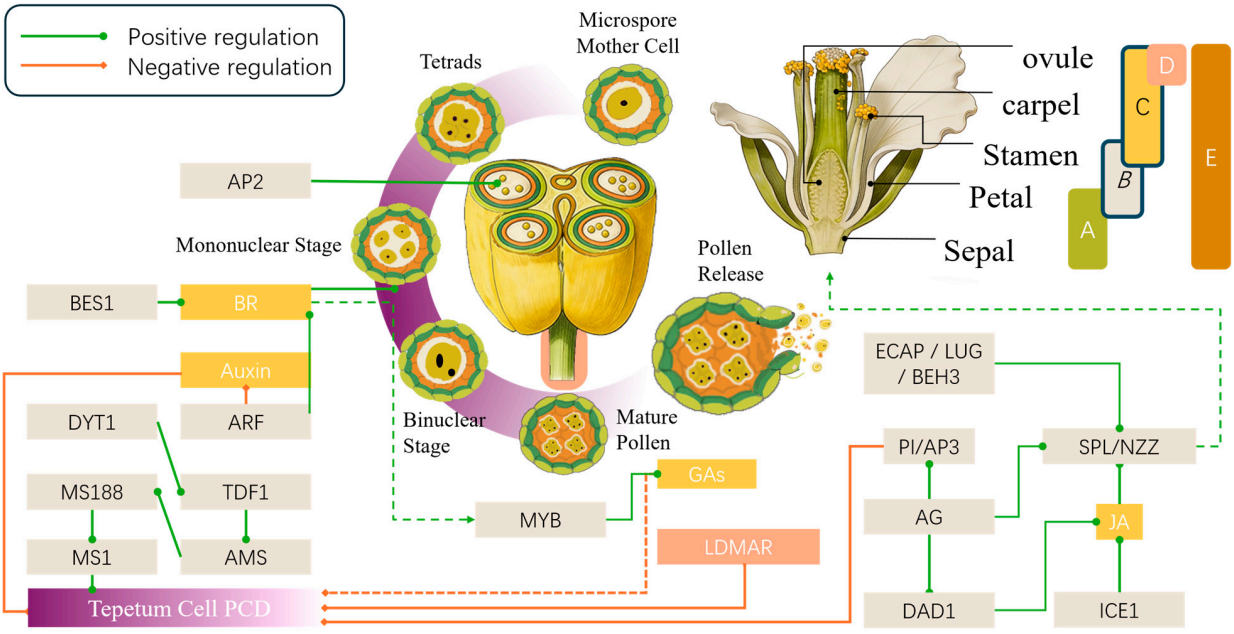


Figure 1. Regulatory networks in plant male organ development and genic male sterility and The ABCDE model of floral organ identity specification in plants. Pollen development is divided into five stages: microspore mother cell, tetrad, uninucleate, binucleate and mature pollen stages. White boxes = regulatory genes, yellow boxes = functional phytohormones. Dotted lines indicate indirect regulatory relationships.

2.1.2. Sporogenous Male Sterility

Plants with this type of male sterility exhibit normal floral-organ development but fail to produce viable and functional pollen grains [14]. This trait is governed by the genotype of the parental sporophyte, indicating that sporophytic genes participate in pollen developmental processes.

Male germline development is initiated by the expression of SPOROCYTELESS/NOZZLE (SPL/NZZ), a core sporogenesis gene activated by AGAMOUS [26]. The epidermis is derived from the L1 layer of the early anther primordium, whose cells divide anticlinally to form the outer protective layer. In the L2 layer cells at the four corners of the stamen

primordium, the transcriptional repressor complex ECAP-LUG-BEH3 sustains SPL/NZZ expression to drive archesporial cell specification. These cells then give rise to primary sporogenous cells (PSCs). Meanwhile, the CLE-BAM1/2 module regulates periclinal divisions of archesporial cells to produce primary parietal cells (PPCs) [31].

The PPCs undergo ordered periclinal divisions, generating the outer and inner secondary parietal cells at stage 4 [32]. At stage 5, the outer secondary parietal cells differentiate into the endothecium, whereas the inner secondary parietal cells give rise to the middle layer and the tapetum [32]. These early divisions and layer-fate decisions are coordinated by the leucine-rich-repeat receptor kinases BAM1/2 and RPK2, together with the CIK coreceptors [33–35]; RPK2 is required for specification of the middle layer, and loss of RPK2 results in an absent middle layer, a hypertrophic tapetum and a defective endothecium [35]. Downstream of SPL/NZZ [27], the bZIP transcription factors TGA9 and TGA10, initially expressed throughout the early anther primordium, become restricted to the middle layer and tapetum during stages 5 and 6 [36] (Figure 2).

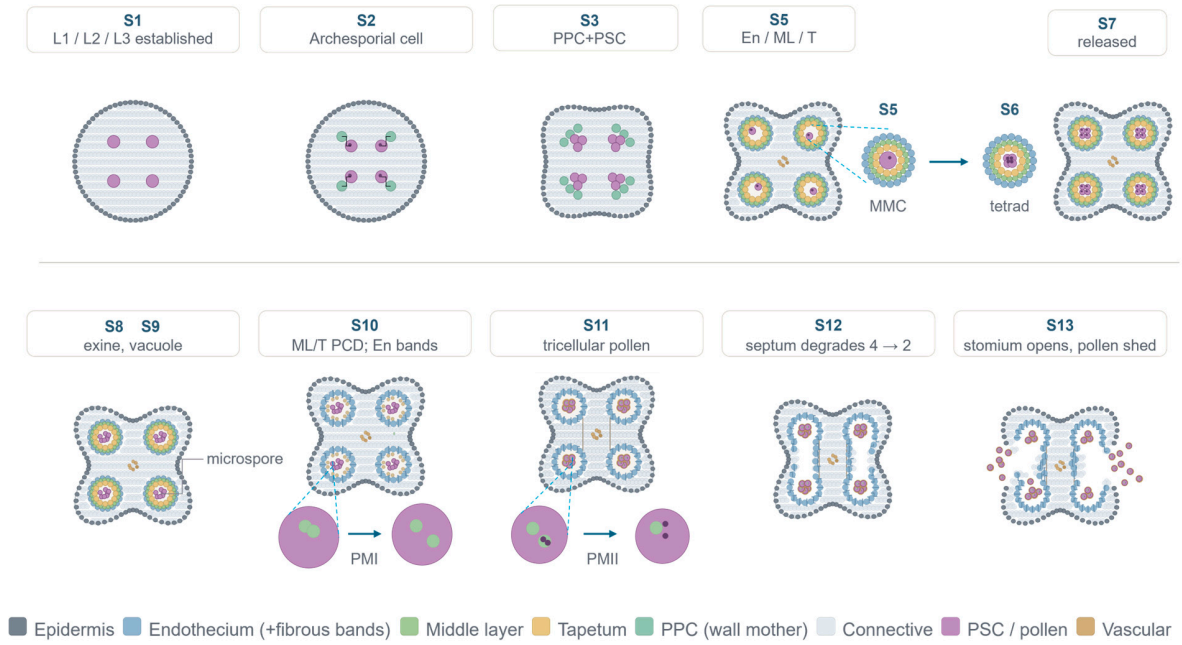


Figure 2. Cellular-resolution cross-sections of Arabidopsis anther development. Each panel shows a transverse section of a single anther at the indicated developmental stage, with all cell layers drawn as individually outlined tessellated cells. Stages 1–4 represent early anther primordium development: at stage 1, the L1, L2 and L3 cell layers are established; at stage 2, archesporial cells divide periclinally into primary parietal cells (PPCs) and primary sporogenous cells (PSCs); at stage 3, the four-lobed anther pattern is established; and at stage 4, the four concentric wall layers and the filament–anther boundary are defined. From stage 5 onward, the four locules are established, each enclosed by the epidermis and three inner wall layers—the endothecium (En), middle layer (ML) and tapetum (T)—with microspore mother cells (MMCs) at the centre. At stage 6, MMCs complete meiosis to form tetrads, while the filament remains short (approximately 20% of its final length). At stage 7, microspores are released from the tetrads; at stage 8 they begin to synthesize the exine wall, and at stage 9 they undergo vacuolation. At stage 10, pollen mitosis I (PMI) produces bicellular pollen, fibrous bands appear in the endothecium, and the middle layer and tapetum undergo programmed cell death (PCD). At stage 11, pollen mitosis II (PMII) yields tricellular pollen. At stage 12, the pollen reaches maturity and the septum degrades, converting the four locules into two. At stage 13, the stomium opens and mature pollen is shed. Circular insets magnify one locule at stage 5 (MMC) and stage 6 (tetrad) to resolve internal cell morphology that is too small to distinguish at whole-anther scale; a leader line at stage 9 indicates a representative vacuolated microspore. Colour coding is given in the legend below each panel.

The PSCs undergo mitotic expansion under the control of EMS1/EXS and differentiate into microspore mother cells [37,38]. EMS1/EXS, together with SERK1/2, encodes leucine-rich repeat receptor-like kinases (LRR-RLKs) [39,40], whereas TPD1 encodes a small protein with a predicted secretory signal peptide [41]. In differentiating microspore mother cells, EMS1/EXS expression declines while TPD1 expression increases. Secreted TPD1 binds to the EMS1/EXS-SERK1/2 receptor complex on the adjacent somatic cell membrane [39]. This binding triggers phosphorylation of BES1, a transcription factor in the brassinosteroid (BR) pathway, and promotes its nuclear import to directly activate DYT1 expression [42].

BES1 also binds to the promoters of SPL/NZZ, TDF1, AMS, MS1, and MS2 [43]. Among these, DYT1 directly activates TDF1, which is essential for tapetum differentiation and function [44]. TDF1 subsequently drives the tapetum toward a secretory cell type. The secretory tapetum supplies carbohydrates, lipids, and proteins to support developing microspores. In tomato, SlTDF1, the ortholog of Arabidopsis TDF1, is required for timely tapetum degradation and pollen development; disrupting SlTDF1 function perturbs tapetal degeneration and pollen formation and yields male-sterile plants, offering a genetic resource for hybrid breeding [45].

At stage 6, each MMC undergoes meiosis to form a tetrad of four haploid microspores enclosed in a callose wall [32]. Callose wall synthesis depends primarily on CalS5, which is positively regulated by ARF17 and CDKG1 and repressed by AtTTP; loss of CalS5 nearly eliminates the callose wall, disrupts exine formation and causes male sterility [46–49]. After meiosis, TDF1 activates AMS [50], which positively regulates TEK; TEK in turn activates the arabinogalactan protein genes AGP6, AGP11, AGP23 and AGP40 to drive formation of the nexine, the inner layer of the exine, in each microspore of the tetrad [51]. In tomato, silencing or CRISPR/Cas9 knockout of SlAMS—and even its over-expression—strongly reduces pollen viability [52].

Following meiosis, degradation of the pectin wall that encloses the four meiotic products releases individual microspores from the tetrad. This process is mediated by the QUARTET (QRT) family, whose three core Arabidopsis members act sequentially: QRT1 encodes a pectin methylesterase that demethylates pectin [53], rendering it accessible to the polygalacturonases encoded by QRT2 and QRT3 [54,55], which cleave α-1,4-glycosidic bonds in the demethylated backbone. The best-characterized member, QRT3 [55], is a direct target of the tapetum master regulator MS188; it is transiently expressed in tapetal cells coincident with microspore release, and the secreted protein degrades the pectin wall surrounding the microspores.

Concurrently, MYB80 (also known as MYB103) directly binds to the A6 promoter and drives tapetum-specific secretion of β-1,3-glucanase. UPEX1, which encodes an arabinogalactan β-1,3-galactosyltransferase, is transcribed in tapetal cells under the direct regulation of AMS [56]. UPEX1 mediates secretion of A6 proteins into the anther locule, where they degrade the callose wall separating the microspores, releasing them from the tetrad into the locule and thereby marking the onset of the male gametophytic phase.

MYB80 regulates downstream target genes involved in sporopollenin biosynthesis, including MS2, PKSA, PKSB, ACOS5, TKPR1, TKPR2 and CYP703A2. ACOS5 activates fatty acids by ligating them to coenzyme A [57], and CYP703A2 catalyzes the hydroxylation of long-chain fatty acids [58]. The resulting hydroxylated products are channelled into two branches: PKSA and PKSB generate triketide and tetraketide pyrones, whereas MS2 produces fatty alcohols [59]. The tetraketide pyrones are then reduced by TKPR1 and TKPR2 to polyhydroxylated tetraketides [60]. Together, these enzymes catalyze a series of fatty acid modification reactions [61,62] that drive sporopollenin biosynthesis in the tapetum and ultimately shape the exine, the outer layer of the pollen wall [63]. Beyond the MYB80 module, numerous bHLH and bZIP transcription factors directly or indirectly

regulate genes involved in lipid precursor supply and polyketide metabolism during pollen wall formation [64–67].

AMS also regulates ABCG26, whose protein product transports sporopollenin precursors from the tapetum into the anther locule [68,69]. AMS further regulates LTP12 and IEF [70]: LTP12 encodes a lipid transfer protein that mediates lipid exchange between membranes, whereas IEF encodes a plasma membrane-localized protein that translocates exine precursors into the anther locule, thereby reinforcing the exine and the layers beneath it [71].

The pollen intine is synthesized by the microspore itself. The transcriptional coactivator NMDM1 regulates arabinogalactan protein (AGP) and pectin methylesterase (PME) genes to control intine formation [72]. Concurrent with this, multiple small vacuoles within the microspore fuse into a single large central vacuole, displacing the nucleus to the cell periphery and thereby generating a polarized microspore.

