**INTRODUCTION**

Plants are constantly exposed to various types of environmental stresses, which negatively affect the productivity of agricultural crops. Complex multicellular organisms must efficiently and rapidly respond to a wide variety of environmental stimuli, both abiotic (e.g., drought or salinity) and biotic (e.g., bacterial infections). These pathways require unique and highly coordinated molecular responses that are achieved through the differential regulation of the genome to generate specific transcriptomes and proteomes for each environmental condition and cell type (Martín et al., 2021).

Stress factors cause numerous perturbations in plant metabolism, and consequently can lead to abnormal development and even death. Plants, as organisms leading a stationary lifestyle, cannot avoid the source of stress, therefore they have developed specific defence mechanisms that lead to reprogramming of cellular metabolism and activation of immune system reactions, which effectively allow them to overcome adverse environmental conditions and...
pathogen infections. The proper course of many cellular processes largely depends on efficient RNA metabolism, in particular RNA maturation and degradation. A key step in the processing of eukaryotic RNAs is pre-mRNA splicing, which in addition to determining the identity and function of RNA molecules plays an essential role in regulating development, cell differentiation, and stress response. In contrast to the large number of studies on alternative splicing (AS) in animals, the role, regulation, and physiological consequences of this mechanism in plants are less recognized. In recent years, the rapid development of high-throughput technologies and bioinformatic tools, most notably next-generation sequencing such as Illumina, PacBio, and Oxford Nanopore direct RNA sequencing, have significantly contributed to broadening the scope of AS events and determining the function of alternative transcripts in plants (Reddy et al., 2020). Analyses of plant transcriptomes using new RNA sequencing (RNA-Seq) technologies significantly improved annotation of plant transcriptomes and uncovered numerous novel transcript isoforms, protein-coding and noncoding genes, and alternative processing and polyadenylation sites (Cui et al., 2020; Li et al., 2020; Parker et al., 2020; Zhang et al., 2020b; Zhao et al., 2019; Zhu et al., 2020).

Multiple studies have shown that in Arabidopsis thaliana AS is widespread and that a large number of genes undergo AS in response to various abiotic and biotic stresses (Howard et al., 2013; Li et al., 2020; Martín et al., 2021; Mine et al., 2018; Rigo et al., 2020; Zhu et al., 2020). Moreover, recent studies have revealed that, although AS in Arabidopsis is predominantly cotranscriptional as in other eukaryotes, it is possible that posttranscriptional removal of alternative introns regulates the expression of stress-responsive genes (Jia et al., 2020). It is likely that a combination of co- and posttranscriptional reprogramming of gene expression is applied in plants for effective adaption to adverse conditions. Therefore, functional analysis of alternative transcripts represents a powerful approach to developing new strategies to improve plant tolerance to environmental stresses, which is especially important for crops.

Because several comprehensive reviews have already been written on the role of AS in abiotic stress response (Cruz et al., 2014; Duque, 2011; Filichkin et al., 2015b; Laloum et al., 2018; Ling et al., 2021; Meyer et al., 2015; Punzo et al., 2020a; Reddy et al., 2013; Reddy & Shad Ali, 2011; Shang et al., 2017; Staiger & Brown, 2013), in this review we will focus on the most important recent advancements related to the biotic stress in the model plant organism Arabidopsis thaliana.

2 | SPlicing in A. Thaliana

AS is a widespread and vital regulatory mechanism in all multicellular eukaryotic organisms. As a result of this process, several different mRNA isoforms can be produced from one pre-mRNA due to the selection of alternative or cryptic 5′ and 3′ splice sites. There are many patterns of AS, leading to the formation of a family of related proteins from one gene, which may differ in enzymatic activity, function, cell localization, and stability. In this way, AS also contributes to proteome diversity. The most common forms of AS events include exon skipping (ES), intron retention (IR), alternative 3′ (A3′SS, acceptor-AltA) or 5′ (A5′SS, donor-AltD) splice site, and mutually exclusive exons (MXE) (Figure 1) (Hatje & Kollmar, 2014; Martín et al., 2020).

