Review

Pre-mRNA Splicing Functions in Plant Sexual Reproduction Development

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Abstract: Precursor messenger RNA (pre-mRNA) splicing is a critical post-transcriptional regulatory mechanism in gene expression. The precise splicing of pre-mRNAs is essential for plant development and responding to genetic and environmental signals. In plant sexual reproduction, gene expression regulation relies on the accurate processing of pre-mRNAs, which is fundamental for coordinating developmental programs. The alternation of generations in plants involves two key phases: gametophyte development, which produces gametes, and fertilization, which leads to the formation of a diploid sporophyte. Gametophyte and embryo development represent essential processes in plant sexual reproduction. This review focuses on summarizing and analyzing the current evidence regarding the role of pre-mRNA splicing in plant sexual reproduction, with an emphasis on its involvement in gametophyte formation and embryo development. Future challenges in understanding RNA splicing regulation in plant sexual reproduction are also discussed, particularly in modulating splicing factor levels and activities and identifying target mRNAs and noncoding RNAs regulated by these factors. This review provides crucial insights into the molecular mechanisms of plant reproductive development and offers a theoretical basis for improving plant fertility and adaptability via RNA splicing regulation.

Keywords: gametophyte formation; embryo development; pre-mRNA splicing; plant sexual reproduction

1. Introduction

Higher plants undergo two distinct multicellular stages in their life cycle: the haploid gametophyte (pollen and embryo sac) and the diploid sporophyte (embryo and seedling). Gametophytes generate gametes, and their fusion gives rise to the diploid sporophyte, marking two critical phases in the alternation of generations. Gene expression regulation plays a key role in gametophyte formation and embryo development. Precursor messenger RNA (pre-mRNA) splicing is a post-transcriptional regulatory mechanism that involves the removal of introns and the joining of exons to produce mature mRNAs for translation. Since plant growth and development rely on precise gene expression, accurate pre-mRNA processing is essential for maintaining normal cellular functions and responding to genetic and environmental signals [1–6]. Studies have shown that pre-mRNA splicing influences plant sexual reproduction. It is noteworthy that many genes involved in plant pre-mRNA splicing are essential genes, and their homozygous mutations often result in embryonic lethality, while defects in heterozygous mutants are particularly pronounced in the haploid gametophyte and early embryogenesis. Therefore, the haploid generation and early embryogenesis stage are ideal for analyzing the role of pre-mRNA splicing in plant sexual

reproduction. This review summarizes the current findings on the role of pre-mRNA splicing in gametophyte formation and embryo development and discusses future challenges in understanding its regulatory impact on plant reproduction.

2. Pre-mRNA Splicing

Introns are non-coding sequences within genes that are removed from precursor RNA during splicing. Based on their splicing mechanisms, introns are classified into four major groups: self-splicing group I and group II introns, tRNA and/or archaeal introns, and spliceosome-catalyzed introns [7–10]. The excision of group I introns occurs through a two-step transesterification reaction that requires exogenous guanosine (exoG) as a cofactor (Figure 1A). On the other hand, group II introns undergo splicing via a different mechanism, forming a lariat structure during intron removal (Figure 1B). The removal of introns from tRNA involves splicing endonuclease and ligase, which process the precursor to generate a functional tRNA (Figure 1C). Spliceosomal introns in nuclear pre-mRNA are excised by the spliceosome, a large ribonucleoprotein complex [11], and their removal follows a mechanism similar to that of group II introns (Figure 1D).
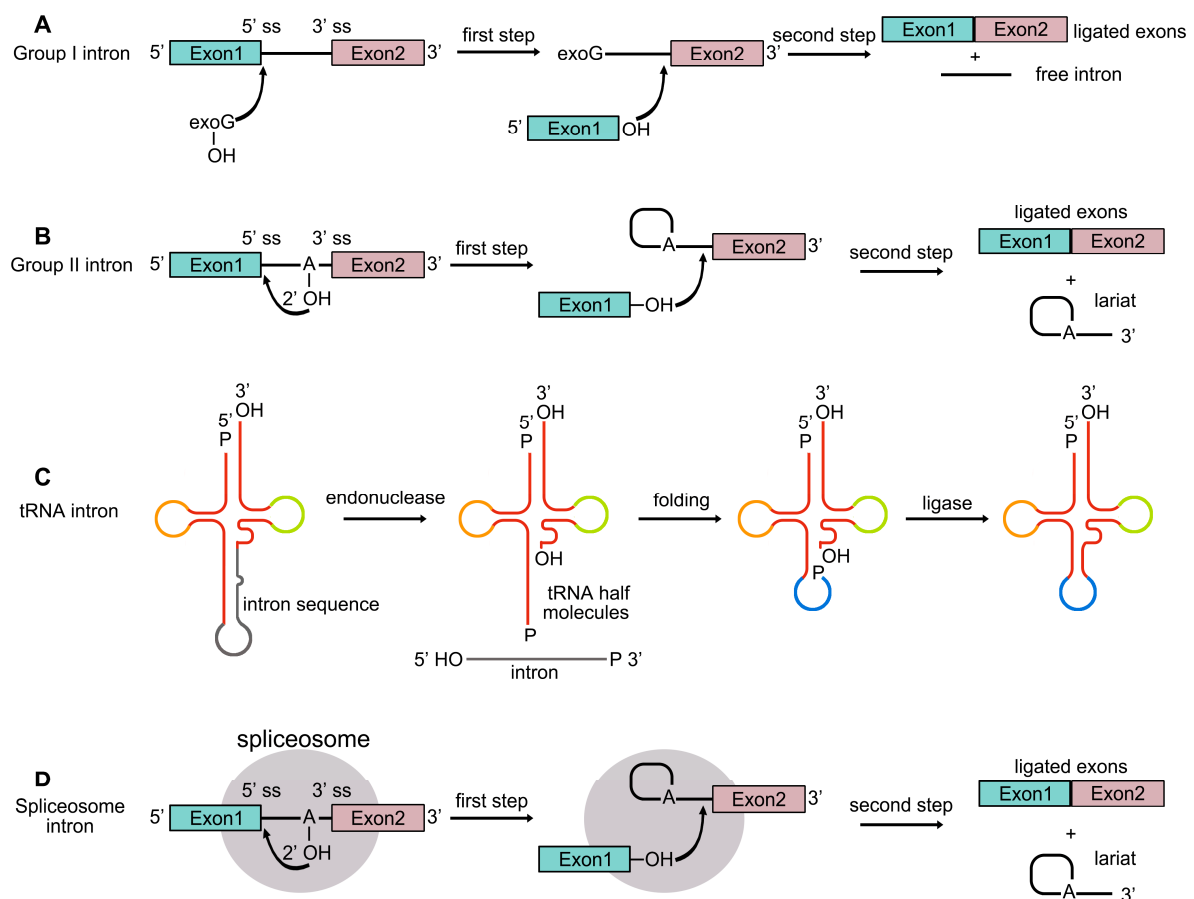

Figure 1. Schematic representation of four pre-mRNA splicing mechanisms. (**A**) In group I introns, splicing is initiated when a cofactor attacks the 5′ splice site (5′ ss) and attaches to the intron, releasing the upstream exon. The 3′ end of the released exon then attacks the 3′ splice site (3′ ss), resulting in exon ligation and intron release. (**B**) Group II introns undergo a similar process, where the 2′-OH of adenosine at the branch site attacks the 5′ ss, leading to cleavage of the 5′ exon and formation of a lariat-3′exon intermediate. The 3′-OH of the cleaved 5′ exon then attacks the 3′ ss, producing ligated exons and a lariat intron. (**C**) tRNA introns are removed by a splicing endonuclease, generating exons with 2′,3′-cyclic phosphate and 5′-OH ends. The exon halves fold into a tRNA-like structure, which is then sealed by a ligase. (**D**) Spliceosome introns follow a two-step transesterification reaction akin to group II introns, but the process is catalyzed by the spliceosome complex.

Studies in metazoans have shown that the spliceosome can be classified into two types based on the introns they process: the U2-type (major) spliceosome and the U12-type (minor) spliceosome [12]. The major spliceosome comprises five small nuclear ribonucleoproteins (snRNPs)—U1, U2, U4/U6, and U5—along with various non-snRNP factors. Spliceosome-mediated splicing is a complex and dynamic process involving the sequential assembly and disassembly of different snRNPs [13]. This process can be broadly divided into two phases, spliceosome assembly and catalysis, ultimately leading to the formation of a mature transcript (Figure 2). During spliceosome assembly, the process begins with U1 snRNP binding to the 5′ splice site through RNA/RNA base pairing. Simultaneously, splicing factor 1 (SF1) and U2 auxiliary factor (U2AF) recognize and bind to the branch point sequence (BPS) and the polypyrimidine tract (PPT), respectively. U2AF consists of two subunits: U2AF65, which binds the PPT, and U2AF35, which associates with the 3′ splice site. This initial assembly forms the E (early) complex. Next, complex A is established as U2 snRNP binds to the BPS. The U4/U5/U6 tri-snRNP then joins, leading to the formation of complex B. The later dissociation of U1 and U4 triggers structural rearrangements, resulting in an activated complex (B_{act}), which then transitions into the catalytically active B* complex. This final complex facilitates intron lariat removal and exon ligation [14]. Furthermore, various non-snRNP protein factors contribute to the splicing process, including the NineTeen Complex (NTC), NTC-associated proteins, serine/arginine (SR)-rich proteins, the REtention and Splicing (RES) complex, and ATPases [15].
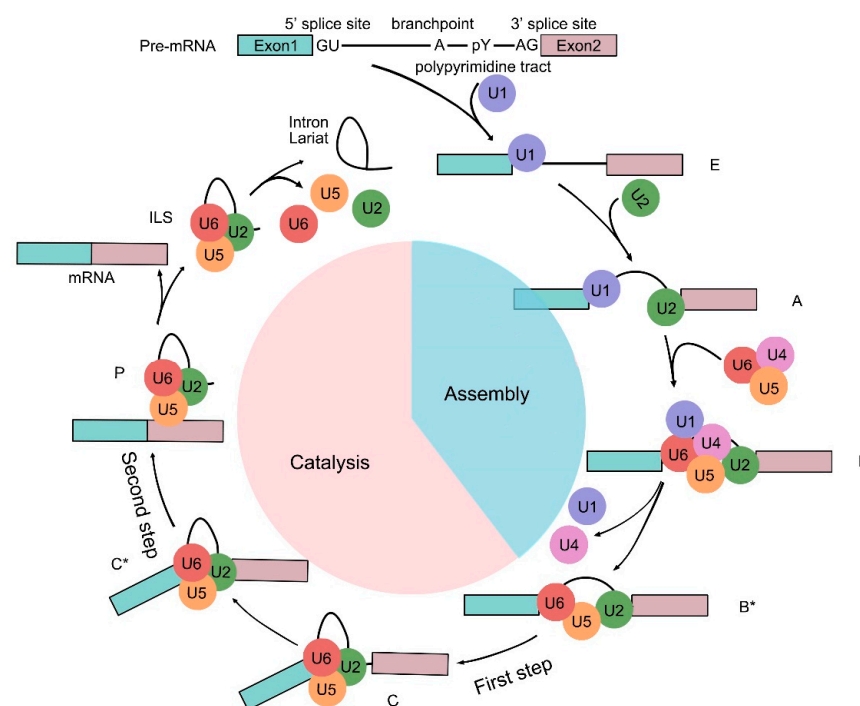