The tapetum undergoes programmed cell death (PCD) governed by the DYT1–TDF1–AMS–MYB80–MS1 transcriptional cascade. This cascade is conserved across flowering plants, including rice and maize (Figure 3), indicating that the ordered bHLH–MYB–PHD regulatory logic is retained across eudicots and monocots [34,35]. Within this cascade, MYB80 regulates the downstream targets UNDEAD and MS1: UNDEAD encodes an aspartic protease that cleaves mitochondrial pro-apoptotic proteins [73], whereas MS1 regulates multiple cysteine protease genes [74]. Independently, MYB2 directly activates CEP1, which encodes a papain-like cysteine protease involved in tapetum degradation [75,76]. Tapetal PCD releases materials required for pollen development [77], including oil bodies and cytoplasmic lipid bodies that contribute to exine formation [78,79]. Conversely, defects in fatty acid transport that perturb reactive oxygen species (ROS) homeostasis suppress activation of this cascade and cause male sterility [80]. A parallel proteolytic module operates in rice, where the AMS orthologue TDR (TAPETUM DEGENERATION RETARDATION) is a positive regulator of tapetal degradation: it forms a complex with its interacting partner TIP2 to activate the downstream bHLH factor EAT1, which induces the aspartic protease genes OsAP25 and OsAP37 and thereby triggers tapetal PCD; disruption of this module delays tapetal degeneration and aborts pollen [81].

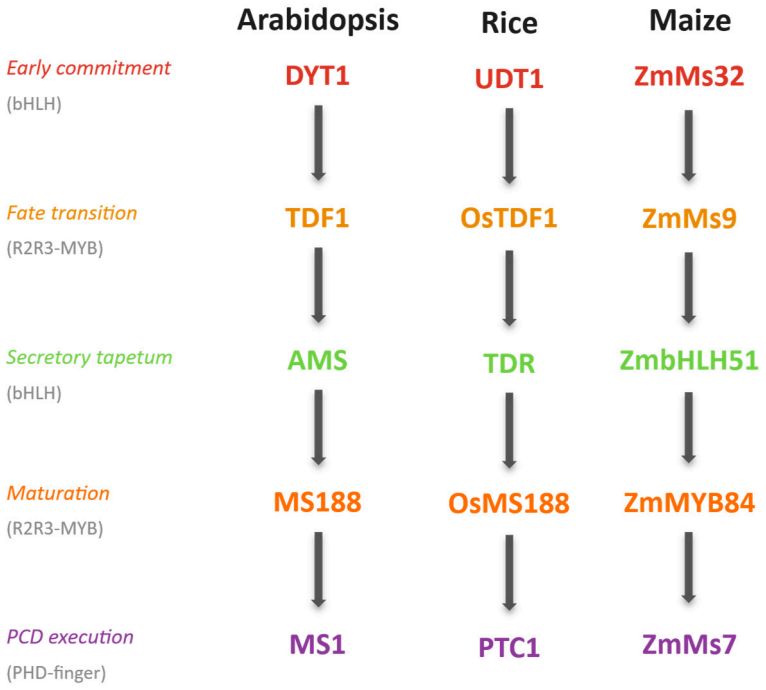


Figure 3. Gene regulatory network in the tapetum of arabidopsis, rice and maize.

The microspore then undergoes two successive mitotic divisions to give rise to a mature male gametophyte. Pollen mitosis I (PMI) requires MYB81: the microspore divides asymmetrically to produce a large vegetative cell and a small generative cell. The generative cell subsequently undergoes pollen mitosis II (PMII), a symmetric division specifically controlled by the MYB transcription factor DUO1. DUO1 acts through its downstream targets DAZ1 and DAZ2, which encode transcriptional repressors, together with the corepressor TOPLESS [82–84]. ARID1 cooperates with the cell-cycle repressor RBR1 to regulate DUO1 at both the transcriptional and post-transcriptional levels, thereby balancing cell specification and cell division during sperm cell formation [85].

2.1.3. Functional Male Sterility

Functional male sterility describes plants that produce viable mature pollen yet fail to complete pollination: mechanical or physiological defects, such as abnormal filament development, indehiscent anthers and impaired pollen release, prevent pollen from reaching the stigma, ultimately causing sterility. Once gametophyte development is complete, the auxin response factors ARF6 and ARF8 transduce developmental cues that drive marked elongation of filament cells, positioning the anthers for effective pollen transfer. In parallel, they activate the expression of DAD1, a key gene in jasmonic acid (JA) biosynthesis [28–30,86]. In addition, the R2R3-MYB transcription factor MYB21 promotes flavonol biosynthesis; flavonols scavenge reactive oxygen species (ROS) and are required for stamen development and filament elongation [87].

At anther stage 12, the tapetum is almost completely degraded, releasing its full complement of flavonoids, alkanes, lipids, proteins and carotenoids into the anther locule. These materials serve as precursors for formation of the pollen wall and pollen coat. Among them, flavonoids are deposited onto the surface of the sporopollenin-based exine, where they protect the haploid pollen from environmental stresses including UV radiation, high temperature and water loss [77,88]; they also contribute to exine assembly [89]. The mature pollen coat is a sticky material that fills the cavities of the exine, protecting pollen from dehydration and mediating pollen–stigma adhesion, recognition and hydration.

The developmental programme of anther cells switches from differentiation to degradation. Anther dehiscence comprises three key events: lignification of the endothecium, degradation of the septum to form the bilocular anther, and rupture of the stomium to release mature pollen grains [3,32]. Secondary wall thickening of the endothecium provides the mechanical driving force for dehiscence, and the NAC transcription factors NST1 and NST2 are central regulators of this process [90]. In response to blue light, Arabidopsis MYC2 and MYC4 act downstream of the CRY1 (CRYPTOCHROME1) photoreceptor: they bind directly to the NST1 promoter and activate its transcription, inducing cellulose synthase and lignin biosynthetic genes required for endothecial secondary wall thickening and hence for anther dehiscence [91]. Consistently, nst1 nst2 double mutants lack secondary wall thickening in the endothecium, fail to dehisce and display functional male sterility [90]. A natural example in tomato is the positional-sterility 2 (ps-2) mutation, in which a polygalacturonase gene defect prevents stomium rupture and produces non-dehiscent anthers [92].

Plants overexpressing TCP5 produce viable pollen yet are sterile, because TCP5 directly downregulates the transcription factor genes MYB26, NST1 and NST2 as well as the cellulose synthase genes IRX3, IRX8 and CESA1 and the lignin biosynthetic genes PAL4, C3H and 4CL1, while upregulating AHP4, a negative regulator of secondary wall thickening. The resulting imbalance causes insufficient lignin deposition and discontinuous fibrous bands in the endothecium, leading to anther indehiscence. This mechanism partially overlaps with that of TCP24, whereas upregulation of CCOAMT appears to be specific to TCP5 [93–95].

Furthermore, stomatal differentiation in the anther epidermis is essential for anther dehydration. ICE1 (INDUCER OF CBF EXPRESSION 1) encodes a MYC-like bHLH transcription factor that binds MYC recognition motifs in the FAMA promoter and activates FAMA expression; FAMA, in turn, acts as a master regulator of guard cell differentiation. Transcriptomic analyses of anther tissue further show that ICE1 modulates a large set of genes involved in water transport and ion exchange. In ice1 mutants, stomatal differentiation in the anther is impaired, accompanied by disturbed water transport and ion exchange; insufficient dehydration of the anther wall ultimately causes anther indehiscence, blocked pollen release and a marked reduction in pollen viability [96]. ICE1 also physically interacts with core regulators of tapetum and pollen development, including DYT1 and AMS, and negatively modulates jasmonic acid (JA) biosynthesis and signalling, thereby coordinating anther dehydration with fertility. ICE1 thus acts as a critical node linking environmental signals to hormonal regulation [97]. See more detail in Table 1.

Table 1. Genes involved in the regulatory pathways of three types of male sterility in *Arabidopsis thaliana*.

Gene Name	Encoded Protein/Family	Biological Function	Associated Male-Sterility Type	Ref.
AP1 (APETALA1)	MADS-box transcription factor (class A)	Specify floral organ identity for sepals and petals	Structural	[1]
AP2	AP2/ERF transcription factor (class A)	Specify floral organ identity for sepals and petals	Structural	[1]
AP3	MADS-box transcription factor (class B)	Determine stamen and petal identity	Structural	[1,19]
PI (PISTILLATA)	MADS-box transcription factor (class B)	Determine stamen and petal identity	Structural	[1,19]
AG (AGAMOUS)	MADS-box transcription factor (class C)	Specify reproductive organ identity; activate SPL/NZZ; regulate JA biosynthesis via DAD1	Structural, sporogenous, functional	[19,26,28]
SEP1–SEP4 (SEPALATA1–4)	MADS-box transcription factor (class E)	Co-regulator, form heterotetrameric complexes with B- and C-class proteins for floral organ specification	Structural	[1,21]
SPL/NZZ (SPOROCYTE-LESS/NOZZLE)	Transcription factor	Master regulator initiating archesporial cell formation and microspore development	Sporogenous	[26,27]
DAD1 (DEFECTIVE IN ANTHER DEHISCENCE 1)	Lipase	Key enzyme in jasmonic acid biosynthesis; control anther dehiscence, pollen maturation and filament elongation	Structural, functional	[28–30,86]
ECAP	Component of transcriptional repressor complex	Maintain SPL/NZZ expression for archesporial cell specification	Sporogenous	[31]
LUG	Component of transcriptional repressor complex	Maintain SPL/NZZ expression for archesporial cell specification	Sporogenous	[31]

Table 1. *Cont.*

Gene Name	Encoded Protein/Family	Biological Function	Associated Male-Sterility Type	Ref.
BEH3	Component of transcriptional repressor complex	Maintain SPL/NZZ expression for archesporial cell specification	Sporogenous	[31]
CLE	CLE peptide ligand	Cooperate with BAM1/2 to control periclinal division of archesporial cells	Sporogenous	[31]
BAM1, BAM2	LRR-RLK	Regulate archesporial division and primary parietal cell formation; early anther layer patterning	Sporogenous	[31,33]
RPK2	LRR-RLK	Specify middle layer fate; mutant lacks middle layer, with hypertrophic tapetum and defective endothecium	Sporogenous	[33–35]
CIK	LRR-RLK co-receptor	Co-receptor for BAM/RPK2 signalling during anther cell layer patterning	Sporogenous	[33–35]
TGA9, TGA10	bZIP transcription factor	Expressed in middle layer and tapetum at anther stages 5–6	Sporogenous	[36]
EMS1/EXS	LRR-RLK	Receptor for TPD1 signal; control fate of microspore mother cells and tapetum	Sporogenous	[37–40]
SERK1, SERK2	LRR-RLK co-receptor	Form receptor complex with EMS1/EXS for TPD1 signal transduction	Sporogenous	[39,40]
TPD1	Secreted small peptide ligand	Ligand of EMS1–SERK complex; trigger downstream brassinosteroid signalling	Sporogenous	[41,42]
BES1	Brassinosteroid-pathway transcription factor	Activate DYT1, SPL/NZZ, TDF1, AMS, MS1 and MS2 after nuclear import	Sporogenous	[42,43]
DYT1	bHLH transcription factor	Top-tier master regulator of the tapetal transcriptional cascade	Sporogenous	[44]
TDF1	MYB transcription factor	Direct tapetum differentiation and secretory identity; activate AMS	Sporogenous	[44,50]
AMS	bHLH transcription factor	Core tapetal regulator; activate TEK, UPEX1, ABCG26, LTP12 and IEF	Sporogenous	[50,56,68,70]
TEK	Transcription factor	Activate AGP6/11/23/40 to direct nexine formation (inner layer of exine)	Sporogenous	[51]
AGP6, AGP11, AGP23, AGP40	Arabinogalactan proteins	Participate in nexine biosynthesis of pollen wall	Sporogenous	[51]
CalS5	Callose synthase 5	Synthesize callose wall surrounding tetrads, essential for normal exine formation	Sporogenous	[46–49]

Table 1. *Cont.*

Gene Name	Encoded Protein/Family	Biological Function	Associated Male-Sterility Type	Ref.
ARF17	Auxin response factor	Positively regulate CalS5 expression	Sporogenous	[47]
CDKG1	Cyclin-dependent kinase G1	Positively regulate CalS5 expression	Sporogenous	[48]
AtTTP	RNA-binding protein (tristetraprolin)	Repress CalS5 expression	Sporogenous	[49]
QRT1	Pectin methylesterase	Demethylate pectin of tetrad wall for subsequent polygalacturonase cleavage	Sporogenous	[53]
QRT2, QRT3	Polygalacturonase	Cleave demethylated pectin; QRT3 is a direct target of MS188 for microspore release	Sporogenous	[54,55]
MYB80 (MYB103)	MYB transcription factor	Tapetum development; activate A6; control sporopollenin biosynthetic genes and tapetal PCD	Sporogenous	[57,73]
A6	β-1,3-glucanase	Degrade callose wall between microspores to release tetrads	Sporogenous	[56]
UPEX1	Arabinogalactan β-1,3-galactosyltransferase	Mediate secretion of A6 protein into anther locule	Sporogenous	[56]
MS2	Fatty acyl reductase	Convert fatty acyl-CoA to fatty alcohols for sporopollenin synthesis	Sporogenous	[57,59]
PKSA, PKSB	Polyketide synthase	Generate triketide and tetraketide pyrones in sporopollenin pathway	Sporogenous	[59]
ACOS5	Acyl-CoA synthetase	Activate fatty acids via CoA ligation to provide sporopollenin precursors	Sporogenous	[57]
TKPR1, TKPR2	Tetraketide α-pyrone reductase	Reduce tetraketide pyrones to polyhydroxylated tetraketides	Sporogenous	[60]
CYP703A2	Cytochrome P450 monooxygenase	Catalyze hydroxylation of long-chain fatty acids for sporopollenin	Sporogenous	[58]
ABCG26	ABC transporter	Export sporopollenin precursors from tapetum to anther locule	Sporogenous	[68,69]
LTP12	Lipid transfer protein	Mediate lipid exchange between membranes during exine assembly	Sporogenous	[70]
IEF	Plasma membrane-localized protein	Translocate exine precursors into anther locule	Sporogenous	[70,71]
NMDM1	Transcriptional coactivator	Regulate AGP and PME genes to control intine formation	Sporogenous	[72]