![FIGURE 1 Most frequent types of simply alternative splicing events based on a high-quality reference transcript dataset for Arabidopsis (AtRTD2).](image)

| Major types of alternative splicing | Arabidopsis thaliana events (%) |
|------------------------------------|---------------------------------|
| Intron retention (IR)              | 10272 (40%)*                    |
| Alternative 3′ splice site (A3′SS acceptor-AltA) | 9264 (24.9%) |
| Alternative 5′ splice site (A5′SS donor-AltD) | 4133 (11.1%) |
| Exon skipping (ES)                 | 2120 (5.7%)                     |
| Mutually exclusive exons (MXE)     | 99 (0.27%)                      |
| Exitrons (EIs)                     | 1130 (4.4%)                     |
| **Total events in AtRTD2**         | **37137**                       |

* including other IR forms such as IR1 (intron 1) or IR2 (intron 2), IR1 and IR2, Alt3′SS and IR, Alt3′SS or IR
Different AS events display diverse molecular functions either by generating distinct protein isoforms with different functional properties or by disrupting the main open reading frame (ORF), which results in the synthesis of truncated proteins and/or mRNA degradation via nonsense-mediated mRNA decay (NMD) (Jabre et al., 2019; Martin et al., 2021). Plants often use AS to fine-tune their metabolism and physiology not only under normal conditions, but also following various abiotic and biotic stresses.

Because splicing is a strictly conserved process, the general mechanism of splicing and structural pre-mRNA features in plants resemble those in animals (Chaudhary et al., 2019; Wang et al., 2008). As in metazoans, the major type of introns in plants that are spliced by the major spliceosome complex is the U2-type, with the invariant GU/AG dinucleotides at the intron boundaries. More than 2000 potential U12-type introns have been identified in A. thaliana (Marquez et al., 2012). These introns contain nonconsensus AU/AC intron termini and are mainly processed by the minor U12-type spliceosome.

However, due to their sessile nature, plants require more complex systems to regulate metabolism and development, and to fend off environmental stress and pathogen attack. One layer of defence regulation is provided by AS. The most spectacular differences in splicing between plants and animals concern the length and nucleotide composition of introns and the frequency of different AS events. Notably, introns in plant genes are relatively short, with an average length of c.170 nucleotides (nt) versus c.5000 nt in humans (Marquez et al., 2015; Sakharov et al., 2004). Considering that recognition of splice sites in short introns probably occurs via intron definition, this model has also been proposed to function in plants (De Conti et al., 2013; Morello & Breviario, 2008). More importantly, plant introns are U- or UA-rich, c.15% richer than exons, and this feature is more pronounced in dicot than in monocot plants. The AU richness defines introns in plants and is crucial for splice site selection and efficient splicing (Marquez et al., 2012; Morello & Breviario, 2008; Reddy, 2007; Reddy et al., 2013). As a consequence, the plant counterpart of the polypyrimidine tract, which is located downstream of the branch point in animal introns, is also largely composed of U residues and the branch point sequence is less recognizable. These differences probably decide that heterologous pre-mRNAs are inefficiently spliced in plant cells (Lorkovic et al., 2000). Another important distinction between plants and animals is related to the type of AS events. In plants, AS regulates 60%–83% of multi-exon genes and a high-quality reference transcript dataset for Arabidopsis (ArRTD2) contains as many as 37,137 AS events (Marquez et al., 2012; Zhang et al., 2017a). Based on high-throughput transcriptomic studies collected in ArRTD2, IR is the most common event (40%), followed by alternative 3′ splice site selection, alternative 5′ splice site selection, ES, and MXE (Figure 1) (Zhang et al., 2017a). Depending on growth conditions and tissue type the extent of IR may even reach 60%, which is in stark contrast to the low level of IR occurrence in human cells (c.5%), where ES is the major type of AS (c.40%) (Chaudhary et al., 2019; Wang et al., 2008). Because the retained plant introns have higher GC content and weaker splicing signals than constitutive introns, their retention is probably due to lower splicing efficiency (Marquez et al., 2012). These introns exhibit more open chromatin, suggesting that an increased RNA polymerase II speed contributes to IR events according to the kinetic model of splicing (Ullah et al., 2018). Finally, a recent study proposes that extensive IR in plants is related to their posttranscriptional splicing (Jia et al., 2020). AS often introduces premature termination codons (PTCs) that trigger degradation of such transcripts by the NMD pathway. Although pre-mRNAs with IR contain PTCs, they are usually not NMD substrates in plants, probably due to their retention in the nucleus (Jia et al., 2020; Kalyna et al., 2012). Interestingly, in Arabidopsis AS appears to have a greater regulatory role than in other organisms because a large number of these events are regulated in response to abiotic and biotic stress conditions and mainly affect the production of functional proteins. In contrast, in animal cells, AS is mostly controlled in a tissue-specific manner and gives rise to multiple protein isoforms, which contributes to increasing proteome complexity (Martin et al., 2021). These observations support the notion that some features of the splicing machinery and regulation of splicing may have a specific character in plants.