Figure 2. A basic model of the RNA splicing mechanism. The schematic diagram represents the spliceosome assembly and catalysis during transcript maturation. The process begins with U1 snRNP binding to the 5′ splice site of the intron in the pre-mRNA, forming complex E, while U2 snRNP binds to the branch site to form complex A. The U4/U5/U6 tri-snRNP then joins complex A to form the B complex. After the dissociation of U1 and U4, the spliceosome undergoes rearrangements, resulting in the activated Bact complex. The catalytically active B* complex is formed, facilitating the two transesterification reactions that remove the intron lariat and ligate the two exons. E: early complex, the early spliceosome. A: A complex, pre-spliceosome. B: B complex, precatalytic spliceosome. B*: B*complex, catalytically activated spliceosome. C: C complex, catalytic step I spliceosome. C*: C* complex, step II catalytically activated spliceosome. P: P complex, post-splicing complex. ILS: intron-lariat spliceosome.

3. Role of Splicing in Pollen Development

Alternative splicing increases functional complexity in plants by producing multiple transcript variants from a single gene, thus improving protein diversity [16–19]. Emerging evidence indicates that alternative splicing isoforms play a role in male gametophyte development (Figure 3, Table 1). *AUXIN RESPONSE FACTOR 8 (ARF8)* produces multiple functionally distinct transcripts through alternative splicing, including *ARF8.1*, *ARF8.2*, and *ARF8.4*, each contributing to different stages and processes of pollen development. The full-length isoform, *ARF8.1*, regulates pollen cell wall formation by directly controlling the expression of key transcription factors—*DEFECTIVE IN TAPETAL DEVELOPMENT (TDF1)*, *ABORTED MICROSPORE (AMS)*, and *MALE STERILITY 188 (MS188)*—in tapetum cells, which are essential for post-meiotic pollen and tapetum development [20]. Anther dehiscence, a crucial step for pollen release and pollination, can be prematurely triggered by the combined action of *ARF8.2* and *ARF8.4*. *ARF8.2* facilitates anther dehiscence by modulating jasmonic acid (JA) biosynthesis through the regulation of *DEFECTIVE IN ANTHER DEHISCENCE1 (DAD1)*. Meanwhile, *ARF8.4* accelerates anther dehiscence by regulating *MYB26*, which influences anther wall lignification. The *ARF8.4* promotes filament elongation by controlling the expression of *INDOLE-3-ACETIC ACID INDUCIBLE19 (AUX/IAA19)*, ensuring optimal pollen positioning for successful pollination [21,22]. Lily 135-ABP is a villin superfamily protein that plays a role in actin cytoskeleton rearrangement. Overexpression of *ABP29*, a splicing variant of *135-ABP*, in lily pollen significantly disrupts actin filament elongation and depolymerization, leading to inhibited pollen germination and pollen tube growth [23]. In *Brassica juncea* var. *tumida*, *T1170* and *T1243* are alternative transcripts of the mitochondrial *T* gene, which is associated with cytoplasmic male sterility. While these isoforms display distinct expression patterns, only *T1243* contributes to the male sterility phenotype [24]. In *Arabidopsis thaliana*, the Jasmonate ZIM-domain 10 (JAZ10) protein functions as a repressor of jasmonate signaling and contains both a Jas domain and a ZIM domain [25]. *JAZ10.4* is a splice variant of *JAZ10* that encodes a protein lacking the Jas domain. Transgenic plants overexpressing *JAZ10.4* show strong JA insensitivity and male sterility due to impaired filament elongation and anther dehiscence [26]. Reactive oxygen species (ROS) signaling plays a crucial role in plant reproduction [27,28]. In *Arabidopsis*, the respiratory-burst oxidase homolog E (RBOHE) is a tapetum-specific NADPH oxidase involved in ROS production, essential for tapetal function and pollen development. *RBOHE* has three splice variants, *RBOHE.1–3*. Overexpression of the *RBOHE.3* splice variant disrupts tapetal ROS homeostasis, accelerates tapetal programmed cell death (PCD), and leads to male sterility [29].

Numerous studies have highlighted the critical role of splicing factors in regulating male gametophyte development (Figure 3, Table 1). U2AF65, a component of U2AF, is essential for recruiting the U2 snRNP complex. It plays a key role in defining 3′ splice sites and regulating alternative splicing [30]. In *Arabidopsis*, two isoforms of *U2AF65*, *U2AF65a* and *U2AF65b*, have been identified. *AtU2AF65b* is involved in the splicing of *FLOWERING LOCUS C* and *ABSCISIC ACID-INSENSITIVE 5*, influencing flowering [31]. The *atu2af65a atu2af65b* double mutants show reduced male transmission and defective pollen tube growth [32]. However, the *AtU2AF65a/b*-spliced genes essential for male gametophyte function and pollen tube growth remain unidentified. Furthermore, a mutation in *GAMETOPHYTIC FACTOR 1 (GFA1)*, which encodes a U5 snRNP-associated protein, disrupts pollen tube growth toward the female gametophyte (FG) [33]. The target genes of GFA1 that regulate pollen tube growth, however, remain unknown.
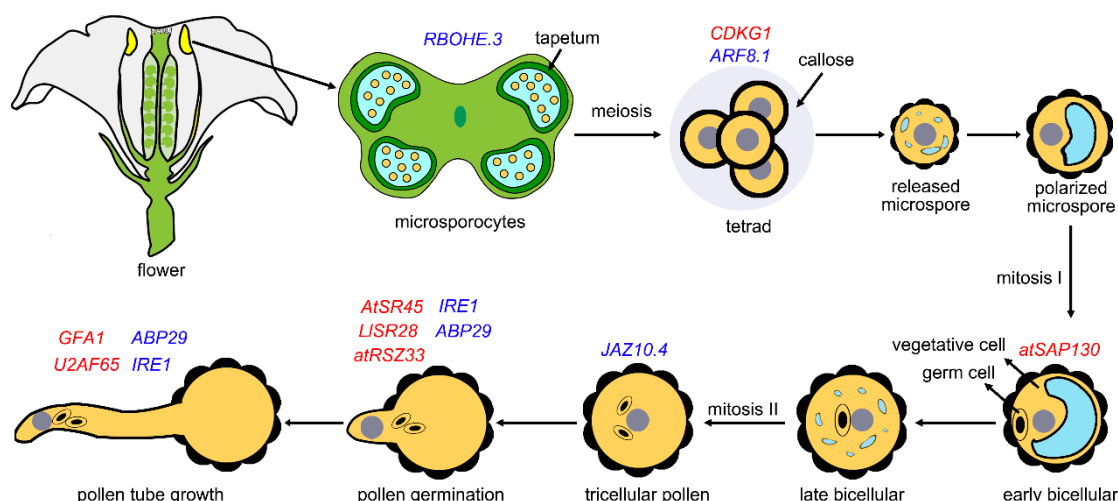
Figure 3. A role of splicing factors in pollen development, germination, and tube growth in *Arabidopsis*. Schematic overview of pollen development, germination, and tube growth in *Arabidopsis*, emphasizing splicing factors crucial for these processes. Pollen development begins within the anther locules, where microsporocytes undergo two meiotic divisions, producing tetrads of four haploid microspores enclosed by callosic cell walls. The tetrads separate as callose is degraded, releasing individual microspores, which then enlarge, polarize, and undergo asymmetric division to form a large vegetative cell and a small generative cell. The generative cell then divides once more to produce two sperm cells, resulting in mature tricellular pollen. Upon germination, pollen grains extend pollen tubes for fertilization. The genes labeled in the figure indicate their functional stages, with red for splicing factors and blue for splicing-related genes.

Table 1. RNA splicing factors involved in male gamete formation.