Table 1. *Cont.*

Gene Name	Encoded Protein/Family	Biological Function	Associated Male-Sterility Type	Ref.
MS188	PHD transcription factor	Member of tapetal regulatory cascade; directly activate QRT3	Sporogenous	[55]
MS1	PHD transcription factor	Member of tapetal regulatory cascade; modulate cysteine proteases for tapetal PCD	Sporogenous	[74]
UNDEAD	Aspartic protease	Cleave mitochondrial pro-apoptotic proteins during tapetal PCD	Sporogenous	[73]
MYB2	MYB transcription factor	Directly activate CEP1 to promote tapetum degradation	Sporogenous	[75,76]
CEP1	Papain-like cysteine protease	Execute tapetal cell degradation during PCD	Sporogenous	[75,76]
MYB81	MYB transcription factor	Required for pollen mitosis I and asymmetric microspore division	Sporogenous	[82]
DUO1	MYB transcription factor	Control pollen mitosis II and sperm cell specification	Sporogenous	[82–84]
DAZ1, DAZ2	Transcriptional repressors	Downstream targets of DUO1, function together with TOPLESS	Sporogenous	[82–84]
TOPLESS	Co-repressor	Co-repressor partnering with DAZ1/DAZ2 during sperm development	Sporogenous	[82–84]
ARID1	ARID transcription factor	Regulate DUO1 at transcriptional and post-transcriptional levels together with RBR1	Sporogenous	[85]
RBR1	Retinoblastoma-related cell-cycle repressor	Cooperate with ARID1 to balance cell specification and division for sperm formation	Sporogenous	[85]
ARF6, ARF8	Auxin response factor	Promote filament elongation; activate JA biosynthetic gene DAD1	Functional	[86]
MYB21	R2R3-MYB transcription factor	Promote flavonol biosynthesis required for stamen development and filament elongation	Functional	[87]
NST1, NST2	NAC transcription factor	Master regulators of endothecial secondary wall thickening for anther dehiscence	Functional	[90]
CRY1	Blue-light photoreceptor	Upstream of MYC2/MYC4 in light-dependent anther dehiscence pathway	Functional	[91]
MYC2, MYC4	bHLH transcription factor	Bind the promoter of NST1 downstream of CRY1 to activate secondary wall programme	Functional	[91]

Table 1. *Cont.*

Gene Name	Encoded Protein/Family	Biological Function	Associated Male-Sterility Type	Ref.
TCP5	TCP transcription factor	Downregulate secondary wall genes; upregulate AHP4, causing insufficient endothecial lignification	Functional	[93–95]
TCP24	TCP transcription factor	Negative regulator of endothecial secondary wall thickening, partially overlapping with TCP5	Functional	[93]
MYB26	MYB transcription factor	Positive regulator of endothecial secondary thickening in anthers	Functional	[93]
IRX3, IRX8, CESA1	Cellulose synthase and secondary wall biosynthetic genes	Cellulose biosynthesis for endothecial secondary wall	Functional	[93]
PAL4, C3H, 4CL, CCOAMT	Lignin biosynthetic enzymes	Lignin deposition in endothecium to support anther dehiscence	Functional	[93–95]
AHP4	Histidine phosphotransfer protein	Negative regulator of secondary wall thickening	Functional	[93]
ICE1	MYC-like bHLH transcription factor	Activate FAMA for anther stomatal differentiation; coordinate anther dehydration and JA signalling	Functional	[96,97]
FAMA	bHLH transcription factor	Master regulator of guard cell differentiation in anther epidermis	Functional	[96]

2.1.4. Environment-Sensitive Genic Male Sterility

Male fertility can also be governed by environmental conditions. Nuclear genes that regulate anther development can condition sterility under particular photoperiod, temperature or humidity regimes, a phenomenon termed environment-sensitive GMS (EGMS), which forms the basis of the two-line hybrid system. In photosensitive GMS (PGMS), plants are male sterile under long days and fertile under short days, whereas in thermo-sensitive GMS (TGMS), sterility occurs above a critical temperature and fertility is restored below it. The first PGMS line, rice Nongken 58S, is controlled by the pms1 and pms3 loci. The pms1 locus encodes a long non-coding RNA, PMS1T, which is targeted by miR2118 to generate 21-nt phased small-interfering RNAs (phasiRNAs); a single nucleotide polymorphism near the miR2118 recognition site alters phasiRNA accumulation under long days, ultimately causing premature tapetal programmed cell death and male sterility [98]. The pms3 locus encodes another long non-coding RNA, LDMAR; under long days, a single nucleotide change increases promoter methylation, reduces LDMAR production and causes pollen abortion [99]. The most widely used TGMS line, tms5, carries a loss-of-function mutation in RNase Z^{S1}, which normally cleaves ubiquitin-fusion ribosomal protein L40 (Ub_{L40}) mRNAs; at high temperature, Ub_{L40} mRNAs over-accumulate and disrupt pollen development [100]. Small-RNA pathways also link temperature to fertility: in maize, DCL5 produces 24-nt reproductive phasiRNAs, and dcl5 mutants are male sterile at high temperature but fertile at low temperature [101]. Across these systems, fertility conversion commonly converges

on reactive oxygen species homeostasis, the timing of tapetal PCD and callose deposition; slowing development or delaying callose degradation can restore fertility in several TGMS lines [12,56]. Because EGMS lines are maintained by selfing under permissive conditions and used for hybrid seed production under restrictive conditions, they simplify hybrid breeding, although they remain vulnerable to sudden weather fluctuations [102,103].

2.2. Cytoplasmic Male Sterility (CMS)

Cytoplasmic male sterility (CMS) arises from a breakdown in coordination between the mitochondrial and nuclear genomes [104–107]. Plant mitochondrial genomes are structurally dynamic, undergoing frequent rearrangement and recombination, and they readily acquire sequences from the nucleus, the plastid and foreign donors [108]. These changes generate novel chimeric genes that confer the CMS phenotype [108,109]. CMS genes have now been identified in more than 150 plant species, including cotton [110], rice [111–113], wheat [114–117], rapeseed [118–120], tomato [121], sunflower [122] and petunia [123,124]. They share several features: they usually lie adjacent to core mitochondrial genes and are frequently co-transcribed with them; most are chimeric open reading frames (ORFs), and their products carry transmembrane domains thought to underpin sterility-inducing activity [108,125].

The CMS/Rf system has become the classical model for dissecting mitonuclear communication in plants [126,127]. This communication is bidirectional. Anterograde (nucleus-to-mitochondrion) regulation reflects the dominance of the nucleus in mitochondrial biogenesis: it encodes most respiratory-chain subunits and nearly the entire machinery for mitochondrial transcription, RNA processing and translation. Retrograde (mitochondrion-to-nucleus) regulation conveys organellar status back to the nucleus, reshaping nuclear transcription whenever mitochondrial homeostasis is disturbed. In CMS lines, a chimeric mitochondrial ORF uncouples these two arms, and fertility is restored only when a nuclear Rf allele re-establishes compatibility [128,129]. Mitonuclear interaction operates at successive levels—from genome organization and transcription to RNA processing, translation and protein assembly—and each offers a potential point of intervention for an Rf gene [130].

Anterograde control is most evident in alloplasmic lines, where substituting the nucleus removes functional Rf alleles and exposes an otherwise cryptic mitochondrial ORF, converting a fertile cytoplasm into a sterile one [129]. Retrograde signalling is best understood at the metabolic interface: CMS proteins impair respiratory complexes, lower ATP output and elevate ROS, and this organellar stress is relayed to the nucleus, where it reprogrammes genes governing alternative respiration, phytohormone signalling and tapetal PCD [126,128]. A chimeric mitochondrial product can also bind directly to—and cripple—a nuclear-encoded component of the respiratory machinery, a more physical route than the diffuse retrograde signal. Non-coding RNAs, both mitochondrial- and nuclear-encoded and including miRNAs and lncRNAs, have likewise been implicated in CMS-associated reprogramming of hormone and energy metabolism [107,131].

Four models are widely invoked to explain how CMS arises: (1) cytotoxicity, (2) energy deficiency, (3) aberrant programmed cell death (PCD) and (4) retrograde regulation, and a single CMS gene may act through more than one route (Figure 4) [108].

2.2.1. Cytotoxicity Model

The cytotoxicity model holds that CMS-encoded proteins are toxic to the cell [128]. In Escherichia coli and yeast, ORF138 encoded by the Brassicaceae CMS gene orf138 oligomerizes within the membrane, causing electrolyte leakage and ultimately inhibiting growth [119]; the maize URF13 protein behaves similarly [132]. Cytotoxicity of CMS proteins has been documented across many other species and cytoplasm types, includ-

ing ORF79 in Boro II rice [133], ORFH79 in Honglian rice [111], ORFH522 in the PET1 cytoplasm of sunflower [122] and ORF346 in rapeseed carrying the Nsa cytoplasm [134]. Although the toxicity of these proteins is well established in heterologous bacterial systems, how they act in the host plant and how that activity causes sterility remain unclear.

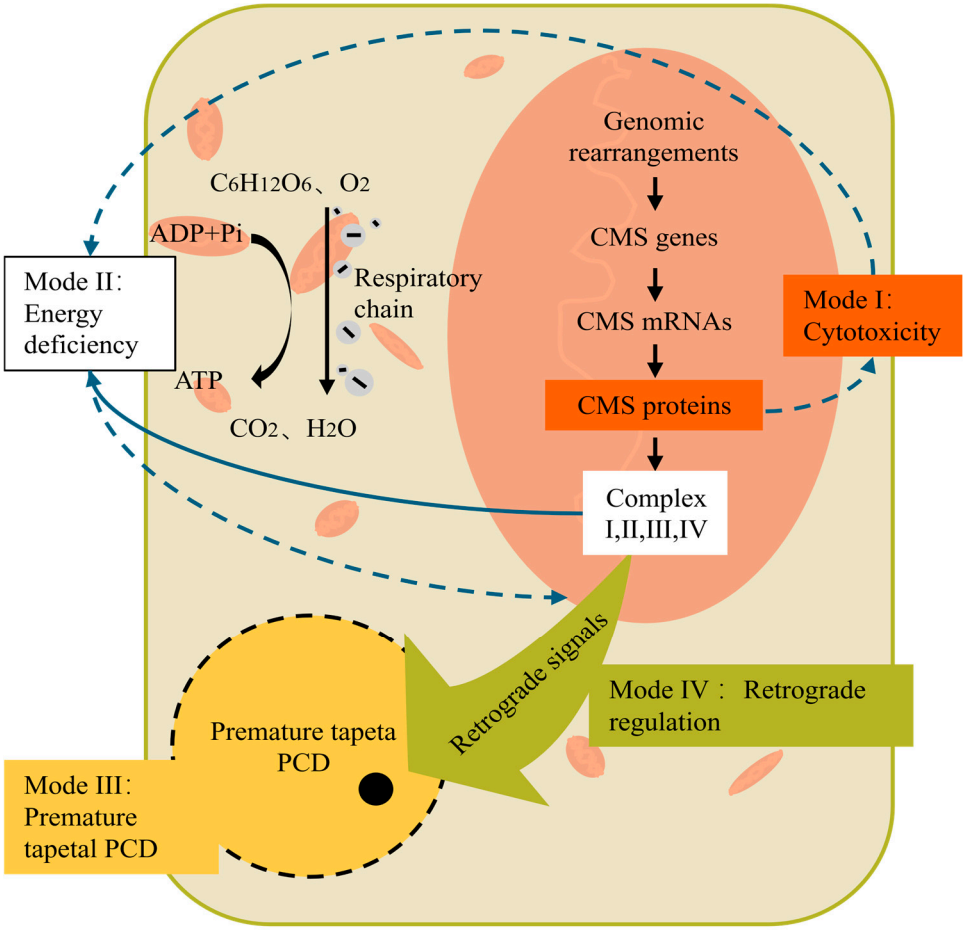


Figure 4. Four mechanistic models for CMS. (I) CMS proteins derived from rearranged mito-chondrial genome exert cytotoxic effects to destroy tapetal cells and induce pollen abortion; (II) CMS proteins damage respiratory complexes to hinder ATP production and cause developmental arrest due to energy shortage; (III) disordered ROS signalling initiates premature tapetal PCD to cut off nutrient supply for microspores; (IV) mitochondrial retrograde signals enter nucleus to alter nuclear gene expression and disrupt pollen development.

2.2.2. Energy Deficiency Model

Most CMS proteins are transmembrane proteins that interfere with ATP synthesis. On the basis of such observations, CMS genes alter mitochondrial function and restrict ATP supply, so that anther development, which has a particularly high energy demand, is arrested [126]. In maize CMS-T, for example, mitochondria proliferate but fail to mature, cannot meet the ATP demand of anther development, and the anther aborts. Some chimeric CMS proteins also disrupt other components of the ATP synthesis machinery (Figure 5) [135,136]. In pepper, orf165 confers CMS by perturbing the activity of energy-metabolism genes: flowers of the sterile line contain markedly less ATP than those of the maintainer line, whereas H^+-ATPase activity is elevated, and the resulting energy imbalance leads to microspore abortion. In the Honglian (HL) CMS system of rice, the mitochondrial protein ORFH79 interacts with P61, a homologue of the QCR10 subunit of complex III; this interaction inhibits complex III, lowers energy production and increases ROS accumula-

tion, ultimately causing male sterility [111]. In the wild-abortive (WA) CMS system of rice, WA352 interacts with COX11, a nuclear-encoded mitochondrial protein, and disrupts its role in peroxide metabolism, thereby triggering premature tapetal PCD and pollen abortion [137].

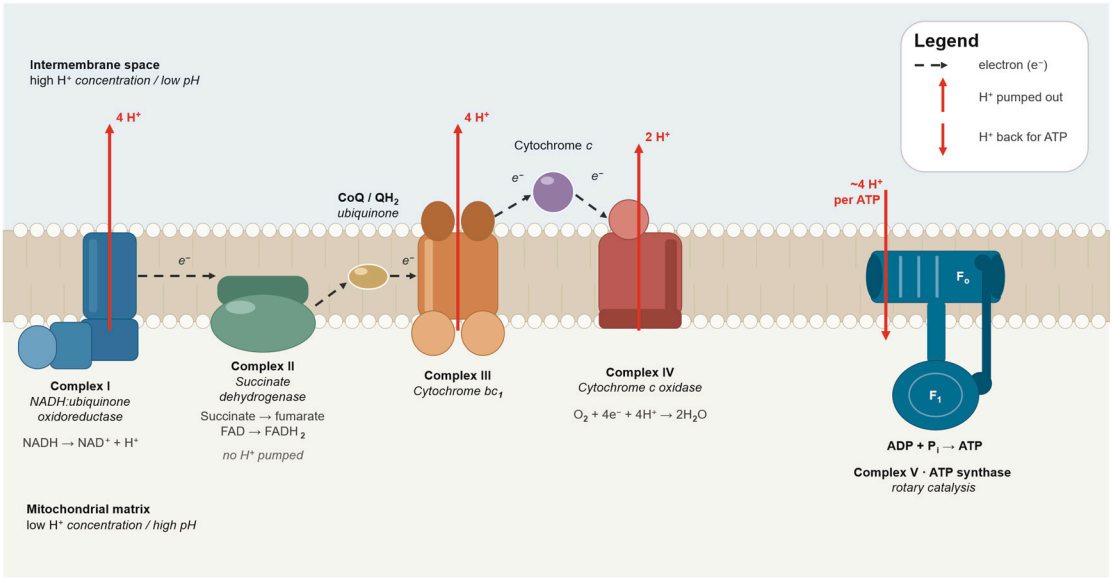


Figure 5. Mitochondrial electron transport chain and oxidative phosphorylation.