Another notable and relatively recently discovered aspect of AS has been the detection of exitrons (exonic introns), which are alternatively spliced internal regions of protein-coding exons (Marquez et al., 2015). Exitrons have been identified in plant and human cells, and differ from standard introns, mainly because they are GC-rich in plants and do not have stop codons, so their retention does not interfere with protein-coding potential. In contrast to transcripts with IR, those with unspliced exitrons represent abundant isoforms, are exported to the cytoplasm, and undergo translation. Consequently, exitrons were postulated not to act as AS events that regulate gene expression but rather that increase proteome complexity by generating protein isoforms with different domain composition and activities. Importantly, exitron processing in plants impacts genes involved in stress response and developmental processes, and is affected by specific stresses, supporting their role in plant adaptation to environmental conditions (Marquez et al., 2015).

### 3 | PROTEINS INVOLVED IN ALTERNATIVE SPlicing IN A. THALIANA

In A. thaliana approximately 430 proteins involved in pre-mRNA splicing have been identified or predicted, including core and specific small nuclear RNA (snRNA) ribonucleoproteins (snRNP), components of the NineTeenComplex (NTC) homolog termed MAC (MOs-4 ASSOCIATED COMPLEX), and the retention and splicing complex (RES), serine/arginine-rich (SR) proteins, heterogeneous nuclear ribonucleoproteins (hnRNP) as well as a large number of RNA-binding proteins and associated regulatory factors (Koncz et al., 2012). Two families, SR and hnRNP proteins, are among the most important regulators of AS and constitutive splicing (Duque, 2011; Meyer et al., 2015).
SR phosphoproteins belong to a highly conserved family in eukaryotes and are involved in RNA–protein and protein–protein interactions during spliceosome assembly (Duque, 2011; Reddy, 2004; Reddy & Shad Ali, 2011). Members of this family are characterized by the presence of one or two RNA recognition motifs (RRMs) in the N-terminal region, which bind to cis-acting regulatory enhancement (ESE/ISE) or silencing (ESS/ISS) sequences in exons (exonic splicing regulators) and/or introns (intronic splicing regulators). In turn, C-terminal domains rich in serine and arginine dipeptides (RS domain) mediate protein–protein interactions with other components of the splicing machinery and also directly contact the pre-mRNA branchpoint. In addition, SR proteins contribute to splice site recognition by recruiting U1 snRNP to the 5′ splice site and U2 snRNP to the branchpoint via promoting the interaction of U2AF with the adjacent 3′ splice site (Reddy & Shad Ali, 2011). In A. thaliana, 19 SR proteins, ranging from 21 to 45 kDa, belong to six different subfamilies. Three families contain orthologs of human ASF/SF2 (SRSF1), 9G8 (SRSF7), and SC35 (SRSF2), while the other three are plant specific (SCL, RS2Z, and RS), giving unique structural features not found in metazoan SR proteins (Barta et al., 2012; Duque, 2011; Reddy, 2004). Most plant SR proteins contain a single RS domain, except for SR45 with two distinct RS domains on either side of the RRM (Reddy, 2004). Interestingly, human SR45 ortholog RNPS1 has been reported to be deposited near the exon–exon junctions and interacts with the UP-FRAMESHIFT (UPF) complex, a main component of the NMD machinery (Lykke-Andersen et al., 2001), thus forming a link between splicing and RNA quality control. These functions are probably conserved in plant SR45 (Zhang & Mount, 2009; Zhang et al., 2017b).