Species	Biological Process	Type of Splicing Factor	Splicing Factor	Targeted Genes	References
Arabidopsis thaliana	Male gametophyte transmission, pollen tube growth	Non-snRNP, one subunit of U2AF	U2AF65	Unknown	[32]
A. thaliana	Male gametophyte transmission	DEAH-box RNA-dependent ATPase Prp16	CUV	Genes involved in auxin biosynthesis, polar auxin transport, and auxin perception and signaling	[34]
A. thaliana	Pollen tube growth	U5 snRNP component	GFA1	Unknown	[33,35,36]
A. thaliana	Pollen wall formation	Cyclin-dependent protein kinases (SR protein)	CDKG1	*CalS5*	[37]
A. thaliana	Male fertility at modestly ET (elevated temperature)	A major component of the UPR signaling pathway	IRE1	*bZIP60*	[38]
Lilium longiflorum	Pollen germination	SR protein	LlSR28	*AtVLN1*	[39]
A. thaliana	Pollen germination	SR protein	AtSR45	*AtVLN1*	[39]
A. thaliana	Pollen germination, pollen tube growth	SR protein	atRSZ33	Unknown	[40]
A. thaliana	Pollen development	A U2 snRNP-associated protein	AtSAP130	*QRT1* and *QRT3*	[41]
A. thaliana	Male gametophyte transmission	A U4/U6 snRNP associated protein	RDM16	Unknown	[42]
A. thaliana	Male gametophyte development	U5 snRNP component	PRP8A, PRP8B	Unknown	[43]

CYCLIN-DEPENDENT KINASE G1 (CDKG1), an SR motif-containing protein, is recruited to U1 snRNP through its interaction with an arginine/serine-rich splicing factor, facilitating the efficient splicing of *Callose synthase 5* (*CalS5*) [37]. *CalS5* encodes the primary enzyme responsible for synthesizing the callose layer during microspore development [44].

As a result, the *Arabidopsis CDKG1* loss-of-function mutant shows impaired male fertility, characterized by defective pollen wall formation at the tetrad stage [37]. Further studies have established the role of *CDKG1* in meiosis, particularly under high-temperature conditions, where it is essential for homologous chromosome pairing and recombination during male meiosis. Specifically, CDKG1, in complex with CYCLINL, maintains chromosome pairing stability and crossover events, ensuring proper meiotic progression. While *cdkg1* mutants display normal vegetative growth, they become sterile under high temperatures [45]. The findings of a recent study indicate that the long isoform of *CDKG1* (*CDKG1L*) is required for fertility maintenance under high-temperature conditions, whereas the short isoform (*CDKG1S*) cannot rescue fertility in the *cdkg1* mutant [46]. This suggests that the *CDKG1* function is determined not only by its kinase activity but also by the expression and role of its splice isoforms.

Arabidopsis SR45 (AtSR45) is an SR-rich protein that regulates the alternative splicing of multiple *SR* gene mRNAs [47]. In the *atsr45* mutant, the full-length transcript of *VILLIN1* increases, while the short transcript decreases, resulting in reduced F-actin levels in hydrated pollen [39]. The lily LlSR28 shares structural similarities with AtSR45. In *atsr45* mutants, pollen germination occurs earlier than in the wild type, whereas *LlSR28* overexpression in *Arabidopsis* completely inhibits pollen germination. These findings suggest that *LlSR28* and *AtSR45* regulate filamentous actin dynamics by modulating the alternative splicing of *VILLIN1*, therefore influencing pollen germination [39]. Similarly, *Arabidopsis* RSZ33 (AtRSZ33) is an SR protein involved in the splicing of *AtSRp30* and *AtSRp34/SR1*. The overexpression of *AtRSZ33* leads to impaired pollen germination [40]. However, the specific target genes regulated by *AtRSZ33* remain unidentified.

4. Splicing and Female Gametogenesis

Splicing factors also play a crucial role in the normal development of female gametophytes. Mutations in spliceosome-associated genes frequently lead to defects in female gametophyte development (Figure 4, Table 2). For example, GFA1 directly interacts with two U5 snRNP components, AtBRR2 and AtPRP8, as well as the RNA helicase ROOT INITIATION DEFECTIVE 1 (RID1), a homolog of the yeast splicing factor Prp22 in *Arabidopsis* [33,35,36]. *GFA1* and *RID1* are essential for embryo sac development, as they participate in the pre-mRNA splicing of key genes required for female gametophyte formation, including *EMBRYO SAC DEVELOPMENT ARREST 26* and *4*, *FOLYLPOLYGLUTAMATE SYNTHETASE ISOFORM 3*, and *GAST1 PROTEIN HOMOLOG 4* [36,48]. ATROPOS (ATO), a homolog of the yeast pre-mRNA processing factor 9 (PRP9), is a splicing factor associated with U2 snRNP-binding pre-mRNA. Mutation in *ATO* leads to fertility defects, resulting in the production of supernumerary egg cells during female gametophyte development in *Arabidopsis* [49]. *LACHESIS* (*LIS*) encodes an *Arabidopsis* homolog of yeast PRP4, an RNA splicing factor with seven WD40 repeats essential for early spliceosome assembly. In *lis* mutants, female gametophytes show impaired pollen tube attraction, and many accessory cells differentiate into extra egg cells [50,51]. CWC15 is a potential component of the NTC, and its loss of function leads to widespread alterations in splice sites, primarily characterized by increased intron retention. The *cwc15-2* T-DNA insertion mutant displays defective double fertilization, mainly due to significantly reduced female gametophyte transmission efficiency [52]. *Arabidopsis RRC1* encodes a protein similar to the human splicing factor SR140, which is involved in splicing regulation. The null allele *rrc1-4* results in developmental defects in the female gametophyte; however, the specific target genes responsible for this phenotype remain unknown [53].
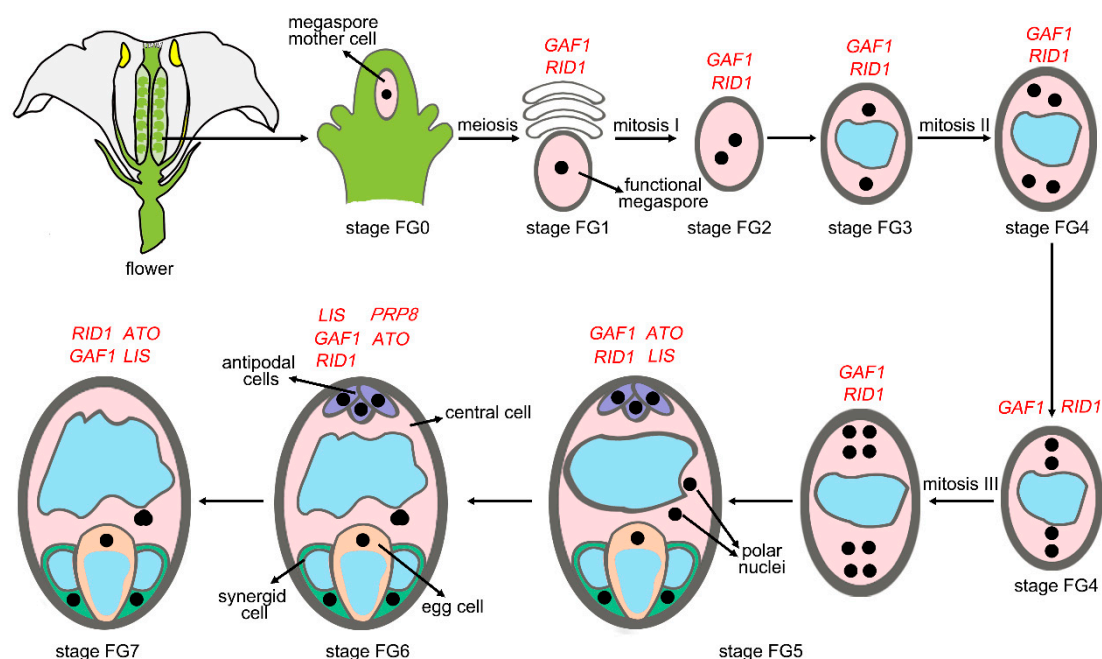
Figure 4. *Arabidopsis* female gametophyte (FG) development highlighting splicing-related factors essential for FG formation. The figure shows *Arabidopsis* female gametophyte (FG) development, highlighting splicing-related factors involved in this process. *Arabidopsis* FG development is categorized into eight stages based on morphological changes. The archesporial cell differentiates into the megaspore mother cell (MMC), which undergoes meiosis to produce a tetrad of haploid megaspores, of which only one survives as the functional megaspore (FM) at stage FG1. The FM undergoes three rounds of mitosis, forming an eight-nucleate coenocyte with four nuclei at each pole separated by a central vacuole. By late FG5, the female gametophyte develops into a seven-celled, eight-nucleate embryo sac with unfused polar nuclei, which fuse at FG6. At FG7, three antipodal cells degenerate, forming a four-celled female gametophyte. At FG8, one synergid cell degrades, resulting in a three-celled female gametophyte. Red-labeled genes represent splicing factors that play roles in different stages of FG development.

Table 2. RNA splicing factors involved in female gamete formation.