2.2.3. Premature Tapetal PCD

The third model invokes premature tapetal PCD, emphasizing the crosstalk between gametophytic and sporophytic cells in the control of tapetum degeneration: when tapetal cells undergo ectopic or mistimed PCD, male sterility ensues. Linking this to metabolism, proteomic comparisons between sterile and maintainer lines have implicated proteins required for pollen exine formation [138]. The key differentially abundant proteins include malate dehydrogenase (MDH), pyruvate dehydrogenase (PDH), aldehyde dehydrogenase (ALDH), acetyl-CoA synthetase (ACS) and pyruvate decarboxylase (PDC); these enzymes act in the tricarboxylic acid (TCA) cycle of tapetal cells and have been linked to the PCD programme [138]. Aberrant PCD is also accompanied by altered transcript levels of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), and by altered ROS-scavenging capacity. In maize CMS-C, the mitochondrial gene atp6c confers male sterility: during assembly of the F_1F_0-ATP synthase complex, its product ATP6C interacts more strongly with ATP8 and ATP9 than does the normal ATP6, reducing the abundance and activity of the synthase, causing protons to accumulate in the intermembrane space, and thereby triggering an ROS burst, premature tapetal PCD and pollen abortion [117].

2.2.4. Retrograde Regulation Model

Retrograde signalling between mitochondrial CMS genes and nuclear genes is central to the CMS/fertility-restoration (Rf) system. Under the retrograde model, dysfunctional mitochondria emit retrograde signals that reprogramme the expression of nuclear genes encoding mitochondrial proteins. One such protein, RETROGRADE-REGULATED MALE STERILITY (RMS), modulates nuclear gene expression [139], perturbs stamen-development pathways and inhibits pollen germination, thereby causing sterility [126]. Unusually for an Rf gene product, RMS is a non-PPR (pentatricopeptide repeat) protein. The co-evolution of CMS genes and Rf genes across different cytoplasms and crop species is well documented.

In the rice CMS-CW system, for example, mitochondrial genes regulate nuclear genes that are preferentially expressed in sterile lines and are associated with alternative oxidases, and up-regulation of these nuclear genes in the anther leads to male sterility [139,140]. In maize, ZmDREB1.7 is highly expressed in sterile microspores and activates the CMS-associated mitochondrial gene orf355 to induce sterility; orf355 in turn reinforces mitochondrial retrograde signalling and further up-regulates ZmDREB1.7, forming a positive feedback loop [141].

2.2.5. Advances in Restorer-of-Fertility Genes

The nuclear side of this interaction is dominated by restorer-of-fertility (Rf) genes, most of which encode mitochondria-targeted pentatricopeptide repeat (PPR) proteins. Each PPR motif forms a helix-turn-helix unit, and tandem arrays fold into a superhelical groove that reads RNA in a base-specific manner, enabling PPR proteins to govern the cleavage, editing, splicing, stability and translation of organellar transcripts. Most Rf-active members fall within a distinct, rapidly diversifying Rf-like (RFL) clade of the P-class PPR family, whose hallmark is elevated non-synonymous substitution precisely at the RNA-contacting residues [142,143]. Since the first Rf-PPR gene, Rf-PPR592, was cloned in petunia [144], the same molecular logic, a nucleus-encoded RNA-binding protein silencing a mitochondrial ORF, has been confirmed crop after crop. The radish Rfo gene of Ogura CMS carries a cluster of PPRs and suppresses accumulation of the ORF138 protein [145]. In Boro II rice, the clustered Rf1a and Rf1b genes silence the dicistronic B-atp6/orf79 mRNA by endonucleolytic cleavage and by transcript degradation, respectively, with RF1A epistatic to RF1B and additionally promoting atp6 editing independently of its cleavage activity [133]. In T-CMS wheat, the RFL proteins RFL79 (Rf1) and RFL29a (Rf3) bind the mitochondrial orf279 transcript and induce its cleavage [116], and in soybean, CRISPR/Cas9 knockout of the RFL gene GmPPR576 converted a fertile line into a male-sterile one, confirming its identity as the restorer [146]. In rapeseed, Rfp and Rfn, the restorers for pol and nap CMS, represent alternate haplotypes of a single RFL-rich locus and act on distinct mitochondrial transcripts, illustrating how one genomic region can serve several cytoplasms [147].

Restorers nevertheless act at every level of mitonuclear gene expression, and the minority that have been cloned do not all encode PPR proteins. At the level of genome organization, the common bean Fr locus eliminates the pvs-orf239-bearing subgenomic molecule from the mitochondrial genome of reproductive tissues instead of acting on its transcript; the effect is dosage-sensitive, homozygotes being fully fertile and heterozygotes only partially so [148,149]. At the translational level, the radish Ogura restorer Rfo, whose active product, PPR-B (ORF687), is itself a PPR protein, leaves orf138 mRNA accumulation unchanged but blocks ORF138 synthesis in the tapetum, so the sterility protein never accumulates [150]. At the post-translational level, sugar beet Rf1 encodes an OMA1-like metallopeptidase, bvORF20, that binds the CMS-specific preSATP6 protein and dismantles the 250 kDa complex in which it resides, generating 200 and 150 kDa species; restoration strength scales with Rf1 expression and with the corresponding depletion of the residual 250 kDa complex [151–153]. The maize T-CMS restorer Rf2 provides the clearest metabolic example: its cloned functional allele Rf2a encodes a mitochondrial aldehyde dehydrogenase whose catalytic activity, rather than any effect on the urf13 transcript or on URF13 accumulation, is required for fertility [154,155]. Most recently, a member of the mitochondrial transcription termination factor (mTERF) family has been identified as a non-PPR restorer in G-type CMS rye, widening the range of protein classes that can be recruited for restoration [156]. Together, these cases argue for classifying restorers by their molecular level of action rather than by the presence or absence of PPRs [143].

Comparative genomics explains why RFL genes supply restorers so disproportionately often. RFL genes reside in chromosomal clusters, undergo frequent duplication and recombination, and show elevated non-synonymous-to-synonymous substitution rates, with diversifying selection concentrated on the PPR residues that make base-specific contacts with RNA. This signature is read as the product of sustained coevolution between a rapidly rearranging mitochondrial genome and nuclear RNA-binding proteins that must continually acquire new sequence specificities [142,157]. Clustering and copy-number variation recur at RFL loci across angiosperms, though the underlying expansion mechanism differs by lineage; tandem duplication and homologous recombination between clustered paralogues dominate in rice, whereas the Brassica Rf region is dispersed and has amplified largely through retrotransposition, but in both cases recombination, rather than point substitution, is the principal generator of novel RNA-recognition profiles [147,157]. The same evolutionary logic extends beyond CMS: in Arabidopsis, RFL8 was selected not to suppress a sterility ORF but to enable translation of ccmFN2, a fragment produced by the Brassicaceae-specific split of the ancestral mitochondrial ccmFN gene; rfl8 is embryo-lethal rather than male-sterile, demonstrating that RFL proteins are recruited to compensate for lineage-specific mitochondrial gene fragmentation as readily as they are to silence CMS ORFs [158].

These advances are now converging on breeding practice. Fine mapping combined with comparative RFL genomics and anther transcriptomics accelerates candidate identification even in polyploid genomes such as wheat, and genome editing both validates candidate RFL genes; knockout of GmPPR576 converted a fertile soybean line into a male-sterile one, and creates novel alleles directly, as when promoter editing of RMS generated artificial restorer lines [116,130,140,146]. Pyramiding several Rf genes, or driving a single Rf from a strong promoter, improves the completeness and environmental stability of restoration, and marker-assisted transfer of Rf alleles remains the operational backbone of three-line hybrid breeding [116]. Several open questions still constrain rational design. Although the PPR–RNA recognition code is now sufficiently well understood to permit designer PPR proteins, predicting the native target of a given RFL protein in vivo remains imperfect, and off-target effects are a documented risk. Many restorers act through protein partners that are still uncharacterized, so the specificity linking one Rf to its cognate S-orf cannot yet be read off from sequence alone. Closing these gaps, refining the recognition code, resolving the complexes through which non-PPR restorers act, and placing ncRNA-mediated retrograde regulation within the CMS/Rf network will be required before fertility restoration can be engineered rather than selected. See more detail in Table 2.

Table 2. Restorer-of-fertility (Rf) genes and their species, CMS systems, protein products, and molecular mechanisms.

Rf Gene	Species	CMS System	Protein Product	Molecular Mechanism	Ref.
Rf-PPR592	*Petunia hybrida*	Petunia CMS	PPR protein (P-class)	Silences mitochondrial sterility ORF; first cloned Rf-PPR gene	[144]
Rfo (PPR-B/ORF687)	*Raphanus sativus*	Ogura CMS	PPR protein (RFL)	Does not alter orf138 mRNA accumulation but blocks ORF138 protein translation in tapetum	[145, 150]
Rf1a	*Oryza sativa*	Boro II CMS	PPR protein (Rf)	Endoribonuclease cleavage of dicistronic B-atp6/orf79 mRNA; also promotes atp6 editing	[133]

Table 2. *Cont.*

Rf Gene	Species	CMS System	Protein Product	Molecular Mechanism	Ref.
Rf1b	*Oryza sativa*	Boro II CMS	PPR protein (Rf)	Silences orf79 mRNA via transcript degradation; Rf1a is epistatic to Rf1b	[133]
RF5	*Oryza sativa*	Honglian CMS (HL-CMS)	PPR protein	Interacts with processing complex; cleaves atp6-orfH79 transcript	[112]
RFL79 (Rf1)	*Triticum aestivum*	T-CMS wheat	RFL protein	Binds mitochondrial orf279 transcript and induces cleavage	[116]
RFL29a (Rf3)	*Triticum aestivum*	T-CMS wheat	RFL protein	Binds mitochondrial orf279 transcript and induces cleavage	[116]
GmPPR576	*Glycine max*	Soybean CMS	RFL PPR protein	CRISPR knockout converts fertile line to sterile, confirming restorer identity	[146]
Rfp	*Brassica napus*	pol CMS	RFL-enriched haplotype	Acts on specific mitochondrial transcripts	[147]
Rfn	*Brassica napus*	nap CMS	RFL-enriched haplotype	Alternative haplotype at same locus as Rfp; acts on different mitochondrial transcripts	[147]
Fr locus	*Phaseolus vulgaris*	CMS (pvs)	Non-PPR (genome level)	Eliminates subgenomic molecule carrying pvs-orf239 from reproductive tissue mitochondria; dosage-sensitive	[148, 149]
Rf1 (bvORF20)	*Beta vulgaris*	Sugar beet CMS (N CMS)	OMA1-like metalloprotease	Binds CMS-specific preSATP6 protein; disassembles 250 kDa complex into 200/150 kDa	[151–153]
Rf2 (Rf2a)	*Zea mays*	T-CMS maize	Mitochondrial aldehyde dehydrogenase	Catalytic activity (not urf13 transcript regulation) restores fertility; metabolic restoration	[154, 155]
mTERF member	*Secale cereale*	G-type CMS rye	mTERF-family protein	First non-PPR restorer identified in rye G-type CMS	[156]

3. Perspectives

Research on the molecular mechanisms of plant male sterility has shifted from the functional characterization of individual genes toward a systems-level, multi-omics dissection of the regulatory networks that govern fertility, thereby establishing a solid theoretical foundation for the exploitation of crop heterosis. Propelled by rapid advances in high-throughput sequencing, genome editing, and artificial intelligence, the field is now entering an era in which mechanistic discovery and breeding application are becoming deeply intertwined.

At the mechanistic level, the wider adoption of single-cell RNA sequencing and spatial transcriptomics is enabling cell-atlas reconstruction of anther development at unprecedented resolution. Single-cell transcriptome atlases spanning multiple developmental stages have already been established in crops such as maize [159], alfalfa [160] and cotton [161], capturing gene-expression dynamics in pivotal cell types including the tapetum and microspore mother cells. Most of these studies, however, remain largely descriptive. Two questions stand out as demanding conceptual breakthroughs: how mitochondrial chimeric genes reprogram nuclear gene expression through retrograde signalling [139], and how restorer genes recognize and neutralize sterility-inducing factors [157]. The regulatory roles of non-coding RNAs, particularly phasiRNAs and lncRNAs, in anther development have only begun to be systematically characterized in recent years [106,107], and both their target networks and modes of action remain to be defined. Equally unresolved are the molecular pathways by which environmental cues such as light, temperature, and humidity drive fertility conversion [162]. Deciphering the crosstalk between these environmental signals and endogenous hormone pathways will provide the theoretical foundation for developing environmentally stable male-sterile lines.

At the technological level, CRISPR/Cas9 and its derivative technologies, notably base and prime editing, have enabled the targeted creation of male-sterile lines across crops, including rice [163], maize [164], cotton [165], tomato [166], and soybean [167]. Genotype dependence, low editing efficiency in polyploids, and off-target effects, however, continue to constrain their widespread adoption. Future efforts should prioritize more efficient and precise delivery and editing platforms that permit direct modification of elite inbred lines, thereby streamlining or even bypassing the conventional backcross-based pipeline. Turning to fertility-control systems, conventional photoperiod- and thermo-sensitive sterile lines remain vulnerable to environmental fluctuations. Conditional sterility systems triggered by humidity offer more stable fertility control and broader applicability [162], whereas those triggered by exogenous chemicals provide an additional and well-established route to pollination control [6]. Multiplex genome editing is expected to further enable composite systems responsive to multiple cues, enhancing the environmental robustness of these lines. Apomixis offers a route to fixing heterosis, although its efficiency and agronomic performance still require optimization [168]. Finally, coupling haploid induction with genome editing should accelerate the production of homozygous male-sterile lines and compress breeding cycles considerably [169].