The other factors important for AS belong to the hnRNP family of RNA-binding proteins that bind to nascent RNA polymerase II transcripts and contribute to pre-mRNA processing (Han et al., 2010; Meyer et al., 2015). All hnRNPs contain RRMs or other domains with similar function (e.g., KH domain, glycine-rich, quasi-RRMs) (Han et al., 2010; Lorkovic & Barta, 2002). hnRNPs bind to numerous specific RNA sequences and their function and interacting partners differ depending on the binding site (Wachter et al., 2012). Some of the best-known proteins from the hnRNP family are polyypyrimidine tract-binding proteins (PTBs), which interact with CU-rich motifs located within the 3′ splice site (Meyer et al., 2015; Simpson et al., 2014; Wachter et al., 2012). Additionally, these proteins may interact or compete with other hnRNPs or SR proteins, acting as positive or negative AS regulators by impacting splice site selection (Meyer et al., 2015). Out of three A. thaliana PTB homologs, PTB1 and PTB2, but not PTB3, have been shown to affect AS events, mainly the choice of 5′ alternative splice sites, IR, and exon inclusion. Several of their target genes encode factors that control plant development, for example seed germination and flowering (Meyer et al., 2015; Rühl et al., 2012; Simpson et al., 2014). The hnRNP family also contains glycine-rich RNA-binding proteins (GRPs) with the N-terminal RRM or cold-shock domain, followed by a C-terminal glycine-rich domain (Ciuzan et al., 2015; Mangeon et al., 2010). These proteins regulate not only AS but also translation and mRNA degradation, contributing to plant adaptation to abiotic and biotic stress (Ciuzan et al., 2015; Lambermon et al., 2000). Two A. thaliana GRPs, GRP7 and partially also its close paralog GRP8, were demonstrated to bind RNA and have a role in different RNA-related processes, including AS by affecting the usage of 5′ AS sites (Ciuzan et al., 2015; Streitner et al., 2012; Wachter et al., 2012).

The level of AS factors is often controlled by physiological conditions (e.g., GRP7 and GRP8 by the circadian clock or RRM25 by abscisic acid) and/or auto- and cross-regulated via AS-coupled NMD as is the case for PTBs and GRPs (Stauffer et al., 2010; Streitner et al., 2008; Wachter et al., 2012; Zhan et al., 2015). Moreover, these proteins form an intricate network of interactions with other components of the spliceosome (Lorkovic et al., 2000; Meyer et al., 2015; Reddy et al., 2013), strengthening the notion that the AS landscape in plants is an outcome of the complex interplay between regulatory factors.

4 ALTERNATIVE SPlicing IN RESPONSE TO BIOTIC STRESS

Plants, like animals, are constantly exposed to various types of stress. Depending on the type of stimuli, stress is divided into abiotic and biotic. Abiotic stress is induced by environmental factors such as drought, heat, cold, salinity, and light, whereas biotic stress is elicited by living organisms such as bacteria, viruses, fungi, oomycetes, and insects. Disturbances caused by stress factors often inhibit the growth and development of the plant, can cause tissue damage, diseases and even lead to plant death. In the course of evolution, plants have developed specific defence mechanisms that allow for effective protection against stress factors. These response pathways are modulated by a wide variety of molecular processes, including AS.

Because IR is the most frequent AS event in plants, this type is prevalent among genes that are differentially regulated by abiotic and biotic stresses. However, it has been reported recently that in Arabidopsis also non-IR events, including ES, are overrepresented among stress-responsive genes (Martín et al., 2021). Moreover, in response to stress these events may introduce upstream ORFs (uORF) by extending 5′ untranslated region sequences and thus affect mRNA stability and translation. Finally, stress-related transcripts that are regulated by AS are probably targeted by NMD and have faster turnover than is expected for this type of genes. In turn, exitron splicing contributes to protein diversity, is regulated by stress conditions, and affects many genes involved in the stress response (Marquez et al., 2015). Although generation of functional proteins by alternatively spliced exitrons is still poorly documented, there are examples of exitron-spliced protein isoforms involved in stress response. These include ABI3 variants, which regulate seed abscisic acid sensitivity (Punzo et al., 2020b), FLS2-derived suppressors of signalling mediated by the pathogen effector flagellin (flg22) (Cheng et al., 2020), or MBD4L isoforms generated during heat stress that have different subnuclear localization (Cecchini et al., 2022). All
these observations support AS-mediated stress adaptation of plant transcriptomes and proteomes.