Species	Biological Process	Type of Splicing Factor	Splicing Factor	Targeted Genes	References
A. thaliana	Female gametophyte development	DEAH-box RNA-dependent ATPase Prp22	RID1	*EDA26, EDA4, FGPS3, GASA4*	[36,48]
A. thaliana	Megagametogenesis	U5 snRNP component	GFA1	*EDA26, EDA4, FGPS3, GASA4*	[33,35,36,49]
A. thaliana	Female gametophyte development	U2 snRNP-associated protein	ATO	Unknown	[49]
A. thaliana	Supernumerary egg cells, female gametophytes development	U4/U6 snRNP-associated protein	LIS	Unknown	[50,51]
A. thaliana	Female gametophyte development	A U2 snRNP-associated protein	AtSAP130	Unknown	[41]
A. thaliana	Female gametophyte transmission	A U4/U6 snRNP associated protein	RDM16	Unknown	[42]
A. thaliana	A functional redundancy between *PRP8A* and *PRP8B* in female gametophyte development	U5 snRNP component	PRP8A; PRP8B	*EDA9, EDA30, EDA35, MEE29*	[43]
A. thaliana	Female gametophyte transmission	Potential component of the NTC	CWC15	Unknown	[52]
A. thaliana	Female gametophyte transmission	RS domain (SR-like) protein	RRC1	Unknown	[53]

Many splicing factors are constitutively expressed in plants, and their mutations often lead to defects in the development and function of male and female reproductive organs. *Arabidopsis* SPLICEOSOME-ASSOCIATED PROTEIN 130 (AtSAP130) is a subunit of splicing factor 3b (SF3b), which is essential for pre-spliceosome assembly and pre-mRNA splicing [54]. The RNAi-mediated knockdown of *AtSAP130* results in functional impairments in female reproductive organs and disrupts pollen transition from the microspore to the bicellular stage. AtSAP130 is likely involved in the splicing of *QRT1* and *QRT3*, genes associated with post-tetrad pollen development [41,55]. *Arabidopsis* RDM16 is a U4/U6 snRNP-associated component essential for pre-mRNA splicing. Loss-of-function mutants of *RDM16* show increased intron retention events, reduced viability in both male and female gametophytes, and decreased transmission efficiency through gametes [42]. PRP8 is a core component of the snRNP complex, contributing to the formation of the larger catalytically active spliceosomal B complex [56]. The *Arabidopsis* genome encodes two copies of PRP8, PRP8A and PRP8B, which are involved in the splicing of spliceosome factors and key functional genes required for embryo sac development, including *EMBRYO SAC DEVELOPMENT ARREST 9* (*EAD9*), *EAD30*, *EAD35*, and *MATERNAL EFFECT EMBRYO ARREST 29* (*MEE29*). In the *prp8a prp8b* double mutant, ovules fail to attract pollen tubes, and pollen tube perception of ovular attraction signals is impaired [43].

5. Splicing-Regulated Embryo Development

Research has demonstrated that post-transcriptional RNA processing plays a crucial role in embryo development, with mutations affecting splicing leading to abnormal embryogenesis. This highlights the necessity of accurate pre-mRNA splicing for the proper transmission of genetic information during normal plant development (Table 3). *Arabidopsis DEBRANCHING ENZYME 1* (*AtDBR1*) encodes an RNA lariat debranching enzyme, which facilitates the excision of intron lariats during splicing. The mutation of *AtDBR1* causes embryo development to arrest at an early stage [57]. *AtBUD13* encodes an *Arabidopsis* homolog of yeast Bud13, a subunit of the RES complex. Mutations in *AtBUD13* result in early embryonic lethality due to the retention of shorter introns in genes essential for early embryo development [58]. In addition to AtBUD13, GROWTH, DEVELOPMENT AND SPLICING 1 (GDS1), and DAWDLE (DDL) are key components of the RES complex in *Arabidopsis*. Mutations in *GDS1* and *DDL* disrupt the proper splicing of multiple genes involved in cell proliferation and early embryogenesis, leading to developmental defects [59]. Maize *ROUGH ENDOSPERM3* encodes a cofactor of the U2AF35-related protein, influencing alternative splicing and playing a role in embryo and endosperm development as well as their interaction [60]. In *Arabidopsis*, the core splicing factor REPLICATION TERMINATION FACTOR 2 (AtRTF2) is a RING finger-containing protein essential for genome-wide pre-mRNA splicing. Loss of *AtRTF2* function leads to increased intron retention and embryo abortion [61]. LEFKOTHEA (LEFKO), a nuclear-encoded protein, regulates both nuclear and chloroplast pre-mRNA splicing and is crucial for embryogenesis. A *LEFKO* knockout mutation results in embryo lethality due to splicing defects in nuclear and chloroplast gene mRNAs [62]. The direct target genes of LEFKO involved in embryo development regulation remain unidentified. In *Arabidopsis*, AtPRP17, a homolog of the yeast splicing factor PRP17, plays a vital role in embryonic pattern formation. The embryonic lethality observed in *atprp17* mutants is likely due to its involvement in the splicing of genes associated with embryo development [63]. Similarly, JANUS, a homolog of the conserved U2 snRNP assembly factor in yeast and humans, is essential for embryonic pattern formation [64,65]. *Arabidopsis* SM-like (LSM) proteins form two distinct heptameric complexes: LSM1-7 in the cytoplasm and LSM2-8 in the nucleus. The LSM2-8 complex selectively regulates the splicing of specific embryogenesis-related genes, such as *EMBRYO*

DEFECTIVE 2785 (EMB2785) and *EMBRYO DEFECTIVE 2016 (EMB2016)*, by stabilizing U6 snRNA. Splicing defects in *lsm8* mutants have been linked to shorter siliques, reduced seed numbers, and seed abortion [66]. SmD3, a core component of the snRNP complex required for pre-mRNA splicing, is encoded by two genes in *Arabidopsis* (*SmD3-a* and *SmD3-b*). The *smd3-b* mutant represents pleiotropic defects, including 10% ovule abortion, while the inability to generate *smd3-a/smd3-b* double mutants suggests that SmD3 is essential for embryogenesis. However, the specific target genes responsible for this phenotype remain unidentified [67].

Table 3. RNA splicing factors involved in embryo development.

Species	Biological Process	Type of Splicing Factor	Splicing Factor	Targeted Genes	References
Zea mays	Kernel development	RNA lariat debranching enzyme	AtDBR1	Unknown	[57]
A. thaliana	Embryogenesis	Nucleus-encoded RNA-binding protein	LEFKO	Unknown	[62]
A. thaliana	Early embryogenesis	One composition of the RES complex	AtBUD13	Early embryo developmental genes, e.g., *ZYG1/APC11*, *PFI* and *ATML1*	[58]
A. thaliana	Early embryogenesis	One composition of the RES complex	DDL	Genes associated with cell proliferation and early embryo development e.g., *AGO10/ZLL*, *ATML1*	[59]
A. thaliana	Early embryogenesis	One composition of the RES complex	GDS1	Genes associated with cell proliferation and early embryo development e.g., *AGO10/ZLL*, *ATML1*	[59]
A. thaliana	Embryogenesis	U5 snRNP component	PRP8A	Unknown	[61,68]
Z. mays	Kernel development	U2AF35-related protein	Rgh3	Unknown	[60]
Z. mays	Kernel development	Mitochondrion-targeted P-type PRP protein	DEK43/DEK41	Mitochondrial *nad4* introns 1 and 3	[69,70]
Z. mays	Kernel development	Mitochondria-targeted P-type PPR protein	EMP8	*nad1* intron 4, *nad4* intron 1 and *nad2* intron 1	[71]
Z. mays	Kernel development	Mitochondria-targeted P-type PPR protein	DEK35	Mitochondrial *nad4* intron 1	[72]
A. thaliana	Embryogenesis	Mitochondria-targeted P-type PPR protein	MID1	Mitochondrial *nad2* intron 1	[73]
A. thaliana	Seed development	Mitochondria-targeted P-type PPR protein	OTP43	Mitochondrial *nad1* Intron 1	[74]
A. thaliana	Seed development	Mitochondria-targeted P-type PPR protein	SLO3	Mitochondrial *nad7* Intron 2	[75]
A. thaliana	Embryogenesis	Rtf2-domain splicing-related protein	AtRTF2	Unknown	[61]
Oryza sativa	Grain development	Mitochondrion-targeted P-type PRP protein	RL1	Mitochondrial *nad4* intron 1	[76]
O. sativa	Grain development	Nucleolus-localized PPR protein	FLO14/OsNPPR3	*nad 1–2* and *nad 2*	[77]
A. thaliana	Embryogenesis	DEAH-box RNA-dependent ATPase Prp16	CUV	Genes involved in auxin biosynthesis, polar auxin transport, and auxin perception and signaling	[34]
A. thaliana	Embryogenesis	U5 snRNP component	GFA1	Unknown	[35]
A. thaliana	Embryogenesis	SR protein	AtRSZ33	Unknown	[40]
A. thaliana	Embryogenesis	A homolog of the yeast splicing factor PRP17	AtPRP17	Unknown	[63]
A. thaliana	Embryonic pattern formation	U2 snRNP assembly factor	JANUS	Unknown	[64,65]
A. thaliana	Embryogenesis	U6 SnRNP component (SM-like protein)	LSM	Genes involved in embryo development e.g., *EMB2785, EMB2016*	[66]
A. thaliana	Embryogenesis	RS domain (SR-like) protein	RRC1	Unknown	[53]
A. thaliana	Embryogenesis	SnRNP core protein	SmD3	Unknown	[67]

In higher plants, the splicing of introns in organelle genes relies on a large family of pentatricopeptide repeat (PPR) proteins [78]. These proteins contain 2–30 tandem repeats of a degenerate 31- to 36-amino acid motif, which plays a role in post-transcriptional reg-

ulation, including RNA splicing in plant organelles [79]. PPR proteins are classified into two types: the P-type, which contains PPR (P) motifs, and the PLS-type, which includes P motifs along with longer (L) or shorter (S) variants. A subset of P-type PPR proteins has been identified as essential for the splicing of group II introns in organelle genes [80]. MITOCHONDRION-MEDIATED GROWTH DEFECT 1 (MID1) is a P-type PPR protein in *Arabidopsis* that facilitates the splicing of intron 1 in the mitochondrial *NADH dehydrogenase 2* gene by interacting with MITOCHONDRIAL INTRON SF1. Loss-of-function mutations in *MID1* result in arrested embryogenesis [73]. Several P-type PPR proteins in *Arabidopsis*, maize, and rice, including *DEFECTIVE KERNELS and EMPTY PERICARPS*, are involved in the intron splicing of *NADH dehydrogenase* genes encoding subunits of the mitochondrial respiratory chain complex. Mutations in these proteins often lead to mitochondrial dysfunction, adversely affecting embryo and pollen development [69–72,74–77,81,82].