At the application level, male-sterility technology is steadily moving from laboratory research to large-scale, standardized field production. Three-line and two-line hybrid systems built on male sterility are now well established in staple crops such as rice and maize [128]. In wheat, sterility-based seed production is still constrained by high costs, unstable fertility, and poor compatibility with mechanization [170]. Similar constraints persist in vegetable crops [6]. In forest trees, the creation of sterile lines is slowed by long generation times and the need to evaluate sterility across multiple years [171]. Future work should remain closely aligned with practical breeding needs, combining continued screening and improvement of male-sterile and restorer germplasm to identify materials with broad environmental adaptability and reliable outcrossing seed set. The integration of phenomics, artificial intelligence, and the Internet of Things will enable high-throughput, standardized phenotyping of fertility, improving both the efficiency of sterility screening and the precision of flowering-time synchronization. Advances in agricultural machinery and field management tailored to sterility-based seed production will accelerate the commercialization of crops with lagging hybrid systems, hybrid wheat above all, and extend male-sterility technology to cash crops and ecologically important forest trees. Male sterility can further underpin

transgene containment and biosafety management, thereby supporting the responsible deployment of biotech-based breeding [172].

Overall, research on the molecular mechanisms of plant male sterility continues to advance rapidly, leaving substantial room for deeper theoretical insight and broader application. Through the multi-omics dissection of fertility-regulatory networks, the precise creation of male-sterile lines via genome editing, and the refinement of field-deployment systems and industry standards, current limitations, including fragmented mechanistic understanding, insufficient technical robustness, and limited commercial readiness, can be progressively overcome. This will establish an integrated technical framework spanning fundamental discovery, germplasm development, and seed production. Continued innovation in the theory and technology of plant male sterility will provide a critical foundation for broadening crop breeding resources, enhancing hybrid breeding efficiency, promoting high-quality development of the seed industry, and safeguarding food security.

Author Contributions: Conceptualization, Z.L. and X.Z.; literature curation, Z.L., X.Z., C.Z. and Y.W.; formal analysis, Z.L. and X.Z.; writing—original draft preparation, Z.L. and X.Z.; writing—review and editing, Z.L., X.Z., X.J., C.Z., Y.W. and L.C.; supervision, L.C.; project administration, L.C.; funding acquisition, L.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Modern Agricultural Industrial Park Project, entitled Ginseng Resource Conservation and Breeding Technology Innovation Project (grant number RSCYY2025-01). The Article Processing Charge (APC) was funded by the above project. The sponsors had no role in the design of the study, data collection, analysis, writing, or decision to publish the manuscript.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Cardarelli, M.; Cecchetti, V. Auxin polar transport in stamen formation and development: How many actors? *Front. Plant Sci.* **2014**, *5*, 333. [CrossRef] [PubMed]
2. Ma, H. Molecular genetic analyses of microsporogenesis and microgametogenesis in flowering plants. *Annu. Rev. Plant Biol.* **2005**, *56*, 393–434. [CrossRef] [PubMed]
3. Goldberg, R.B.; Beals, T.P.; Sanders, P.M. Anther development: Basic principles and practical applications. *Plant Cell* **1993**, *5*, 1217–1229. [CrossRef]
4. Blackmore, S.; Worden, A.H.; Barnes, S.H. Pollen wall development in flowering plants. *New Phytol.* **2007**, *174*, 483–498. [CrossRef] [PubMed]
5. Dobritsa, A.A.; Shrestha, J.; Morant, M.; Pinot, F.; Matsuno, M.; Swanson, R.; Møller, B.L.; Preuss, D. CYP704B1 is a long-chain fatty acid ω-hydroxylase essential for sporopollenin synthesis in pollen of Arabidopsis. *Plant Physiol.* **2009**, *151*, 574–589. [CrossRef] [PubMed]
6. Colombo, N.; Galmarini, C.R. The use of genetic, manual and chemical methods to control pollination in vegetable hybrid seed production: A review. *Plant Breed.* **2017**, *136*, 287–299. [CrossRef]
7. Singh, S.; Dey, S.S.; Bhatia, R.; Kumar, R.; Behera, T.K. Current understanding of male sterility systems in vegetable Brassicas and their exploitation in hybrid breeding. *Plant Reprod.* **2019**, *32*, 231–256. [CrossRef] [PubMed]
8. Singh, S.; Vidyasagar. Effect of common salt (NaCl) sprays to overcome the self- incompatibility in the s-allele lines of *Brassica oleracea* var. *Capitata* L. *SABRAO J. Breed. Genet.* **2012**, *44*, 339–348.
9. Sehgal, N.; Singh, S. Progress on deciphering the molecular aspects of cell-to-cell communication in Brassica self-incompatibility response. *3 Biotech* **2018**, *8*, 347. [CrossRef] [PubMed]
10. Dey, S.S.; Singh, S.; Munshi, A.D.; Behera, T.K. Classical genetics and traditional breeding. In *The Cucumber Genome. Compendium of Plant Genomes*; Pandey, S., Weng, Y., Behera, T.K., Bo, K., Eds.; Springer: Cham, Switzerland, 2022.
11. Thakur, P.; Vidyasagar; Singh, S. Evaluation of cytoplasmic male sterile (CMS) progenies and maintainer lines for yield and horticultural traits in cabbage (*Brassica oleracea var. capitata* L.). *SABRAO J. Breed. Genet.* **2015**, *47*, 29–39.

12. Zhu, J.; Lou, Y.; Shi, Q.-S.; Zhang, S.; Zhou, W.-T.; Yang, J.; Zhang, C.; Yao, X.-Z.; Xu, T.; Liu, J.-L.; et al. Slowing development restores the fertility of thermo-sensitive male-sterile plant lines. *Nat. Plants* **2020**, *6*, 360–367. [CrossRef] [PubMed]
13. Kaul, M.L.H. *Male Sterility in Higher Plants*; Springer: Berlin, Germany, 1988.
14. Gorman, M.; McCormick, S. Male gametophyte development. *Plant Cell* **1997**, *9*, 1221–1230. [CrossRef]
15. Smyth, D.R.; Bowman, J.L.; Meyerowitz, E.M. Early flower development in Arabidopsis. *Plant Cell* **1990**, *2*, 755–767. [CrossRef] [PubMed]
16. Taylor, P.; Glover, J.A.; Lavithis, M.; Craig, S.; Singh, M.B.; Knox, R.B.; Dennis, E.S.; Chaudhury, A.M. Genetic control of male fertility in Arabidopsis thaliana: Structural analyses of postmeiotic developmental mutants. *Planta* **1998**, *205*, 492–505. [CrossRef] [PubMed]
17. Theißen, G.; Melzer, R.; Rümpler, F. MADS-domain transcription factors and the floral quartet model of flower development: Linking plant development and evolution. *Development* **2016**, *143*, 3259–3271. [CrossRef] [PubMed]
18. Becker, A.; Theißen, G. The major clades of MADS-box genes and their role in the development and evolution of flowering plants. *Mol. Phylogenet. Evol.* **2003**, *29*, 464–489. [CrossRef] [PubMed]
19. Bowman, J.L.; Smyth, D.R.; Meyerowitz, E.M. Genes directing flower development in Arabidopsis. *Plant Cell* **1989**, *1*, 37–52. [CrossRef] [PubMed]
20. Rounsley, S.D.; Ditta, G.S.; Yanofsky, M.F. Diverse roles for MADS box genes in Arabidopsis development. *Plant Cell* **1995**, *7*, 1259–1269. [CrossRef] [PubMed]
21. Pelaz, S.; Ditta, G.; Baumann, E.; Wisman, E.; Yanofsky, M.F. B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature* **2000**, *405*, 200–203. [CrossRef] [PubMed]
22. Ditta, G.; Pinyopich, A.; Robles, P.; Pelaz, S.; Yanofsky, M.F. The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. *Curr. Biol.* **2004**, *14*, 193–197. [CrossRef] [PubMed]
23. Gomez, P.; Jamilena, M.; Capel, J.; Zurita, S.; Angosto, T.; Lozano, R. Stamenless, a tomato mutant with homeotic conversions in petals and stamens. *Planta* **1999**, *209*, 172–179. [CrossRef] [PubMed]
24. Quinet, M.; Bataille, G.; Dobrev, P.I.; Capel, C.; Gómez, P.; Capel, J.; Lutts, S.; Motyka, V.; Angosto, T.; Lozano, R. Transcriptional and hormonal regulation of petal and stamen development by STAMENLESS, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class APETALA3 gene. *J. Exp. Bot.* **2014**, *65*, 2243–2256. [CrossRef] [PubMed]
25. Fonseca, R.; Capel, C.; Lebrón, R.; Ortiz-Atienza, A.; Yuste-Lisbona, F.J.; Angosto, T.; Capel, J.; Lozano, R. Insights into the functional role of tomato TM6 as a transcriptional regulator of flower development. *Hortic. Res.* **2024**, *11*, uhae019. [CrossRef] [PubMed]
26. Ito, T.; Wellmer, F.; Yu, H.; Das, P.; Ito, N.; Alves-Ferreira, M.; Riechmann, J.L.; Meyerowitz, E.M. The homeotic protein AGAMOUS controls microsporogenesis by regulation of SPOROCYTELESS. *Nature* **2004**, *430*, 356–360. [CrossRef] [PubMed]
27. Wei, B.; Zhang, J.; Pang, C.; Yu, H.; Guo, D.; Jiang, H.; Ding, M.; Chen, Z.; Tao, Q.; Gu, H.; et al. The molecular mechanism of SPOROCYTELESS/NOZZLE in controlling Arabidopsis ovule development. *Cell Res.* **2015**, *25*, 121–134. [CrossRef] [PubMed]
28. Ishiguro, S.; Kawai-Oda, A.; Ueda, J.; Nishida, I.; Okada, K. The DEFECTIVE IN ANTHER DEHISCENCE1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis. *Plant Cell* **2001**, *13*, 2191–2209. [CrossRef]
29. Wang, K.; Guo, Q.; Froehlich, J.E.; Hersh, H.L.; Zienkiewicz, A.; Howe, G.A.; Benning, C. Two Abscisic Ac-id-Responsive Plastid Lipase Genes Involved in Jasmonic Acid Biosynthesis in Arabidopsis thaliana. *Plant Cell* **2018**, *30*, 1006–1022. [CrossRef] [PubMed]
30. Ito, T.; Ng, K.H.; Lim, T.S.; Yu, H.; Meyerowitz, E.M. The homeotic protein AGAMOUS controls late stamen development by regulating a jasmonate biosynthetic gene in Arabidopsis. *Plant Cell* **2007**, *19*, 3516–3529. [CrossRef] [PubMed]
31. Shi, L.; Li, C.; Lv, G.; Li, X.; Feng, W.; Bi, Y.; Wang, W.; Wang, Y.; Zhu, L.; Tang, W.; et al. The adaptor protein ECAP, the corepressor LEUNIG, and the transcription factor BEH3 interact and regulate microsporocyte generation in Arabidopsis. *Plant Cell* **2024**, *36*, 2531–2547. [CrossRef] [PubMed]
32. Sanders, P.M.; Bui, A.Q.; Weterings, K.; McIntire, K.N.; Hsu, Y.C.; Lee, P.Y.; Truong, M.T.; Beals, T.P.; Goldberg, R.B. Anther developmental defects in Arabidopsis thaliana male-sterile mutants. *Sex. Plant Reprod.* **1999**, *11*, 297–322. [CrossRef]
33. Hord, C.L.H.; Chen, C.; DeYoung, B.J.; Clark, S.E.; Ma, H. The BAM1/BAM2 Receptor-Like Kinases Are Important Regulators of Arabidopsis Early Anther Development. *Plant Cell* **2006**, *18*, 1667–1680. [CrossRef] [PubMed]
34. Gómez, J.F.; Talle, B.; Wilson, Z.A. Anther and pollen development: A conserved developmental pathway. *J. Integr. Plant Biol.* **2015**, *57*, 876–891. [CrossRef] [PubMed]
35. Wilson, Z.A.; Zhang, D.B. From Arabidopsis to rice: Pathways in pollen development. *J. Exp. Bot.* **2009**, *60*, 1479–1492. [CrossRef] [PubMed]
36. Murmu, J.; Bush, M.J.; DeLong, C.; Li, S.; Xu, M.; Khan, M.; Malcolmson, C.; Fobert, P.R.; Zachgo, S.; Hepworth, S.R. Arabidopsis basic leucine-zipper transcription factors TGA9 and TGA10 interact with floral glutaredoxins ROXY1 and ROXY2 and are redundantly required for anther development. *Plant Physiol.* **2010**, *154*, 1492–1504. [CrossRef] [PubMed]