One of the most extensively studied plant pathogens, used as a model organism to understand molecular mechanisms of bacterial pathogenicity and plant-microbial interactions, is the hemibiotrophic *Pseudomonas syringae* pv. *tomato* DC3000 (Pst) (Mansfield et al., 2012). As an extracellular pathogen, Pst propagates in the apoplastic space without entering cells and uses a type III secretion system to introduce a wide variety of effector factors into the host's cytoplasm (Xin & He, 2013; Xin et al., 2018). In the first stage of the plant innate immune response, plants detect conserved molecular patterns associated with pathogens (PAMPs) through receptors from the pattern recognition receptor family on the cell surface. This leads to the activation of intracellular signalling pathways that result in local PAMP-triggered immunity (PTI). The second immune system, called the effector-triggered immunity (ETI) response, is based on the detection of bacterial effectors (Avr) by disease resistance R proteins (Xin & He, 2013). Most R proteins belong to the NBS-LRR family and contain nucleotide-binding site (NBS) and leucine-rich-repeat (LRR) domains, which are involved in the activation of the signalling cascade and recognition of the pathogen effector proteins, respectively (Kapos et al., 2019; Li et al., 2015; Nguyen et al., 2021; Yang et al., 2014).

Many genes encoding factors involved in the defence response undergo AS and, more importantly, the extent of this process is affected by pathogen infection. The biotic stress-response AS genes described here are listed in Table S1. These include, for example, TIR-NBS-LRR R genes *RPS4* (RESISTANCE TO *Pseudomonas syringae* 4), *SNC1* (SUPPRESSOR OF npr1-1, CONSTITUTIVE 1), *RPS6*, *RPP5* (RECOGNITION OF PERONOSPORA PARASITICA 5), and *RAC1* (RECOGNITION OF ALBUGO CANDIDA 1) as well as receptor-like kinases *SNC4* and *CERK1* (CHITIN ELICITOR RECEPTOR KINASE 1), pathogen-induced transcription factor *WRKY40*, receptor-like kinase *FRK1* (FLG22-INDUCED RECEPTOR-LIKE KINASE 1), a central regulator of plant immunity *EDS1* (ENGHANCED DISEASE SUSCEPTIBILITY 1), and a negative regulator of *EDS1*-mediated disease response *NUDT6* (NUCLEOSIDE DIPHOSPHATE LINKED X 6) (Table S1) (van der Biezen et al., 2002; Howard et al., 2013; Kim et al., 2009; Meyer et al., 2015; Staiger & Brown, 2013; Wang et al., 2013a; Yang et al., 2014; Zhang & Gassmann, 2003; Zhang et al., 2014; Zhu et al., 2010). While the effect of AS events related to these factors on plant resistance has not been established in most cases, there are examples where effective counteraction of pathogen invasion requires the synthesis of protein isoforms from alternatively spliced transcripts. The best described is AS of the R gene *RPS4*. *RPS4* confers resistance to the AvrRps4 effector protein expressed by the avirulent Pst. AS of *RPS4* yields several transcript isoforms resulting from retention of introns 2 and/or 3 or splicing of a cryptic intron in exon 3 (Marquez et al., 2012; Zhang & Gassmann, 2003, 2007). Although these AS events generate PTCs, these IR isoforms are probably NMD-insensitive and are strongly up-regulated in response to the AvrRps4 effector. Notably, expression of the truncated variants lacking some LRRs probably regulates the function of *RPS4* because transgenic *RPS4* forms devoid of either of these introns are unable to complement *RPS4* deficiency, even when the fully spliced major form is produced.

An interesting case of the impact of AS on plant immunity concerns *CPK28* (CALCIUM-DEPENDENT PROTEIN KINASE 28), encoding a negative regulator of the immune response (Figure 2). *CPK28* phosphorylates BIK1 (BOTRYTIS-INDUCED KINASE 1) receptor-like cytoplasmic kinase that is activated by pattern recognition receptors and acts as a positive regulator of PTI, as well as E3 ligases PUB25/PUB26 (PLANT U-BOX 25/26) that target BIK1 for degradation (Monaghan et al., 2014; Wang et al., 2018). *CPK28* mRNA undergoes AS as a result of immune activation by plant elicitor peptides, producing an intron-retained PTC-containing variant that encodes a truncated protein with a decreased activity. This in turn leads to BIK1 stabilization and amplification of the immune defence (Dressano et al., 2020). This interplay between *CPK28* and BIK1 is additionally complicated by the fact that *CPK28* activity requires phosphorylation at a single Ser318 residue, which is carried out not only by *CPK28* itself but also by BIK1 (Bredow et al., 2021).