Embryo development begins with egg cell fertilization; therefore, defects in female gametophyte development often compromise embryogenesis. For instance, a partial loss-of-function mutation in *GFA1* not only disrupts female gametophyte development but also arrests embryo development before the octant stage [35]. In the *cwc15-2* mutant, impaired female gametophyte function significantly reduces fertilization success. Even when fertilization occurs, abnormal gene expression in *cwc15-2* leads to early embryonic arrest, resulting in an embryonic lethal phenotype [52]. Similarly, the *rrc1-4* mutant shows reduced female gametophyte transmission efficiency while maintaining normal male transmission. The low frequency of *rrc1-4* homozygotes in progeny from self-fertilized *rrc1-4* heterozygotes suggests additional defects in embryogenesis following fertilization [53]. *CLUMSY VEIN* (*CUV*) encodes an *Arabidopsis* ortholog of yeast Prp16, an RNA splicing factor involved in the second-step transesterification reaction during splicing. *CUV* regulates the splicing of genes associated with auxin biosynthesis, transport, perception, and signaling. Phenotypic analysis of *cuv* mutants revealed developmental defects typically linked to auxin, including altered leaf venation, defective gynoecium and stamen development, and arrested embryogenesis [34]. As previously mentioned, the *prp8a prp8b* double mutant disrupts embryo sac attraction for the pollen tube [43], while the *prp8a* mutant alone is embryo-lethal [61,68].

6. Conclusions and Future Prospects

This review explores the critical role of pre-mRNA splicing in plant reproductive development, summarizing the functional diversity of various transcripts during reproductive processes and highlighting the essential roles of splicing factors in both female and male gametophytes, as well as embryonic development. These studies demonstrate that the precise regulation of splicing is crucial for the proper progression of plant reproductive development. As a model organism, *Arabidopsis* has laid a crucial foundation for studying pre-mRNA splicing regulation in plant reproductive development. From an evolutionary perspective, the core mechanisms of pre-mRNA splicing, such as the basic composition of the spliceosome, the removal of introns, and splice site recognition, are relatively conserved in higher plants [19,83]. Nevertheless, pre-mRNA splicing regulation networks may vary among different plant species, and this diversity could enable plants to adapt to different environmental conditions and influence their reproductive strategies [19]. Therefore, future research should focus on investigating the role of pre-mRNA splicing in plant reproductive development across more species, exploring potential species-specific pre-mRNA splicing regulatory networks to provide a more comprehensive understanding of plant reproductive development.

Beyond the classic splicing factors, an increasing number of studies show that non-splicing factors also significantly impact plant reproductive development by influencing

splicing. For example, *Arabidopsis* INOSITOL-REQUIRING ENZYME 1 (IRE1), a dual-function protein kinase/ribonuclease [84,85], plays a vital role in the splicing of *bZIP60* mRNA at elevated temperatures, which results in reduced male fertility due to interference with pollen coat deposition and normal pollen grain dispersal. Furthermore, the overexpression of *SEC31A*, a gene transcriptionally activated by bZIP60, rescues the temperature-sensitive sterility in *ire1a ire1b* mutants [38]. *SHOOT REDIFFERENTIATION DEFECTIVE 2* (*SRD2*), which encodes a nuclear protein homologous to human SNAP50 that activates snRNA transcription, also influences splicing. Loss of *SRD2* function reduces snRNA levels, impairing splicing and causing reproductive defects, such as failed pollen tube attraction and embryo arrest before the globular stage [86–88].

In conclusion, the precise regulation of pre-mRNA splicing, involving both classical and non-classical splicing factors, is essential for plant reproductive development, with these factors influencing splicing through various mechanisms. RNA splicing regulation serves as a crucial mechanism for post-transcriptional gene expression control in plants, offering a faster response to developmental signals compared to the slower processes of transcriptional activation and pre-mRNA accumulation. Many splicing factors play a role in the splicing of key genes involved in plant gametophyte (Tables 1 and 2) and embryo development (Table 3). However, the mechanisms by which developmental signals—such as hormones and environmental cues—regulate the levels and activities of these splicing factors remain unclear. Investigating how splicing factor levels and activities are modulated by these signals could provide valuable insights into the regulation of splicing during gametophyte and embryo development in plants. Studies have suggested that hormones, epigenetic modifications, and environmental signals influence mRNA splicing [89–95], but the specific regulation of splicing factors in plant gametophyte and embryo development is yet to be fully explored. Another challenge is identifying the direct mRNA targets of splicing factors involved in these processes. Recent advances in techniques can aid in this task. For example, RNA immunoprecipitation sequencing (RIP-seq) can detect the mRNA targets of splicing factors with RNA-recognition motifs, although it does not reveal binding site information. On the other hand, cross-linking immunoprecipitation (CLIP) coupled with high-throughput sequencing and its variants—individual nucleotide resolution CLIP (iCLIP) and photoactivatable-ribonucleoside-enhanced CLIP (PAR-CLIP)—not only identify the mRNA targets of splicing factors but also provide insights into their binding sites on target mRNAs [96]. Non-coding RNAs (ncRNAs), including microRNA (miRNA), small interfering RNA (siRNA), and long non-coding RNA (lncRNA), play a key role in regulating gene expression both at the transcriptional and post-transcriptional levels [97–99]. Multiple studies have shown that ncRNAs are direct targets of splicing factors [100–103]. Moreover, ncRNAs are involved in plant sexual reproduction and plants' responses to environmental changes [104–112]. Identifying the ncRNA targets of splicing factors could improve our understanding of the role of splicing in plant gametophyte and embryo development.

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References

1. Ning, M.; Li, Q.; Wang, Y.; Li, Q.; Tao, Y.; Zhang, F.; Hu, F.; Huang, L. Alternative splicing drives the functional diversification of a bHLH transcription factor in the control of growth and drought tolerance in rice. *Sci. Bull.* **2024**, *70*, 153–156. [CrossRef] [PubMed]

2. Li, Y.; Du, Y.; Huai, J.; Jing, Y.; Lin, R. The RNA helicase UAP56 and the E3 ubiquitin ligase COP1 coordinately regulate alternative splicing to repress photomorphogenesis in *Arabidopsis*. *Plant Cell* **2022**, *34*, 4191–4212. [CrossRef] [PubMed]

3. Wang, Y.Y.; Xiong, F.; Ren, Q.P.; Wang, X.L.; Wellmer, F. Regulation of flowering transition by alternative splicing: The role of the U2 auxiliary factor. *J. Exp. Bot.* **2020**, *71*, 751–758. [CrossRef] [PubMed]

4. Muhammad, S.; Xu, X.; Zhou, W.; Wu, L. Alternative splicing: An efficient regulatory approach towards plant developmental plasticity. *WIREs RNA* **2023**, *14*, e1758. [CrossRef]

5. Liu, Y.; Do, S.; Huynh, H.; Li, J.X.; Liu, Y.G.; Du, Z.Y.; Chen, M.X. Importance of pre-mRNA splicing and its study tools in plants. *Adv. Biotechnol.* **2024**, *2*, 4. [CrossRef]

6. Deng, X.; Cao, X. Roles of pre-mRNA splicing and polyadenylation in plant development. *Curr. Opin. Plant Biol.* **2017**, *35*, 45–53. [CrossRef]

7. Haugen, P.; Simon, D.M.; Bhattacharya, D. The natural history of group I introns. *Trends Genet.* **2005**, *21*, 111–119. [CrossRef]

8. Wilkinson, M.E.; Charenton, C.; Nagai, K. RNA splicing by the spliceosome. *Annu. Rev. Biochem.* **2020**, *89*, 359–388. [CrossRef]

9. Pyle, A.M. Group II intron self-splicing. *Annu. Rev. Biophys.* **2016**, *45*, 183–205. [CrossRef]

10. Schmidt, C.A.; Matera, A.G. tRNA introns: Presence, processing, and purpose. *WIREs RNA* **2019**, *11*, e1583. [CrossRef]

11. Wahl, M.C.; Will, C.L.; Luhrmann, R. The spliceosome: Design principles of a dynamic RNP machine. *Cell* **2009**, *136*, 701–718. [CrossRef] [PubMed]

12. Burge, P.A.; Burge, C.B. Classification of introns: U2-type or U12-type. *Cell* **1997**, *91*, 875–879. [CrossRef]

13. Matera, A.G.; Wang, Z. A day in the life of the spliceosome. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 108–121. [CrossRef] [PubMed]

14. Will, C.L.; Luhrmann, R. Spliceosome structure and function. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a003707. [CrossRef]

15. Shi, Y. Mechanistic insights into precursor messenger RNA splicing by the spliceosome. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 655–670. [CrossRef]

16. Thatcher, S.R.; Zhou, W.; Leonard, A.; Wang, B.B.; Beatty, M.; Zastrow-Hayes, G.; Zhao, X.; Baumgarten, A.; Li, B. Genome-wide analysis of alternative splicing in *zea mays*: Landscape and genetic regulation. *Plant Cell* **2014**, *26*, 3472–3487. [CrossRef]

17. Lee, B.M.C. A genomic view of alternative splicing. *Nat. Genet.* **2002**, *30*, 13–19. [CrossRef]

18. Reddy, A.S.N.; Marquez, Y.; Kalyna, M.; Barta, A. Complexity of the alternative splicing landscape in plants. *Plant Cell* **2013**, *25*, 3657–3683. [CrossRef]

19. Shang, X.; Cao, Y.; Ma, L. Alternative splicing in plant genes: A means of regulating the environmental fitness of plants. *Int. J. Mol. Sci.* **2017**, *18*, 432. [CrossRef]