37. Yang, S.L.; Jiang, L.; Puah, C.S.; Xie, L.-F.; Zhang, X.-Q.; Chen, L.-Q.; Yang, W.-C.; Ye, D. Overexpression of TAPETUM DETERMINANT1 alters the cell fates in the Arabidopsis carpel and tapetum via genetic interaction with excess microsporocytes1/extra sporogenous cells. *Plant Physiol.* **2005**, *139*, 186–191. [CrossRef] [PubMed]
38. Canales, C.; Bhatt, A.M.; Scott, R.; Dickinson, H. EXS, a putative LRR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in Arabidopsis. *Curr. Biol.* **2002**, *12*, 1718–1727. [CrossRef] [PubMed]
39. Albrecht, C.; Russinova, E.; Hecht, V.; Baaijens, E.; de Vries, S. The Arabidopsis thaliana SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES1 and 2 Control Male Sporogenesis. *Plant Cell* **2005**, *17*, 3337–3349. [CrossRef] [PubMed]
40. Colcombet, J.; Boisson-Dernier, A.; Ros-Palau, R.; Vera, C.E.; Schroeder, J.I. Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASES1 and 2 Are Essential for Tapetum Development and Microspore Maturation. *Plant Cell* **2005**, *17*, 3350–3361. [CrossRef] [PubMed]
41. Yang, S.L.; Xie, L.F.; Mao, H.Z.; Puah, C.S.; Yang, W.C.; Jiang, L.; Sundaresan, V.; Ye, D. TAPETUM DETERMINANT1 Is Required for Cell Specialization in the Arabidopsis Anther. *Plant Cell* **2003**, *15*, 2792–2804. [CrossRef] [PubMed]
42. Ye, Q.; Zhu, W.; Li, L.; Zhang, S.; Yang, Y.; Mao, H.; Wang, X. Brassinosteroids control male fertility by regulating the expression of key genes involved in Arabidopsis anther and pollen development. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 6100–6105. [CrossRef] [PubMed]
43. Chen, W.; Lv, M.; Wang, Y.; Wang, P.-A.; Cui, Y.; Li, M.; Wang, R.; Gou, X.; Li, J. BES1 is activated by EMS1-TPD1-SERK1/2-mediated signaling to control tapetum development in Arabidopsis thaliana. *Nat. Commun.* **2019**, *10*, 4164. [CrossRef] [PubMed]
44. Zhu, J.; Chen, H.; Li, H.; Gao, J.F.; Jiang, H.; Wang, C.; Guan, Y.; Yang, Z. Defective in Tapetal development and function 1 is essential for anther development and tapetal function for microspore maturation in Arabidopsis. *Plant J.* **2008**, *55*, 266–277. [CrossRef] [PubMed]
45. Sun, Z.; Cheng, B.; Zhang, Y.; Meng, L.; Yao, Y.; Liang, Y. SlTDF1: A Key Regulator of Tapetum Degradation and Pollen Development in Tomato. *Plant Sci.* **2025**, *351*, 112321. [CrossRef] [PubMed]
46. Dong, X.; Hong, Z.; Sivaramakrishnan, M.; Mahfouz, M.; Verma, D.P.S. Callose synthase (CalS5) is required for exine formation during microgametogenesis and for pollen viability in Arabidopsis. *Plant J.* **2005**, *42*, 315–328. [CrossRef] [PubMed]
47. Yang, J.; Tian, L.; Sun, M.X.; Huang, X.-Y.; Zhu, J.; Guan, Y.-F.; Jia, Q.-S.; Yang, Z.-N. AUXIN RESPONSE FACTOR17 is essential for pollen wall pattern formation in Arabidopsis. *Plant Physiol.* **2013**, *162*, 720–731. [CrossRef] [PubMed]
48. Huang, X.-Y.; Niu, J.; Sun, M.-X.; Zhu, J.; Gao, J.-F.; Yang, J.; Zhou, Q.; Yang, Z.-N. CDKG1 is associated with the spliceosome to regulate CALLOSE SYNTHASE5 splicing and pollen wall formation in Arabidopsis. *Plant Cell* **2013**, *25*, 637–648. [CrossRef] [PubMed]
49. Shi, Z.-H.; Zhang, C.; Xu, X.-F.; Zhu, J.; Zhou, Q.; Ma, L.-J.; Niu, J.; Yang, Z.-N. Overexpression of AtTTP affects ARF17 expression and leads to male sterility in Arabidopsis. *PLoS ONE* **2015**, *10*, e0117317. [CrossRef] [PubMed]
50. Lou, Y.; Zhou, H.S.; Han, Y.; Zeng, Q.Y.; Zhu, J.; Yang, Z.N. Positive regulation of AMS by TDF1 and the formation of a TDF1-AMS complex are required for anther development in Arabidopsis thaliana. *New Phytol.* **2018**, *217*, 378–391. [CrossRef] [PubMed]
51. Jia, Q.S.; Zhu, J.; Xu, X.F.; Lou, Y.; Zhang, Z.L.; Zhang, Z.P.; Yang, Z.-N. Arabidopsis AT-hook protein TEK positively regulates the expression of arabinogalactan proteins for nexine formation. *Mol. Plant* **2015**, *8*, 251–260. [CrossRef] [PubMed]
52. Bao, H.; Ding, Y.; Yang, F.; Zhang, J.; Xie, J.; Zhao, C.; Du, K.; Zeng, Y.; Zhao, K.; Li, Z.; et al. Gene silencing, knockout and over-expression of a transcription factor ABORTED MICROSPORES (SlAMS) strongly affects pollen viability in tomato (*Solanum lycopersicum*). *BMC Genom.* **2022**, *23*, 346. [CrossRef] [PubMed]
53. Francis, K.E.; Lam, S.Y.; Copenhaver, G.P. Separation of Arabidopsis pollen tetrads is regulated by QUARTET1, a pectin methylesterase gene. *Plant Physiol.* **2006**, *142*, 1004–1013. [CrossRef] [PubMed]
54. Ogawa, M.; Kay, P.; Wilson, S.; Swain, S.M. ADPG1, ADPG2, and QUARTET2 are polygalacturonases required for cell separation during reproductive development in Arabidopsis. *Plant Cell* **2009**, *21*, 256–273. [CrossRef] [PubMed]
55. Rhee, S.Y.; Osborne, E.; Poindexter, P.D.; Somerville, C.R. Microspore separation in the quartet 3 mutants of Arabidopsis is impaired by a defect in a developmentally regulated polygalacturonase required for pollen mother cell wall degradation. *Plant Physiol.* **2003**, *133*, 1170–1180. [CrossRef] [PubMed]
56. Wang, K.Q.; Yu, Y.H.; Jia, X.L.; Zhou, S.D.; Zhang, F.; Zhao, X.; Zhai, M.; Gong, Y.; Lu, J.; Guo, Y.; et al. Delayed callose degradation restores the fertility of multiple P/TGMS lines in Arabidopsis. *J. Integr. Plant Biol.* **2022**, *64*, 717–730. [CrossRef] [PubMed]
57. de Azevedo Souza, C.; Kim, S.S.; Koch, S.; Kienow, L.; Schneider, K.; McKim, S.M.; Haughn, G.W.; Kombrink, E.; Douglas, C.J. A novel fatty acyl-CoA synthetase is required for pollen development and sporopollenin biosynthesis in Arabidopsis. *Plant Cell* **2009**, *21*, 507–525. [CrossRef] [PubMed]
58. Morant, M.; Jørgensen, K.; Schaller, H.; Pinot, F.; Møller, B.L.; Werck-Reichhart, D.; Bak, S. CYP703 is an ancient cytochrome P450 in land plants catalyzing in-chain hydroxylation of lauric acid to provide building blocks for sporopollenin synthesis in pollen. *Plant Cell* **2007**, *19*, 1473–1487. [CrossRef] [PubMed]
59. Aarts, M.; Dirkse, W.; Stiekema, W.; Pereira, A. Transposon tagging of a male sterility gene in Arabidopsis. *Nature* **1993**, *363*, 715–717. [CrossRef] [PubMed]

60. Grienenberger, E.; Kim, S.S.; Lallemand, B.; Geoffroy, P.; Heintz, D.; Souza, C.D.A.; Heitz, T.; Douglas, C.J.; Legrand, M. Analysis of TETRAKETIDE α-PYRONE REDUCTASE function in *Arabidopsis thaliana* reveals a previously unknown, but conserved, biochemical pathway in sporopollenin monomer biosynthesis. *Plant Cell* **2010**, *22*, 4067–4083. [CrossRef] [PubMed]
61. Kim, S.S.; Grienenberger, E.; Lallemand, B.; Colpitts, C.; Kim, S.Y.; de Azevedo Souza, C.; Geoffroy, P.; Heintz, D.; Krahn, D.; Kaiser, M.; et al. LAP6/POLYKETIDE SYNTHASE A and LAP5/POLYKETIDE SYNTHASE B encode hydroxyalkyl α-pyrone synthases required for pollen development and sporopollenin biosynthesis in *Arabidopsis thaliana*. *Plant Cell* **2010**, *22*, 4045–4066. [CrossRef] [PubMed]
62. Shi, J.; Cui, M.; Yang, L.; Kim, Y.J.; Zhang, D. Genetic and biochemical mechanisms of pollen wall development. *Trends Plant Sci.* **2015**, *20*, 741–753. [CrossRef] [PubMed]
63. Wang, K.; Guo, Z.L.; Zhou, W.T.; Zhang, C.; Zhang, Z.Y.; Lou, Y.; Xiong, S.-X.; Yao, X.-Z.; Fan, J.-J.; Zhu, J.; et al. The regulation of sporopollenin biosynthesis genes for rapid pollen wall formation. *Plant Physiol.* **2018**, *178*, 283–294. [CrossRef] [PubMed]
64. Honys, D.; Twell, D. Transcriptome analysis of haploid male gametophyte development in Arabidopsis. *Genome Biol.* **2004**, *5*, R85. [CrossRef] [PubMed]
65. Gibalová, A.; Reňák, D.; Matczuk, K.; Dupl'áková, N.; Cháb, D.; Twell, D.; Honys, D. AtbZIP34 is required for Arabidopsis pollen wall patterning and the control of several metabolic pathways in developing pollen. *Plant Mol. Biol.* **2009**, *70*, 581–601. [CrossRef] [PubMed]
66. Gibalová, A.; Réinová, V.; Štorchová, H. Anther and pollen development transcriptomes with emphasis on the co-expression of TDF1 and AMS. *Front. Plant Sci.* **2017**, *8*, 1887.
67. Lai, Z.; Wang, J.; Peng, S.; Chang, F. BHLH010/089 transcription factors control pollen wall development via specific transcriptional and metabolic networks in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **2022**, *23*, 11683. [CrossRef] [PubMed]
68. Choi, H.; Jin, J.Y.; Choi, S.; Hwang, J.U.; Kim, Y.Y.; Suh, M.C.; Lee, Y. An ABCG/WBC-type ABC transporter is essential for transport of sporopollenin precursors for exine formation in developing pollen. *Plant J.* **2011**, *65*, 181–193. [CrossRef] [PubMed]
69. Quilichini, T.; Samuels, L.; Douglas, C. ABCG26-mediated polyketide trafficking and hydroxycinnamoyl spermidines contribute to pollen wall exine formation in Arabidopsis. *Plant Cell* **2014**, *26*, 4483–4498. [CrossRef] [PubMed]
70. Xu, J.; Yang, C.; Yuan, Z.; Zhang, D.; Gondwe, M.; Ding, Z.; Liang, W.; Zhang, D.; Wilson, Z.A. The ABORTED MICROSPORES regulatory network is required for postmeiotic male reproductive development in *Arabidopsis thaliana*. *Plant Cell* **2010**, *22*, 91–107. [CrossRef] [PubMed]
71. Wang, K.; Zhao, X.; Pang, C.; Zhou, S.; Qian, X.; Tang, N.; Xu, P.; Xu, X.; Gao, J. IMPERFECTIVE EXINE FORMATION (IEF) is required for exine formation and male fertility in Arabidopsis. *Plant Mol. Biol.* **2021**, *105*, 625–635. [CrossRef] [PubMed]
72. Mi, L.; Mo, A.; Yang, J.; Liu, H.; Ren, D.; Che, W.; Long, H.; Jiang, N.; Zhang, T.; Lu, P. Arabidopsis novel microgametophyte defective mutant 1 is required for pollen viability via influencing intine development in Arabidopsis. *Front. Plant Sci.* **2022**, *13*, 814870. [CrossRef] [PubMed]
73. Phan, H.A.; Iacuone, S.; Li, S.; Parish, R. The MYB80 transcription factor is required for pollen development and the regulation of tapetal programmed cell death in *Arabidopsis thaliana*. *Plant Cell* **2011**, *23*, 2209–2224. [CrossRef] [PubMed]
74. Yang, C.; Vizcay-Barrena, G.; Conner, K.; Wilson, Z.A. MALE STERILITY1 is required for tapetal development and pollen wall biosynthesis. *Plant Cell* **2007**, *19*, 3530–3548. [CrossRef] [PubMed]
75. Zhang, D.; Liu, D.; Lv, X.; Wang, Y.; Xun, Z.; Liu, Z.; Li, F.; Lu, H. The cysteine protease CEP1, a key executor involved in tapetal programmed cell death, regulates pollen development in Arabidopsis. *Plant Cell* **2014**, *26*, 2939–2961. [CrossRef] [PubMed]
76. Guo, X.; Li, L.; Liu, X.; Zhang, C.; Yao, X.; Xun, Z.; Zhao, Z.; Yan, W.; Zou, Y.; Liu, D.; et al. MYB2 is important for tapetal PCD and pollen development by directly activating protease expression in Arabidopsis. *Int. J. Mol. Sci.* **2022**, *23*, 3563. [CrossRef] [PubMed]
77. Hsieh, K.; Huang, A.H.C. Tapetosomes in Brassica tapetum accumulate endoplasmic reticulum–derived flavonoids and alkanes for delivery to the pollen surface. *Plant Cell* **2007**, *19*, 582–596. [CrossRef] [PubMed]
78. Wu, S.S.; Cheung, A.Y. Hydroxyproline-rich glycoproteins in plant reproductive tissues: Structure, functions and regulation. *Sex. Plant Reprod.* **2000**, *13*, 131–138.
79. Parish, R.W.; Li, S.F. Death of a tapetum: A programme of developmental altruism. *Plant Sci.* **2010**, *178*, 73–89. [CrossRef]
80. Zhu, L.; He, S.; Liu, Y.; Shi, J.; Xu, J. Arabidopsis FAX1 mediated fatty acid export is required for the transcriptional regulation of anther development and pollen wall formation. *Plant Mol. Biol.* **2020**, *104*, 187–201. [CrossRef] [PubMed]
81. Niu, N.; Liang, W.; Yang, X.; Jin, W.; Wilson, Z.A.; Hu, J.; Zhang, D. EAT1 promotes tapetal cell death by regulating aspartic proteases during male reproductive development in rice. *Nat. Commun.* **2013**, *4*, 1445. [CrossRef] [PubMed]
82. Brownfield, L.; Hafidh, S.; Borg, M.; Sidorova, A.; Mori, T.; Twell, D. A plant germline-specific integrator of sperm specification and cell cycle progression. *PLoS Genet.* **2009**, *5*, e1000430. [CrossRef] [PubMed]
83. Borg, M.; Twell, D. Pollen: Structure and development. In *eLS*; Wiley: Hoboken, NJ, USA, 2011.