Another key aspect of plant immunity is provided by regulation of AS of defence-related factors, including *R* genes. It transpires that several components of spliceosome or spliceosome-associated complexes affect AS of these genes in response to pathogen infection. For example, MAC core proteins MAC3A and MAC3B (paralogs of human and yeast PRP19), MACSA and MACSB (MO54-ASSOCIATED COMPLEX SUBUNIT 5A, 5B), MOS4 (MODIFIER OF SNC1, 4), Myb-transcription factor CDC5 (CELL DIVISION CYCLE 5), and WD-40 repeat protein PRL1 (PLEIOTROPIC REGULATORY LOCUS 1), although not essential for general splicing, have been shown to be required for basal and *R* gene-mediated defence (Table S1) (Koncz et al., 2012; Meyer et al., 2015; Monaghan et al., 2010; Yang et al., 2014). Mutants in these factors, identified in a screening for suppressors of the snc1-mediated autoimmunity, have a constitutive defence response and enhanced susceptibility to pathogen infection (Monaghan et al., 2009; Palma et al., 2007). Moreover, the AS pattern of SNC1 and *RPS4* is altered in mos4, cdc5, and mac3a mac3b plants as well as a mutant in MAC-associated MOS12 arginine-rich protein that also suppresses snc1 phenotypes (Xu et al., 2012). A spliceosome component SKIP (SKI-INTERACTING PROTEIN) is another protein that interacts with MAC3A, MAC3B, and PRL1, and is responsible for AS of an overlapping set of genes (Li et al., 2019). SKIP regulates AS via splice site recognition and processing, but also has a role as a general splicing factor (Feng et al., 2015). Although in *Arabidopsis* it has been shown to regulate circadian clock and abiotic stress genes (Feng et al., 2015; Li et al., 2019; Wang et al., 2012), SKIP1b silencing in tomato leads to increased susceptibility to *Pst* and *Botrytis cinerea* (Zhang et al., 2020a). It is therefore possible that the defence-related function of MAC and MAC-associated proteins is conferred via AS of *R* genes. Interestingly, SKIP also appears to be a key regulator mediating signalling of jasmonic acid, a key phytohormone in response to pathogens (Feng et al., 2020). SKIP has been reported to interact with transcription factors and kinases involved in jasmonate signalling and to regulate AS of potential target genes, such as NUDX9 (NUDEX HYDROLASE 9) and NRT1.8 (NITRATE
Other examples relate to two conserved splicing factors, SUA (SUPPRESSOR OF ABI3-5) and RSN2 (REQUIRED FOR SNC4-1D2), that have been shown to regulate AS of two receptor-like kinases important for PAMP perception, SNC4 and CERK1 (Zhang et al., 2014). Both SUA, a homolog of human RBM5, and RSN2, a homolog of metazoan SPF45, associate with U2 snRNP and are involved in splice site selection. Mutations in SUA and RSN2 lead to IR, which generates a short insertion and premature stop codon in SNC4 and CERK1 transcripts, respectively. Both sua and rsn2 mutants exhibit decreased resistance against nonpathogenic bacteria suggesting that AS of SNC4 and CERK1 is required for PTI (Zhang et al., 2014).