20. Ghelli, R.; Brunetti, P.; Marzi, D.; Cecchetti, V.; Costantini, M.; Lanzoni-Rossi, M.; Scaglia Linhares, F.; Costantino, P.; Cardarelli, M. The full-length Auxin Response Factor 8 isoform ARF8.1 controls pollen cell wall formation and directly regulates *TDF1*, *AMS* and *MS188* expression. *Plant J.* **2023**, *113*, 851–865. [CrossRef]

21. Ghelli, R.; Brunetti, P.; Napoli, N.; De Paolis, A.; Cecchetti, V.; Tsuge, T.; Serino, G.; Matsui, M.; Mele, G.; Rinaldi, G.; et al. A newly identified flower-specific splice variant of *AUXIN RESPONSE FACTOR8* regulates stamen elongation and endothecium lignification in *Arabidopsis*. *Plant Cell* **2018**, *30*, 620–637. [CrossRef] [PubMed]

22. Lockhart, J. Fresh as an exitron: A flower-specific splice variant of *AUXIN RESPONSE FACTOR8* helps shape the stamen. *Plant Cell* **2018**, *30*, 518–519. [CrossRef] [PubMed]

23. Xiang, Y.; Huang, X.; Wang, T.; Zhang, Y.; Liu, Q.; Hussey, P.J.; Ren, H. ACTIN BINDING PROTEIN29 from *Lilium* pollen plays an important role in dynamic actin remodeling. *Plant Cell* **2007**, *19*, 1930–1946. [CrossRef]

24. Pei, Y.X.; Zengjie, Q.; Xuejun, C.; Zhujun, C.; Jiashu, C.; Xin, Y.; Lihua, M.; Xiaohui, L. *T1243*, an alternative transcript of the mitochondrial *T* gene in *Brassica juncea* var. *tumida, causes pollen abortion in Arabidopsis thaliana*. *Plant Sci.* **2008**, *175*, 793–798. [CrossRef]

25. Ju, L.; Jing, Y.; Shi, P.; Liu, J.; Chen, J.; Yan, J.; Chu, J.; Chen, K.M.; Sun, J. JAZ proteins modulate seed germination through interaction with ABI5 in bread wheat and *Arabidopsis*. *New Phytol.* **2019**, *223*, 246–260. [CrossRef]

26. Chung, H.S.; Howe, G.A. A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM-domain protein JAZ10 in *Arabidopsis*. *Plant Cell* **2009**, *21*, 131–145. [CrossRef]

27. Gao, X.Q.; Liu, C.Z.; Li, D.D.; Zhao, T.T.; Li, F.; Jia, X.N.; Zhao, X.Y.; Zhang, X.S. The *Arabidopsis* KINβγ subunit of the SNRK1 complex regulates pollen hydration on the stigma by mediating the level of reactive oxygen species in pollen. *PLoS Genet.* **2016**, *12*, e1006228. [CrossRef]

28. Zhang, M.J.; Zhang, X.S.; Gao, X.Q. ROS in the male-female interactions during pollination: Function and regulation. *Front. Plant Sci.* **2020**, *11*, 177. [CrossRef]

29. Xie, H.T.; Wan, Z.Y.; Li, S.; Zhang, Y. Spatiotemporal production of reactive oxygen species by NADPH oxidase is critical for tapetal programmed cell death and pollen development in *Arabidopsis*. *Plant Cell* **2014**, *26*, 2007–2023. [CrossRef]

30. Shao, C.; Yang, B.; Wu, T.; Huang, J.; Tang, P.; Zhou, Y.; Zhou, J.; Qiu, J.; Jiang, L.; Li, H.; et al. Mechanisms for U2AF to define 3′ splice sites and regulate alternative splicing in the human genome. *Nat. Struct. Mol. Biol.* **2014**, *21*, 997–1005. [CrossRef]

31. Xiong, F.; Ren, J.J.; Yu, Q.; Wang, Y.Y.; Lu, C.C.; Kong, L.J.; Otegui, M.S.; Wang, X.L. AtU2AF65b functions in abscisic acid mediated flowering via regulating the precursor messenger RNA splicing of *ABI5* and *FLC* in *Arabidopsis*. *New Phytol.* **2019**, *223*, 277–292. [CrossRef] [PubMed]

32. Park, H.Y.; Lee, H.T.; Lee, J.H.; Kim, J.K. *Arabidopsis U2AF65* regulates flowering time and the growth of pollen tubes. *Front. Plant Sci.* **2019**, *10*, 569. [CrossRef] [PubMed]

33. Coury, D.A.; Zhang, C.; Ko, A.; Skaggs, M.I.; Christensen, C.A.; Drews, G.N.; Feldmann, K.A.; Yadegari, R. Segregation distortion in *Arabidopsis gametophytic factor 1* (*gfa1*) mutants is caused by a deficiency of an essential RNA splicing factor. *Sex. Plant Reprod.* **2007**, *20*, 87–97. [CrossRef]

34. Tsugeki, R.; Tanaka-Sato, N.; Maruyama, N.; Terada, S.; Kojima, M.; Sakakibara, H.; Okada, K. CLUMSY VEIN, the *Arabidopsis* DEAH-box Prp16 ortholog, is required for auxin-mediated development. *Plant J.* **2015**, *81*, 183–197. [CrossRef]

35. Liu, M.; Yuan, L.; Liu, N.Y.; Shi, D.Q.; Liu, J.; Yang, W.C. GAMETOPHYTIC FACTOR 1, involved in pre-mRNA splicing, is essential for megagametogenesis and embryogenesis in *Arabidopsis*. *J. Integr. Plant Biol.* **2009**, *51*, 261–271. [CrossRef]

36. Zhu, D.Z.; Zhao, X.F.; Liu, C.Z.; Ma, F.F.; Wang, F.; Gao, X.Q.; Zhang, X.S. Interaction between RNA helicase ROOT INITIATION DEFECTIVE 1 and GAMETOPHYTIC FACTOR 1 is involved in female gametophyte development in *Arabidopsis*. *J. Exp. Bot.* **2016**, *67*, 5757–5768. [CrossRef]

37. Huang, X.Y.; Niu, J.; Sun, M.X.; Zhu, J.; Gao, J.F.; Yang, J.; Zhou, Q.; Yang, Z.N. CYCLIN-DEPENDENT KINASE G1 is associated with the spliceosome to regulate *CALLOSE SYNTHASE5* splicing and pollen wall formation in *Arabidopsis*. *Plant Cell* **2013**, *25*, 637–648. [CrossRef]

38. Deng, Y.; Srivastava, R.; Quilichini, T.D.; Dong, H.; Bao, Y.; Horner, H.T.; Howell, S.H. IRE1, a component of the unfolded protein response signaling pathway, protects pollen development in *Arabidopsis* from heat stress. *Plant J.* **2016**, *88*, 193–204. [CrossRef]

39. Cao, L.J.; Zhao, M.M.; Liu, C.; Dong, H.J.; Li, W.C.; Ren, H.Y. LlSR28 is involved in pollen germination by affecting filamentous actin dynamics. *Mol. Plant* **2013**, *6*, 1163–1175. [CrossRef]

40. Kalyna, M.; Lopato, S.; Barta, A. Ectopic expression of atRSZ33 reveals its function in splicing and causes pleiotropic changes in development. *Mol. Biol. Cell* **2003**, *14*, 3565–3577. [CrossRef]

41. Aki, S.; Nakai, H.; Aoyama, T.; Oka, A.; Tsuge, T. *AtSAP130/AtSF3b-3* function is required for reproduction in *Arabidopsis thaliana*. *Plant Cell Physiol.* **2011**, *52*, 1330–1339. [CrossRef] [PubMed]

42. Huang, C.F.; Miki, D.; Tang, K.; Zhou, H.R.; Zheng, Z.; Chen, W.; Ma, Z.Y.; Yang, L.; Zhang, H.; Liu, R.; et al. A pre-mRNA-splicing factor is required for RNA-directed DNA methylation in *Arabidopsis*. *PLoS Genet.* **2013**, *9*, e1003779. [CrossRef] [PubMed]

43. Kulichová, K.; Kumar, V.; Steinbachová, L.; Klodová, B.; Timofejeva, L.; Juříček, M.; Honys, D.; Hafidh, S.S. PRP8A and PRP8B spliceosome subunits act coordinately to control pollen tube attraction in *Arabidopsis thaliana*. *Development* **2020**, *147*, dev186742. [CrossRef]

44. 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]

45. Zheng, T.; Nibau, C.; Phillips, D.W.; Jenkins, G.; Armstrong, S.J.; Doonan, J.H. CDKG1 protein kinase is essential for synapsis and male meiosis at high ambient temperature in *Arabidopsis* thaliana. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2182–2187. [CrossRef]

46. Nibau, C.; Dadarou, D.; Kargios, N.; Mallioura, A.; Fernandez-Fuentes, N.; Cavallari, N.; Doonan, J.H. A functional kinase is necessary for Cyclin-Dependent Kinase G1 (CDKG1) to maintain fertility at high ambient temperature in *Arabidopsis*. *Front. Plant Sci.* **2020**, *11*, 586870. [CrossRef]

47. Ali, G.S.; Palusa, S.G.; Golovkin, M.; Prasad, J.; Manley, J.L.; Reddy, A.S.N. Regulation of plant developmental processes by a novel splicing factor. *PLoS ONE* **2007**, *2*, e471. [CrossRef]

48. Ohtani, M.; Demura, T.; Sugiyama, M. *Arabidopsis* ROOT INITIATION DEFECTIVE1, a DEAH-box RNA helicase involved in pre-mRNA splicing, is essential for plant development. *Plant Cell* **2013**, *25*, 2056–2069. [CrossRef]

49. Moll, C.; Von Lyncker, L.; Zimmermann, S.; Kägi, C.; Baumann, N.; Twell, D.; Grossniklaus, U.; Groß-Hardt, R. *CLO/GFA1* and *ATO* are novel regulators of gametic cell fate in plants. *Plant J.* **2008**, *56*, 913–921. [CrossRef]