84. Borg, M.; Rutley, N.; Kagale, S.; Hamamura, Y.; Gherghinoiu, M.; Kumar, S.; Sari, U.; Esparza-Franco, M.A.; Sakamoto, W.; Rozwadowski, K.; et al. An EAR-dependent regulatory module promotes male germ cell division and sperm fertility in Arabidopsis. *Plant Cell* **2014**, *26*, 2098–2113. [CrossRef] [PubMed]
85. Li, L.; Yang, H.; Zhao, Y.; Hu, Q.; Zhang, X.; Jiang, T.; Jiang, H.; Zheng, B. ARID1 is required to regulate and reinforce H3K9me2 in sperm cells in Arabidopsis. *Nat. Commun.* **2024**, *15*, 7078. [CrossRef] [PubMed]
86. Tabata, R.; Ikezaki, M.; Fujibe, T.; Aida, M.; Tian, C.; Ueno, Y.; Yamamoto, K.T.; Machida, Y.; Nakamura, K.; Ishiguro, S. Arabidopsis AUXIN RESPONSE FACTOR6 and 8 regulate jasmonic acid biosynthesis and floral organ development via repression of class 1 KNOX genes. *Plant Cell Physiol.* **2010**, *51*, 164–175. [CrossRef] [PubMed]
87. Zhang, X.; He, Y.; Li, L.; Liu, H.; Hong, G. Involvement of the R2R3-MYB transcription factor MYB21 and its homologs in regulating flavonol accumulation in Arabidopsis stamen. *J. Exp. Bot.* **2021**, *72*, 4319–4332. [CrossRef] [PubMed]
88. Xue, J.S.; Qiu, S.; Jia, X.L.; Shen, S.Y.; Shen, C.W.; Wang, S.; Xu, P.; Tong, Q.; Lou, Y.-X.; Yang, N.-Y.; et al. Stepwise changes in flavonoids in spores/pollen contributed to terrestrial adaptation of plants. *Plant Physiol.* **2023**, *193*, 627–642. [CrossRef] [PubMed]
89. Jia, X.L.; Xue, J.S.; Zhang, F.; Yao, C.; Shen, S.Y.; Sui, C.X.; Peng, Y.-J.; Xu, Q.-L.; Feng, Y.-F.; Hu, W.-J.; et al. A dye combination for the staining of pollen coat and pollen wall. *Plant Reprod.* **2021**, *34*, 91. [CrossRef] [PubMed]
90. Mitsuda, N.; Seki, M.; Shinozaki, K.; Ohme-Takagi, M. The NAC transcription factors NST1 and NST2 of Arabidopsis regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* **2005**, *17*, 2993–3006. [CrossRef] [PubMed]
91. Zhang, Q.; Xie, Z.; Zhang, R.; Xu, P.; Liu, H.T.; Yang, H.Q.; Doblin, M.S.; Bacic, A.; Li, L.G. Blue light regulates secondary cell wall thickening via MYC2/MYC4 activation of the NST1-directed transcriptional network in Arabidopsis. *Plant Cell* **2018**, *30*, 2512–2528. [CrossRef] [PubMed]
92. Gorguet, B.; Schipper, D.; Van Lammeren, A.; Visser, R.G.F.; Van Heusden, A.W. Ps-2, the gene responsible for functional sterility in tomato, due to non-dehiscent anthers, is the result of a mutation in a novel polygalacturonase gene. *Theor. Appl. Genet.* **2009**, *118*, 1199–1209. [CrossRef] [PubMed]
93. Jung, K.W.; Oh, S.I.; Kim, Y.Y.; Yoo, K.S.; Cui, M.H.; Shin, J.S. Arabidopsis histidine-containing phosphotransfer factor 4 (AHP4) negatively regulates secondary wall thickening of the anther endothecium during flowering. *Mol. Cells* **2008**, *25*, 294–300. [CrossRef]
94. Viola, I.L.; Gonzalez, D.H. TCP transcription factors in plant reproductive development: Juggling multiple roles. *Biomolecules* **2023**, *13*, 750. [CrossRef] [PubMed]
95. Li, T.; Tian, P.; Wang, X.; Li, M.; Xing, S. Overexpression of TCP5 or its dominant repressor form, TCP5-SRDX, causes male infertility in Arabidopsis. *Int. J. Mol. Sci.* **2025**, *26*, 1813. [CrossRef] [PubMed]
96. Wei, D.; Liu, M.; Chen, H.; Zheng, Y.; Liu, Y.; Wang, X.; Yang, S.; Zhou, M.; Lin, J. INDUCER OF CBF EXPRESSION 1 is a male fertility regulator impacting anther dehydration in Arabidopsis. *PLoS Genet.* **2018**, *14*, e1007695. [CrossRef] [PubMed]
97. Chang, F.; Kuo, J.; Wu, M.; Liang, C.-H.; Chen, S.-J.; Yang, C.-C. ICE1 plays a vital role in the development of pollen grains through regulating tapetum degradation and nutrient mobilization in *Arabidopsis thaliana*. *Plant Mol. Biol.* **2026**, *116*, 49. [CrossRef] [PubMed]
98. Fan, Y.; Yang, J.; Mathioni, S.M.; Yu, J.; Shen, J.; Yang, X.; Wang, L.; Zhang, Q.; Cai, Z.; Xu, C.; et al. PMS1T, producing phased small-interfering RNAs, regulates photoperiod-sensitive male sterility in rice. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 15144–15149. [CrossRef] [PubMed]
99. Ding, J.; Lu, Q.; Ouyang, Y.; Mao, H.; Zhang, P.; Yao, J.; Xu, C.; Li, X.; Xiao, J.; Zhang, Q. A long noncoding RNA regulates photoperiod-sensitive male sterility, an essential component of hybrid rice. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 2654–2659. [CrossRef] [PubMed]
100. Zhou, H.; Zhou, M.; Yang, Y.; Li, J.; Zhu, L.; Jiang, D.; Dong, J.; Liu, Q.; Gu, L.; Zhou, L.; et al. RNase ZS1 processes UbL40 mRNAs and controls thermosensitive genic male sterility in rice. *Nat. Commun.* **2014**, *5*, 4884. [CrossRef] [PubMed]
101. Teng, C.; Zhang, H.; Hammond, R.; Huang, K.; Meyers, B.C.; Walbot, V. Dicer-like 5 deficiency confers temperature-sensitive male sterility in maize. *Nat. Commun.* **2020**, *11*, 2912. [CrossRef] [PubMed]
102. Ku, S.J.; Cho, K.H.; Choi, Y.J.; Baek, W.K.; Kim, S.; Suh, H.S.; Chung, Y.Y. Cytological Observation of Two Environmental Genic Male-Sterile Lines of Rice. *Mol. Cells* **2001**, *12*, 403–406. [CrossRef]
103. Vasupalli, N.; Mogilicherla, K.; Shaik, V.; Rao, K.R.S.S.; Bhat, S.R.; Lin, X. Advances in plant male sterility for hybrid seed production: An overview of conditional nuclear male sterile lines and biotechnology-based male sterile systems. *Front. Plant Sci.* **2025**, *16*, 1540693. [CrossRef] [PubMed]
104. Hu, J.; Huang, W.C.; Huang, Q.; Qin, X.X.; Yu, C.C.; Wang, L.L.; Li, S.Q.; Zhu, R.S.; Zhu, Y.G. Mitochondria and cytoplasmic male sterility in plants. *Mitochondrion* **2014**, *19B*, 282–288. [CrossRef] [PubMed]
105. Bohra, A. Cytoplasmic male sterility (CMS) in hybrid breeding in field crops. *Plant Cell Rep.* **2016**, *35*, 967–993. [CrossRef] [PubMed]
106. Štorchová, H. The role of non-coding RNAs in cytoplasmic male sterility in flowering plants. *Int. J. Mol. Sci.* **2017**, *18*, 2429. [CrossRef] [PubMed]

107. Nie, H.; Cheng, C.; Kong, J.; Li, H.; Hua, J. Plant non-coding RNAs function in pollen development and male sterility. *Front. Plant Sci.* **2023**, *14*, 1109941. [CrossRef] [PubMed]
108. Chen, Z.W.; Zhao, N.; Li, S.S.; Grover, C.E.; Nie, H.S.; Wendel, J.F.; Hua, J.P. Plant mitochondrial genome evolution and cytoplasmic male sterility. *Crit. Rev. Plant Sci.* **2017**, *36*, 55–69. [CrossRef]
109. Kim, S.; Kim, C.W.; Park, M.; Choi, D. Identification of candidate genes associated with fertility restoration of cytoplasmic male-sterility in onion (*Allium cepa* L.). *Theor. Appl. Genet.* **2015**, *128*, 2289–2299. [CrossRef] [PubMed]
110. Zhang, Y.J.; Han, Y.; Zhang, M.; Zhang, X.; Guo, L.; Qi, T.; Li, Y.; Feng, J.; Wang, H.; Tang, H.; et al. The cotton mitochondrial chimeric gene orf610a causes male sterility by disturbing the dynamic balance of ATP synthesis and ROS burst. *Crop J.* **2022**, *10*, 1683–1694. [CrossRef]
111. Wang, K.; Gao, F.; Ji, Y.; Liu, Y.; Dan, Z.; Yang, P.; Zhu, Y.; Li, S. ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice. *New Phytol.* **2013**, *198*, 408–418. [CrossRef] [PubMed]
112. Hu, J.; Wang, K.; Huang, W.; Liu, G.; Gao, Y.; Wang, J.; Huang, Q.; Ji, Y.; Qin, X.; Wan, L.; et al. The rice pentatricopeptide repeat protein RF5 restores fertility in Hong-Lian cytoplasmic male-sterile lines via a complex with the glycine-rich protein GRP162. *Plant Cell* **2012**, *24*, 109–122. [CrossRef] [PubMed]
113. Omukai, S.; Arimura, S.I.; Toriyama, K.; Kazama, T. Disruption of mitochondrial open reading frame 352 partially restores pollen development in cytoplasmic male sterile rice. *Plant Physiol.* **2021**, *187*, 236–246. [CrossRef] [PubMed]
114. Hedgcoth, C.; El-Shehawi, A.M.; Ping, W.P.; Clarkson, M.; Tamalis, D. A chimeric open reading frame associated with cytoplasmic male sterility in alloplasmic wheat with Triticum timopheevi mitochondria. *Curr. Genet.* **2002**, *41*, 357–365. [CrossRef] [PubMed]
115. Li, W.; Liu, Z.; Meng, C.; Jia, Y.; Zhang, L.; Song, X. Identification of genes related to the regulation of anther and pollen development in Mu-type cytoplasmic male sterile wheat by transcriptome analysis. *Crop Pasture Sci.* **2019**, *70*, 306–317. [CrossRef]
116. Melonek, J.; Duarte, J.; Martin, J.; Beuf, L.; Murigneux, A.; Varenne, P.; Comadran, J.; Specel, S.; Levadoux, S.; Bernath-Levin, K.; et al. The genetic basis of cytoplasmic male sterility and fertility restoration in wheat. *Nat. Commun.* **2021**, *12*, 1036. [CrossRef] [PubMed]
117. Yang, H.; Xue, Y.; Li, B.; Lin, Y.; Li, H.; Guo, Z.; Li, W.; Fu, Z.; Ding, D.; Tang, J. The chimeric gene atp6c confers cytoplasmic male sterility in maize by impairing the assembly of the mitochondrial ATP synthase complex. *Mol. Plant* **2022**, *15*, 872–886. [CrossRef] [PubMed]
118. Bellaoui, M.; Martin-Canadell, A.; Pelletier, G.; Budar, F. Low-copy-number molecules are produced by recombination, actively maintained and can be amplified in the mitochondrial genome of Brassicaceae. *Mol. Gen. Genet.* **1998**, *257*, 177–185. [CrossRef] [PubMed]
119. Duroc, Y.; Gaillard, C.; Hiard, S.; Defrance, M.-C.; Pelletier, G.; Budar, F. Biochemical and functional characterization of ORF138, a mitochondrial protein responsible for Ogura cytoplasmic male sterility in Brassiceae. *Biochimie* **2005**, *87*, 1089–1100. [CrossRef] [PubMed]
120. Jing, B.; Heng, S.P.; Tong, D.; Wan, Z.J.; Fu, T.D.; Tu, J.X.; Ma, C.Z.; Shen, J.X. A male sterility-associated cytotoxic protein ORF288 in Brassica juncea causes aborted pollen development. *J. Exp. Bot.* **2012**, *63*, 1285–1295. [CrossRef] [PubMed]
121. Iki, Y.; Harada, I.; Ezura, K.; Mashita, S.; Kuwabara, K.; Takei, H.; Toyoda, A.; Shirasawa, K.; Ariizumi, T. Multiple fertility restorer loci for cytoplasmic male sterility caused by mitochondrial gene orf137 in tomato. *J. Exp. Bot.* **2025**, *76*, 6520–6526. [CrossRef] [PubMed]
122. Horn, R.; Hustedt, J.E.G.; Horstmeyer, A.; Hahnen, J.; Zetsche, K.; Friedt, W. The CMS-associated 16 kDa protein encoded by orfH522 in the PET1 cytoplasm is also present in other male-sterile cytoplasms of sunflower. *Plant Mol. Biol.* **1996**, *30*, 523–538. [CrossRef] [PubMed]
123. Young, E.G.; Hanson, M.R. A fused mitochondrial gene associated with cytoplasmic male sterility is developmentally regulated. *Cell* **1987**, *50*, 41–49. [CrossRef] [PubMed]
124. Carlsson, J.; Leino, M.; Sohlberg, J.; Sundstrom, J.F.; Glimelius, K. Mitochondrial regulation of flower development. *Mitochondrion* **2008**, *8*, 74–86. [CrossRef] [PubMed]
125. Ali, A.; Bakhsh, M.Z.M.; Li, S.; Zhang, X.; Tu, J.; Yi, B. Cytoplasmic male sterility: Sterility induction, fertility restoration and gene-for-gene interaction of CMS/Rf genes. *Plant Sci.* **2025**, *360*, 112721. [CrossRef] [PubMed]
126. Chen, L.; Liu, Y.G. Male sterility and fertility restoration in crops. *Annu. Rev. Plant Biol.* **2014**, *65*, 579–606. [CrossRef] [PubMed]
127. Chase, C.D. Cytoplasmic male sterility: A window to the world of plant mitochondrial–nuclear interactions. *Trends Genet.* **2007**, *23*, 81–90. [CrossRef] [PubMed]
128. Kim, Y.J.; Zhang, D. Molecular control of male fertility for crop hybrid breeding. *Trends Plant Sci.* **2018**, *23*, 53–65. [CrossRef] [PubMed]
129. Toriyama, K. Molecular basis of cytoplasmic male sterility and fertility restoration in rice. *Plant Biotechnol.* **2021**, *38*, 285–295. [CrossRef] [PubMed]