A number of AS factors, for example SR protein SR45 or hnRNP family protein GRP7, as well as UPF1, UPF3, and SMG7 proteins involved in NMD, which is closely associated with AS, have been reported to contribute to plant immunity (Table S1) (Chen et al., 2019; Hackmann et al., 2014; Jeong et al., 2011; Nicaise et al., 2013; Rayson et al., 2012; Riehs-Kearnan et al., 2012; Zhang et al., 2017b). However, their impact is probably not exerted via regulation of AS but through other transcriptional and posttranscriptional processes. The situation is somehow different for Sm proteins, the core components of the spliceosome snRNP. Sm proteins (B/B′, D1, D2, D3, E, F, and G) directly bind snRNAs and are crucial for splicing initiation by contacting pre-mRNA substrate as a part of snRNP (Zhang et al., 2001). It has been recently reported that knockout of SmD3-β and SmD1 leads to a stronger activation of key pathogenesis markers and altered expression of a large number of defence genes (Golisz et al., 2021). More importantly, lack of SmD3-β affects the
AS pattern of many genes involved in pathogen response, including kinases MAPK4, CRK4 (CYSTEINE-RICH RECEPTOR-LIKE KINASE 4), CPK28, and LRR receptor-like kinase BAK1 (BRASSINOSTEROID-ASSOCIATED RECEPTOR KINASE 1), R genes SNC1 and SNC4, and a splicing factor SUA. Sm proteins interact with pCln and PRMT5 components of the methylsosome complex that mediates snRNP assembly, and the spliceosome activating NTC complex (Deng et al., 2016), which act as negative and positive regulators of plant immunity, respectively (Huang et al., 2016; Monaghan et al., 2009, 2010; Palmia et al., 2007; Xu et al., 2012). Based on these observations the core spliceosome was postulated to modulate plant immunity at least to some extent through AS (Golsz et al., 2021).

AS of R genes is regulated not only by splicing factors but also other type of regulators. For example, transportin-SR (TRN-SR) MOS14 impacts the splicing patterns of SNC1 and RPS4 and is required for both basal and RPS4- and sncl-mediated resistance to Pst (Xu et al., 2011). Because MOS14 is an importin-β family transporter of SR proteins, it is conceivable that MOS14 contributes to plant immunity through nuclear import of these proteins and their involvement in AS of R genes. Recently, it has been shown that expression of some R genes (e.g., RPS2, ZAR1, RPP1-like) is directly regulated by a chromatin-remodelling protein SWP73A (SWITCH/SUCROSE NON-FERMENTABLE (SWI/SNF)-ASSOCIATED PROTEIN) that binds to their promoters and represses transcription, while RPS4 and RRS1 (RESISTANCE TO RALSTONIA SOLANACEARUM 1) are regulated indirectly via modulation of CDC5-mediated AS (Huang et al., 2021). Pst (AvrRps4) infection results in SWP73A silencing by pathogen-induced small RNAs, thus weakening its association with the CDC5 and R gene promoters. This leads to increased expression of CDC5 and as a consequence altered AS pattern of RPS4 and RRS1, which stimulates the synthesis of functional transcripts. At the same time other R genes are up-regulated. This intricate mechanism triggers R gene-mediated immune responses by suppressing SWP73A and activating a set of NLR receptors. In turn, because over-accumulation of NLRs may cause autoimmunity, SWP73A acts as a negative regulator of the plant innate immune response to evade this adverse situation in the absence of pathogens.

AS-mediated regulation of expression and activity of defence-related factors other than R genes concerns, for example, CPK28 encoding a key negative immunity regulator that targets BIK1 for degradation (Monaghan et al., 2014; Wang et al., 2018). The canonical splicing of CPK28 and several other defence genes, such as LSD1 (LESION-SIMULATING DISEASE 1) and JAZ4 (JASMONATE-ZIM-DOMAIN PROTEIN 4), is promoted by the RNA-binding protein IRR (IMMUNOREGULATORY RNA-BINDING PROTEIN), which acts as a negative regulator of the immune response (Dressano et al., 2020). Immune activation of PEP RECEPTORS (PEPRs) by plant elicitor peptides triggers IRR dephosphorylation and dissociation from its RNA substrates, resulting in less efficient splicing and accumulation of intron-retained variants (Figure 2). As already described, in the case of CPK28 the IR transcript contains a PTC and generates a truncated, less active protein isoform. CPK28 AS is also regulated by MPK4 (MAP KINASE 4), a mitogen-activated protein kinase that constitutes the PAMP-induced signalling cascade leading to activation of transcription factors controlling the expression of defence genes (Bigeard et al., 2015). A recent genome-wide study of AS response to the pathogen PTI effector flagellin (flg22) revealed that MPK4, but not MPK3 and MPK6, regulated approximately 40% of flg22-triggered differential AS events, including AS of pathogen response genes, several splicing factors phosphorylated by MPK4 (e.g., SCL33, SR30, SR45a, RS40, RS41, U2AF65A, RZ1B, and RZ1C), RNA-binding proteins, helicases, and transcription factors (Bazini et al., 2020). Notably, among genes with flg22-induced isoform-switching events were those of protein kinases involved in PTI, CPK28, the cysteine-rich receptor-like kinases CRK13 and CRK29, and the FLS2 co-receptor SERK4/BKK1 (SOMATIC EMBRYOGENESIS-RELATED KINASE 4). Such events lead to changes in the ratio of alternative isoforms, containing, for example, altered combination of kinase domains, and as a consequence to functional differences of resulting proteins.