50. Groß-Hardt, R.; Carrington, J.C.; Kägi, C.; Baumann, N.; Moore, J.M.; Baskar, R.; Gagliano, W.B.; Jürgens, G.; Grossniklaus, U. *LACHESIS* restricts gametic cell fate in the female gametophyte of *Arabidopsis*. *PLoS Biol.* **2007**, *5*, e47. [CrossRef]

51. Völz, R.; von Lyncker, L.; Baumann, N.; Dresselhaus, T.; Sprunck, S.; Groß-Hardt, R. *LACHESIS*-dependent egg-cell signaling regulates the development of female gametophytic cells. *Development* **2012**, *139*, 498–502. [CrossRef] [PubMed]

52. Slane, D.; Lee, C.H.; Kolb, M.; Dent, C.; Miao, Y.; Franz-Wachtel, M.; Lau, S.; Macek, B.; Balasubramanian, S.; Bayer, M.; et al. The integral spliceosomal component CWC15 is required for development in *Arabidopsis*. *Sci. Rep.* **2020**, *10*, 13336. [CrossRef] [PubMed]

53. Shikata, H.; Shibata, M.; Ushijima, T.; Nakashima, M.; Kong, S.G.; Matsuoka, K.; Lin, C.; Matsushita, T. The RS domain of *Arabidopsis* splicing factor RRC1 is required for phytochrome B signal transduction. *Plant J.* **2012**, *70*, 727–738. [CrossRef]

54. DaS, B.K.; Xia, L.; Palandjian, L.; Palandjian, O.; Chyung, Y.; Reed, R. Characterization of a protein complex containing spliceosomal proteins SAPs 49, 130, 145, and 155. *Mol. Cell Biol.* **1999**, *19*, 6796–6802. [CrossRef]

55. Aki, S.S.; Yura, K.; Aoyama, T.; Tsuge, T. SAP130 and CSN1 interact and regulate male gametogenesis in *Arabidopsis thaliana*. *J. Plant Res.* **2021**, *134*, 279–289. [CrossRef]

56. Maeder, C.; Kutach, A.K.; Guthrie, C. ATP-dependent unwinding of U4/U6 snRNAs by the Brr2 helicase requires the C terminus of Prp8. *Nat. Struct. Mol. Biol.* **2008**, *16*, 42–48. [CrossRef]

57. Wang, H.; Hill, K.; Perry, S.E. An *Arabidopsis* RNA lariat debranching enzyme is essential for embryogenesis. *J. Biol. Chem.* **2004**, *279*, 1468–1473. [CrossRef]

58. Xiong, F.; Ren, J.J.; Yu, Q.; Wang, Y.Y.; Kong, L.J.; Otegui, M.S.; Wang, X.L. *AtBUD13* affects pre-mRNA splicing and is essential for embryo development in *Arabidopsis*. *Plant J.* **2019**, *98*, 714–726. [CrossRef]

59. Xiong, F.; Ren, J.J.; Wang, Y.Y.; Zhou, Z.; Qi, H.D.; Otegui, M.S.; Wang, X.L. An *Arabidopsis* Retention and Splicing complex regulates root and embryo development through pre-mRNA splicing. *Plant Physiol.* **2022**, *190*, 621–639. [CrossRef]

60. Fouquet, R.; Martin, F.; Fajardo, D.S.; Gault, C.M.; Gómez, E.; Tseung, C.W.; Policht, T.; Hueros, G.; Settles, A.M. Maize *Rough Endosperm 3* encodes an RNA splicing factor required for endosperm cell differentiation and has a nonautonomous effect on embryo development. *Plant Cell* **2011**, *23*, 4280–4297. [CrossRef]

61. Sasaki, T.; Kanno, T.; Liang, S.C.; Chen, P.Y.; Liao, W.W.; Lin, W.D.; Matzke, A.J.M.; Matzke, M. An Rtf2 domain-containing protein influences Pre-mRNA splicing and is essential for embryonic development in *Arabidopsis thaliana*. *Genetics* **2015**, *200*, 523–535. [CrossRef] [PubMed]

62. Daras, G.; Rigas, S.; Alatzas, A.; Samiotaki, M.; Chatzopoulos, D.; Tsitsekian, D.; Papadaki, V.; Templalexis, D.; Banilas, G.; Athanasiadou, A.M.; et al. LEFKOTHEA regulates nuclear and chloroplast mRNA splicing in plants. *Dev. Cell* **2019**, *50*, 767–779.e767. [CrossRef] [PubMed]

63. Shao, D.J.; Wei, Y.M.; Yu, Z.Q.; Dai, X.; Gao, X.Q. *Arabidopsis* AtPRP17 functions in embryo development by regulating embryonic patterning. *Planta* **2021**, *254*, 58. [CrossRef] [PubMed]

64. Xiong, F.; Liu, H.H.; Duan, C.Y.; Zhang, B.K.; Wei, G.; Zhang, Y.; Li, S. *Arabidopsis* JANUS regulates embryonic pattern formation through Pol II-mediated transcription of WOX2 and PIN7. *iScience* **2019**, *19*, 1179–1188. [CrossRef]

65. Xiong, F.; Li, S. Spliceosome component JANUS fulfills a role of mediator in transcriptional regulation during *Arabidopsis* development. *Plant Signal Behav.* **2021**, *16*, 1841974. [CrossRef]

66. Perea-Resa, C.; Hernández-Verdeja, T.; López-Cobollo, R.; Castellano, M.d.M.; Salinas, J. LSM proteins provide accurate splicing and decay of selected transcripts to ensure normal *Arabidopsis* development. *Plant Cell* **2012**, *24*, 4930–4947. [CrossRef]

67. Swaraz, A.M.; Park, Y.-D.; Hur, Y. Knock-out mutations of *Arabidopsis SmD3-b* induce pleotropic phenotypes through altered transcript splicing. *Plant Sci.* **2011**, *180*, 661–671. [CrossRef]

68. Schwartz, B.W.; Yeung, E.C.; Meinke, D.W. Disruption of morphogenesis and transformation of the suspensor in abnormal suspensor mutants of *Arabidopsis*. *Development* **1994**, *120*, 3235–3245. [CrossRef]

69. Ren, R.C.; Wang, L.L.; Zhang, L.; Zhao, Y.J.; Wu, J.W.; Wei, Y.M.; Zhang, X.S.; Zhao, X.Y. DEK43 is a p-type pentatricopeptide repeat (PPR) protein responsible for the *cis*-splicing of *nad4* in maize mitochondria. *J. Integr. Plant Biol.* **2020**, *62*, 299–313. [CrossRef]

70. Zhu, C.; Jin, G.; Fang, P.; Zhang, Y.; Feng, X.; Tang, Y.; Qi, W.; Song, R. Maize pentatricopeptide repeat protein DEK41 affects *cis*-splicing of mitochondrial *nad4* intron 3 and is required for normal seed development. *J. Exp. Bot.* **2019**, *70*, 3795–3808. [CrossRef]

71. Sun, F.; Zhang, X.; Shen, Y.; Wang, H.; Liu, R.; Wang, X.; Gao, D.; Yang, Y.Z.; Liu, Y.; Tan, B.C. The pentatricopeptide repeat protein EMPTY PERICARP8 is required for the splicing of three mitochondrial introns and seed development in maize. *Plant J.* **2018**, *95*, 919–932. [CrossRef] [PubMed]

72. Chen, X.; Feng, F.; Qi, W.; Xu, L.; Yao, D.; Wang, Q.; Song, R. *Dek35* encodes a PPR protein that affects *cis*-splicing of mitochondrial *nad4* intron 1 and seed development in maize. *Mol. Plant* **2017**, *10*, 427–441. [CrossRef] [PubMed]

73. Zhao, P.; Wang, F.; Li, N.; Shi, D.Q.; Yang, W.C. Pentatricopeptide repeat protein MID1 modulates *nad2* intron 1 splicing and *Arabidopsis* development. *Sci. Rep.* **2020**, *10*, 2008. [CrossRef]

74. de Longevialle, A.o.F.; Meyer, E.H.; Andrés, C.; Taylor, N.L.; Lurin, C.; Millar, A.H.; Small, I.D. The pentatricopeptide repeat gene *OTP43* is required for *trans*-splicing of the mitochondrial *nad1* intron 1 in *Arabidopsis thaliana*. *Plant Cell* **2007**, *19*, 3256–3265. [CrossRef]

75. Hsieh, W.Y.; Liao, J.C.; Chang, C.Y.; Harrison, T.; Boucher, C.; Hsieh, M.H. The SLOW GROWTH3 pentatricopeptide repeat protein is required for the splicing of mitochondrial *NADH dehydrogenase subunit7* intron 2 in *Arabidopsis*. *Plant Physiol.* **2015**, *168*, 490–501. [CrossRef]

76. Wu, M.W.; Zhao, H.; Zhang, J.D.; Guo, L.; Liu, C.M. *RADICLELESS 1* (*RL1*)-mediated *nad4* intron 1 splicing is crucial for embryo and endosperm development in rice (*Oryza sativa* L.). *Biochem. Biophys. Res. Commun.* **2020**, *523*, 220–225. [CrossRef] [PubMed]

77. Xue, M.; Liu, L.; Yu, Y.; Zhu, J.; Gao, H.; Wang, Y.; Wan, J. Lose-of-function of a rice nucleolus-localized pentatricopeptide repeat protein is responsible for the *floury endosperm14* mutant phenotypes. *Rice* **2019**, *12*, 100. [CrossRef]

78. Barkan, A.; Small, I. Pentatricopeptide pepeat proteins in plants. *Annu. Rev. Plant Biol.* **2014**, *65*, 415–442. [CrossRef]