130. Bhattacharya, J.; Nitnavare, R.B.; Bhatnagar-Mathur, P.; Reddy, P.S. Cytoplasmic male sterility-based hybrids: Mechanistic insights and biotechnological interventions for crop improvement. *Planta* **2024**, *260*, 53.
131. Stone, J.D.; Kolouškova, P.; Sloan, D.B.; Štorchová, H. Non-coding RNA may be associated with cytoplasmic male sterility in Silene vulgaris. *J. Exp. Bot.* **2017**, *68*, 1599–1612. [CrossRef] [PubMed]
132. Korth, K.L.; Kaspi, C.I.; Siedow, J.N.; Levings, C.S., III. URF13, a maize mitochondrial pore-forming protein, is oligomeric and has a mixed orientation in Escherichia coli plasma membranes. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 10865–10869. [CrossRef] [PubMed]
133. Wang, Z.; Zou, Y.; Li, X.; Zhang, Q.; Chen, L.; Wu, H.; Su, D.; Chen, Y.; Guo, J.; Luo, D.; et al. Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. *Plant Cell* **2006**, *18*, 676–687. [CrossRef] [PubMed]
134. Sang, S.; Cheng, H.; Hao, M.; Ding, B.; Mei, D.; Wang, H.; Wang, W.; Liu, J.; Fu, L.; Liu, K.; et al. Mitochondrial localization of ORF346 causes pollen abortion in alloplasmic male sterility. *Crop J.* **2021**, *9*, 1320–1329. [CrossRef]
135. Saumitou-Laprade, P.; Cuguen, J.; Vernet, P. Cytoplasmic male sterility in plants: Molecular evidence and the nucleocytoplasmic conflict. *Trends Ecol. Evol.* **1994**, *9*, 431–435. [CrossRef] [PubMed]
136. Chase, C.D.; Gabay-Laughnan, S. Cytoplasmic male sterility and fertility restoration by nuclear genes. In *Molecular Biology and Biotechnology of Plant Organelles*; Springer: Dordrecht, The Netherlands, 2004; pp. 593–622.
137. Luo, D.; Xu, H.; Liu, Z.; Guo, J.; Li, H.; Chen, L.; Fang, C.; Zhang, Q.; Bai, M.; Yao, N.; et al. A detrimental mitochondrial-nuclear interaction causes cytoplasmic male sterility in rice. *Nat. Genet.* **2013**, *45*, 573–577. [CrossRef] [PubMed]
138. Guo, J.; Wang, P.; Cheng, Q.; Sun, L.; Wang, H.; Wang, Y.; Kao, L.; Li, Y.; Qiu, T.; Yang, W.; et al. Proteomic analysis reveals strong mitochondrial involvement in cytoplasmic male sterility of pepper (*Capsicum annuum* L.). *J. Proteom.* **2017**, *168*, 15–27. [CrossRef] [PubMed]
139. Fujii, S.; Komatsu, S.; Toriyama, K. Retrograde regulation of nuclear gene expression in CW-CMS of rice. *Plant Mol. Biol.* **2007**, *63*, 405–417. [CrossRef] [PubMed]
140. Suketomo, C.; Kazama, T.; Toriyama, K. Fertility restoration of Chinese wild rice-type cytoplasmic male sterility by CRISPR/Cas9-mediated genome editing of nuclear-encoded RETROGRADE-REGULATED MALE STERILITY. *Plant Biotechnol.* **2020**, *37*, 285–292. [CrossRef] [PubMed]
141. Xiao, S.L.; Zang, J.; Pei, Y.R.; Liu, J.; Liu, J.; Song, W.; Shi, Z.; Su, A.; Zhao, J.; Chen, H. Activation of mitochondrial orf355 gene expression by a nuclear-encoded DREB transcription factor causes cytoplasmic male sterility in maize. *Mol. Plant* **2020**, *13*, 1270–1283. [CrossRef] [PubMed]
142. Fujii, S.; Bond, C.S.; Small, I.D. Selection patterns on restorer-like genes reveal a conflict between nuclear and mitochondrial genomes throughout angiosperm evolution. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 1723–1728. [CrossRef] [PubMed]
143. Kubo, T.; Arakawa, T.; Honma, Y.; Kitazaki, K. What does the molecular genetics of different types of restorer-of-fertility genes imply? *Plants* **2020**, *9*, 361. [CrossRef] [PubMed]
144. Bentolila, S.; Alfonso, A.A.; Hanson, M.R. A pentatricopeptide repeat-containing gene restores fertility to cytoplasmic male-sterile plants. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10887–10892. [CrossRef] [PubMed]
145. Brown, G.G.; Formanová, N.; Jin, H.; Wargachuk, R.; Dendy, C.; Patil, P.; Laforest, M.; Zhang, J.; Cheung, W.Y.; Landry, B.S. The radish Rfo restorer gene of Ogura cytoplasmic male sterility encodes a protein with multiple pentatricopeptide repeats. *Plant J.* **2003**, *35*, 262–272. [CrossRef] [PubMed]
146. Wang, T.; He, T.; Ding, X.; Zhang, Q.; Yang, L.; Nie, Z.; Zhao, T.; Gai, J.; Yang, S. Confirmation of GmPPR576 as a fertility restorer gene of cytoplasmic male sterility in soybean. *J. Exp. Bot.* **2021**, *72*, 7729–7742. [CrossRef] [PubMed]
147. Gaborieau, L.; Brown, G.G. Comparative genomic analysis of the compound Brassica napus Rf locus. *BMC Genom.* **2016**, *17*, 834. [CrossRef] [PubMed]
148. Mackenzie, S.A.; Chase, C.D. Fertility restoration is associated with loss of a portion of the mitochondrial genome in cytoplasmic male-sterile common bean. *Plant Cell* **1990**, *2*, 905–915. [CrossRef]
149. Janska, H.; Sarria, R.; Woloszynska, M.; Arrieta-Montiel, M.; Mackenzie, S.A. Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. *Plant Cell* **1998**, *10*, 1163–1180. [CrossRef] [PubMed]
150. Uyttewaal, M.; Arnal, N.; Quadrado, M.; Martin-Canadell, A.; Vrielynck, N.; Hiard, S.; Gherbi, H.; Bendahmane, A.; Budar, F.; Mireau, H. Characterization of Raphanus sativus pentatricopeptide repeat proteins encoded by the fertility restorer locus for Ogura cytoplasmic male sterility. *Plant Cell* **2008**, *20*, 3331–3345. [CrossRef] [PubMed]
151. Matsuhira, H.; Kagami, H.; Kurata, M.; Kitazaki, K.; Matsunaga, M.; Hamaguchi, Y.; Hagihara, E.; Ueda, M.; Harada, M.; Muramatsu, A.; et al. Unusual and typical features of a novel restorer-of-fertility gene of sugar beet (*Beta vulgaris* L.). *Genetics* **2012**, *192*, 1347–1358. [CrossRef] [PubMed]

152. Kitazaki, K.; Arakawa, T.; Matsunaga, M.; Yui-Kurino, R.; Matsuhira, H.; Mikami, T.; Kubo, T. Post-translational mechanisms are associated with fertility restoration of cytoplasmic male sterility in sugar beet (*Beta vulgaris*). *Plant J.* **2015**, *83*, 290–299. [CrossRef] [PubMed]
153. Arakawa, T.; Ue, S.; Sano, C.; Matsunaga, M.; Kagami, H.; Yoshida, Y.; Kuroda, Y.; Taguchi, K.; Kitazaki, K.; Kubo, T. Identification and characterization of a semi-dominant restorer-of-fertility 1 allele in sugar beet (*Beta vulgaris*). *Theor. Appl. Genet.* **2020**, *133*, 427–440. [CrossRef] [PubMed]
154. Cui, X.; Wise, R.P.; Schnable, P.S. The rf2 nuclear restorer gene of male-sterile T-cytoplasm maize. *Science* **1996**, *272*, 1334–1336. [CrossRef] [PubMed]
155. Liu, F.; Cui, X.; Horner, H.T.; Weiner, H.; Schnable, P.S. Mitochondrial aldehyde dehydrogenase activity is required for male fertility in maize. *Plant Cell* **2001**, *13*, 1063–1078. [CrossRef] [PubMed]
156. Vendelbo, N.M.; Mahmood, K.; Sarup, P.; Kristensen, P.S.; Orabi, J.; Jahoor, A. Genomic scan of male fertility restoration genes in a Gülzow-type hybrid breeding system of rye (*Secale cereale* L.). *Int. J. Mol. Sci.* **2021**, *22*, 9277. [CrossRef] [PubMed]
157. Melonek, J.; Stone, J.D.; Small, I. Evolutionary plasticity of restorer-of-fertility-like proteins in rice. *Sci. Rep.* **2016**, *6*, 35152. [CrossRef] [PubMed]
158. Nguyen, T.N.; Planchard, N.; Dahan, J.; Arnal, N.; Balzergue, S.; Benamar, A.; Bertin, P.; Brunaud, V.; Dargel-Graffin, C.; Macherel, D.; et al. A case of gene fragmentation in plant mitochondria fixed by the selection of a compensatory restorer-of-fertility-like PPR gene. *Mol. Biol. Evol.* **2021**, *38*, 2831–2845. [CrossRef] [PubMed]
159. Nelms, B.; Walbot, V. Defining the developmental program leading to meiosis in maize. *Science* **2019**, *364*, 52–56. [CrossRef] [PubMed]
160. Zhang, S.; Zhang, L.; Cai, H.; Zhang, J.; Yang, Z.; Yan, Q.; Wu, F.; Wang, Y.; Xu, B. Single-Cell Transcriptome Atlas and Dynamic Regulatory Mechanisms of Anther Development in Alfalfa. *Plant Biotechnol. J.* **2025**, *23*, 4966–4980. [CrossRef] [PubMed]
161. Li, Y.; Ma, H.; Wu, Y.; Ma, Y.; Yang, J.; Li, Y.; Yue, D.; Zhang, R.; Kong, J.; Lindsey, K.; et al. Single-Cell Transcriptome Atlas and Regulatory Dynamics in Developing Cotton Anthers. *Adv. Sci.* **2024**, *11*, 2304017. [CrossRef] [PubMed]
162. Wang, D.; Xie, H.; Zhang, Y.; Zhang, M.; Tang, X.; Yu, Y.; Zhu, M. Advances in the Mechanistic Study of Environment-Sensitive Genic Male Sterility in Rice. *Rice* **2026**, *19*, 11. [CrossRef] [PubMed]
163. Chang, Z.; Chen, Z.; Wang, N.; Xie, G.; Lu, J.; Yan, W.; Zhou, J.; Tang, X.; Deng, X.W. Construction of a male sterility system for hybrid rice breeding and seed production using a nuclear male sterility gene. *PNAS* **2016**, *113*, 14145–14150. [CrossRef] [PubMed]
164. Svitashev, S.; Schwartz, C.; Lenderts, B.; Young, J.K.; Cigan, A.M. Genome editing in maize directed by CRISPR-Cas9 ribonucleoprotein complexes. *Nat. Commun.* **2016**, *7*, 13274. [CrossRef] [PubMed]
165. Ramadan, M.; Alariqi, M.; Ma, Y.; Li, Y.; Liu, Z.; Zhang, R.; Jin, S.; Min, L.; Zhang, X. Efficient CRISPR/Cas9 mediated pooled-sgRNAs assembly accelerates targeting multiple genes related to male sterility in cotton. *Plant Methods* **2021**, *17*, 16. [CrossRef] [PubMed]
166. Du, M.; Zhou, K.; Liu, Y.; Deng, L.; Zhang, X.; Lin, L.; Zhou, M.; Zhao, W.; Wen, C.; Xing, J.; et al. A biotechnology-based male-sterility system for hybrid seed production in tomato. *Plant J.* **2020**, *102*, 1090–1100. [CrossRef] [PubMed]
167. Nadeem, M.; Chen, A.; Hong, H.; Li, D.; Li, J.; Zhao, D.; Wang, W.; Wang, X.; Qiu, L. GmMs1 encodes a kinesin-like protein essential for male fertility in soybean (*Glycine max* L.). *J. Integr. Plant Biol.* **2021**, *63*, 1054–1064. [CrossRef] [PubMed]
168. Khanday, I.; Skinner, D.; Yang, B.; Mercier, R.; Sundaresan, V. A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature* **2019**, *565*, 91–95. [CrossRef] [PubMed]
169. Kelliher, T.; Starr, D.; Su, X.; Tang, G.; Chen, Z.; Carter, J.; Wittich, P.E.; Dong, S.; Green, J.; Burch, E.; et al. One-step genome editing of elite crop germplasm during haploid induction. *Nat. Biotechnol.* **2019**, *37*, 287–292. [CrossRef] [PubMed]
170. Abdullah, M.; Sheraz, U.; Tul Ain, A.; Nasir, B.; Hammad, S.; Shokat, S. Exploring the Strategies of Male Sterility for Hybrid Development in Hexaploid Wheat: Prevailing Methods and Potential Approaches. *Agriculture* **2025**, *15*, 807.
171. Fritsche, S.; Klocko, A.L.; Boron, A.; Brunner, A.M.; Thorlby, G. Strategies for Engineering Reproductive Sterility in Plantation Forests. *Front. Plant Sci.* **2018**, *9*, 1671. [CrossRef] [PubMed]
172. Zhang, C.; Norris-Caneda, K.H.; Rottmann, W.H.; Gulledge, J.E.; Chang, S.; Kwan, B.Y.-H.; Thomas, A.M.; Mandel, L.C.; Kothera, R.T.; Victor, A.D.; et al. Control of Pollen-Mediated Gene Flow in Transgenic Trees. *Plant Physiol.* **2012**, *159*, 1319–1334. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.