79. Zhang, Y.; Lu, C. The enigmatic roles of PPR-SMR proteins in plants. *Adv. Sci.* **2019**, *6*, 1900361. [CrossRef]

80. Wang, Y.; Tan, B.C. Pentatricopeptide repeat proteins in plants: Cellular functions, action mechanisms, and potential applications. *Plant Commun.* **2025**, *6*, 101203. [CrossRef]

81. Zheng, P.; Liu, Y.; Liu, X.; Huang, Y.; Sun, F.; Wang, W.; Chen, H.; Jan, M.; Zhang, C.; Yuan, Y.; et al. OsPPR939, a *nad5* splicing factor, is essential for plant growth and pollen development in rice. *Theor. Appl. Genet.* **2021**, *134*, 923–940. [CrossRef] [PubMed]

82. Meng, L.; Du, M.; Zhu, T.; Li, G.; Ding, Y.; Zhang, Q. PPR proteins in plants: Roles, mechanisms, and prospects for rice research. *Front. Plant Sci.* **2024**, *15*. [CrossRef]

83. Lorković, Z.J.; Wieczorek Kirk, D.A.; Lambermon, M.H.; Filipowicz, W. Pre-mRNA splicing in higher plants. *Trends Plant Sci.* **2000**, *5*, 160–167. [CrossRef]

84. Maurel, M.; Chevet, E.; Tavernier, J.; Gerlo, S. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* **2014**, *39*, 245–254. [CrossRef] [PubMed]

85. Nagashima, Y.; Mishiba, K.I.; Suzuki, E.; Shimada, Y.; Iwata, Y.; Koizumi, N. *Arabidopsis* IRE1 catalyses unconventional splicing of *bZIP60* mRNA to produce the active transcription factor. *Sci. Rep.* **2011**, *1*, 29. [CrossRef] [PubMed]

86. Ohtani, M.; Demura, T.; Sugiyama, M. Differential requirement for the function of SRD2, an snRNA transcription activator, in various stages of plant development. *Plant Mol. Biol.* **2007**, *66*, 303–314. [CrossRef]

87. Ohtani, M.; Sugiyama, M. Involvement of SRD2-mediated activation of snRNA transcription in the control of cell proliferation competence in *Arabidopsis*. *Plant J.* **2005**, *43*, 479–490. [CrossRef]

88. Ohtani, M.; Demura, T.; Sugiyama, M. Particular significance of SRD2-dependent snRNA accumulation in polarized pattern generation during lateral root development of *Arabidopsis*. *Plant Cell Physiol.* **2010**, *51*, 2002–2012. [CrossRef]

89. Liu, H.; Ma, X.; Han, H.N.; Hao, Y.J.; Zhang, X.S. AtPRMT5 Regulates Shoot Regeneration through Mediating Histone H4R3 Dimethylation on *KRPs* and Pre-mRNA splicing of *RKP* in *Arabidopsis*. *Mol. Plant* **2016**, *9*, 1634–1646. [CrossRef]

90. El Arbi, N.; Nardeli, S.M.; Šimura, J.; Ljung, K.; Schmid, M. The *Arabidopsis* splicing factor PORCUPINE/SmE1 orchestrates temperature-dependent root development via auxin homeostasis maintenance. *New Phytol.* **2024**, *244*, 1408–1421. [CrossRef]

91. Chong, G.L.; Foo, M.H.; Lin, W.D.; Wong, M.M.; Verslues, P.E. Highly ABA-Induced 1 (HAI1)-Interacting protein HIN1 and drought acclimation-enhanced splicing efficiency at intron retention sites. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 22376–22385. [CrossRef]

92. Butt, H.; Bazin, J.; Prasad, K.V.S.K.; Awad, N.; Crespi, M.; Reddy, A.S.N.; Mahfouz, M.M. The rice serine/arginine splicing factor RS33 regulates Pre-mRNA splicing during abiotic stress responses. *Cells* **2022**, *11*, 1796. [CrossRef]

93. Zhao, J.; Yao, B.; Peng, Z.; Yang, X.; Li, K.; Zhang, X. Splicing defect of StDRO2 intron 1 promotes potato root growth by disturbing auxin transport to adapt to drought stress. *Hortic. Plant J.* **2025**, *11*, 706–720. [CrossRef]

94. Fu, D.; Song, Y.; Wu, S.; Peng, Y.; Ming, Y.; Li, Z.; Zhang, X.; Song, W.; Su, Z.; Gong, Z.; et al. Regulation of alternative splicing by CBF-mediated protein condensation in plant response to cold stress. *Nat. Plants* **2025**, *11*, 505–517. [CrossRef]

95. Lev Maor, G.; Yearim, A.; Ast, G. The alternative role of DNA methylation in splicing regulation. *Trends Genet.* **2015**, *31*, 274–280. [CrossRef] [PubMed]

96. Huppertz, I.; Attig, J.; D'Ambrogio, A.; Easton, L.E.; Sibley, C.R.; Sugimoto, Y.; Tajnik, M.; König, J.; Ule, J. iCLIP: Protein–RNA interactions at nucleotide resolution. *Methods* **2014**, *65*, 274–287. [CrossRef]

97. Ohtani, M. Expanding the plant non-coding RNA world. *J. Plant Res.* **2016**, *130*, 3–5. [CrossRef]

98. Han, X.; Lin, Y.; Gao, X.Q.; Wang, X.L. Non-canonical functions of splicing factors in RNA metabolism. *Crit. Rev. Plant Sci.* **2020**, *39*, 493–513. [CrossRef]

99. Waititu, J.K.; Zhang, C.; Liu, J.; Wang, H. Plant non-coding RNAs: Origin, biogenesis, mode of action and their roles in abiotic stress. *Int. J. Mol. Sci.* **2020**, *21*, 8401. [CrossRef]

100. Li, S.; Liu, K.; Zhou, B.; Li, M.; Zhang, S.; Zeng, L.; Zhang, C.; Yu, B. MAC3A and MAC3B, two core subunits of the MOS4-associated complex, positively influence miRNA biogenesis. *Plant Cell* **2018**, *30*, 481–494. [CrossRef]

101. Zhang, S.; Dou, Y.; Li, S.; Ren, G.; Chevalier, D.; Zhang, C.; Yu, B. DAWDLE Interacts with DICER-LIKE proteins to mediate small RNA biogenesis. *Plant Physiol.* **2018**, *177*, 1142–1151. [CrossRef] [PubMed]

102. Yan, K.; Liu, P.; Wu, C.A.; Yang, G.D.; Xu, R.; Guo, Q.H.; Huang, J.G.; Zheng, C.C. Stress-induced alternative splicing provides a mechanism for the regulation of microRNA processing in *Arabidopsis thaliana*. *Mol. Cell* **2012**, *48*, 521–531. [CrossRef] [PubMed]

103. Tripathi, V.; Ellis, J.D.; Shen, Z.; Song, D.Y.; Pan, Q.; Watt, A.T.; Freier, S.M.; Bennett, C.F.; Sharma, A.; Bubulya, P.A.; et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. *Mol. Cell* **2010**, *39*, 925–938. [CrossRef]

104. Gai, Y.P.; Yuan, S.S.; Zhao, Y.N.; Zhao, H.N.; Zhang, H.L.; Ji, X.L. A novel lncRNA, MuLnc1, associated with environmental stress in mulberry (*Morus multicaulis*). *Front. Plant Sci.* **2018**, *9*, 669. [CrossRef] [PubMed]

105. Kong, X.; Zhang, M.; Xu, X.; Li, X.; Li, C.; Ding, Z. System analysis of microRNAs in the development and aluminium stress responses of the maize root system. *Plant Biotechnol. J.* **2014**, *12*, 1108–1121. [CrossRef]

106. Liu, F.; Xu, Y.; Chang, K.; Li, S.; Liu, Z.; Qi, S.; Jia, J.; Zhang, M.; Crawford, N.M.; Wang, Y. The long noncoding RNA *T5120* regulates nitrate response and assimilation in *Arabidopsis*. *New Phytol.* **2019**, *224*, 117–131. [CrossRef]

107. Lu, J.; Zhang, C.; Baulcombe, D.C.; Chen, Z.J. Maternal siRNAs as regulators of parental genome imbalance and gene expression in endosperm of *Arabidopsis* seeds. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 5529–5534. [CrossRef]

108. Wang, Y.; Luo, X.; Sun, F.; Hu, J.; Zha, X.; Su, W.; Yang, J. Overexpressing lncRNA *LAIR* increases grain yield and regulates neighbouring gene cluster expression in rice. *Nature Commun.* **2018**, *9*, 3516. [CrossRef]

109. Qin, T.; Zhao, H.; Cui, P.; Albesher, N.; Xiong, L. A nucleus-localized long non-coding RNA enhances drought and salt stress tolerance. *Plant Physiol.* **2017**, *175*, 1321–1336. [CrossRef]

110. Vashisht, I.; Dhaka, N.; Jain, R.; Sood, A.; Sharma, N.; Sharma, M.K.; Sharma, R. Non-coding RNAs-mediated environmental surveillance determines male fertility in plants. *Plant Physiol. Biochem.* **2023**, *203*, 108030. [CrossRef]

111. Sharif, R.; Zhu, Y.; Huang, Y.; Sohail, H.; Li, S.; Chen, X.; Qi, X. MicroRNA regulates cytokinin induced parthenocarpy in cucumber (*Cucumis sativus* L.). *Plant Physiol. Biochem.* **2024**, *212*, 108681. [CrossRef] [PubMed]

112. Cao, W.; Yang, L.; Zhuang, M.; Lv, H.; Wang, Y.; Zhang, Y.; Ji, J. Plant non-coding RNAs: The new frontier for the regulation of plant development and adaptation to stress. *Plant Physiol. Biochem.* **2024**, *208*, 108435. [CrossRef] [PubMed]