The last example is represented by a less common type of AS regulator, which is not a protein but a long noncoding RNA (lncRNA). In the last decade IncRNAs have emerged as versatile and potent modulators of gene expression through various mechanisms in all eukaryotic organisms, including plants, where they have also been reported to regulate the response to abiotic and biotic stress (Nejat & Mantri, 2018; Sun et al., 2018). In Arabidopsis, ASCO (ALTERNATIVE SPlicing COMPETITOR) IncRNA was initially shown to interact with AtNSRs (NUCLEAR SPECKLE RNA-BINDING PROTEINS) AS regulators and compete for binding with their mRNA substrates, thus altering their splicing pattern, especially in response to auxin (Figure 3) (Bardou et al., 2014). Interestingly, ASCO also interacts with SmD1b and PRP8a core spliceosome components and influences the accumulation or AS of the subset of their mRNA substrates, including genes involved in plant defence related to flg22 response (Rigo et al., 2020). The differentially spliced flg22-regulatory genes include NBS-LRR R genes RPP4 and RLM3 (RESISTANCE TO LEPTOSPHAERIA MACULANS 3), SR34, SNC4, and NUDT7 (van der Biezen et al., 2002; Staal et al., 2008). ASCO is probably one of many as yet unrecognized IncRNAs that may contribute to plant immunity through modulation of AS or other posttranscriptional processes.

Another relevant question is how AS is triggered by biotic stress, but unlike abiotic stress, biotic stress-induced AS responses in plants are largely unexplored. One of the first RNA-Seq analyses of P. syringae-infected Arabidopsis revealed that over 90% of expressed genes underwent AS and approximately 40% of them were novel AS events (Howard et al., 2013). Pathogen challenge has been also reported to trigger accumulation of the IR PTC-containing isoforms of the master circadian clock regulators CCA1 (CIRCADIAN CLOCK ASSOCIATED 1) and LHY (LATE ELONGATED HYPOCOTYL) (Filichkin et al., 2015a). In addition, the retained CCA1 intron specifically binds SR45 protein in vitro, suggesting that this splicing factor could be involved in regulation of IR, possibly also during pathogen attack. Because the circadian clock has been postulated to synchronize the timing of infection, pathogen-induced changes in the CCA1 and LHY AS variants may contribute to this option of defence response...
regulation (Filichkin et al., 2015a). In turn, flg22 alone has been reported to induce AS of more than 500 genes, with IR as the most frequent event, followed by alternative 3’ splice site selection, alternative 5’ splice site selection, ES, and a single MXE event (Bazin et al., 2020). Importantly, flg22-induced AS appears to be regulated by MPK4, one of the kinases that are key mediators of PAMP-triggered immunity (Bigeard et al., 2015). It is therefore likely that MPK4 activation during PTI represents one of the signalling mechanisms that orchestrate the contribution of AS to the biotic stress response.

5 | THE ROLE OF COUPLING BETWEEN TRANSCRIPTION AND ALTERNATIVE SPlicing IN PLANT IMMUNITY

Another important aspect of the regulation of stress response by splicing that has been addressed recently is the co- and posttranscriptional character of this process (Jabre et al., 2019; Punzo et al., 2020a; Reddy et al., 2020). A growing body of evidence has revealed that splicing in metazoa and yeast is largely cotranscriptional (Herzel et al., 2017). Recent transcriptomic studies of nascent or chromatin-bound transcripts have demonstrated that in plants the vast majority of pre-mRNAs are spliced cotranscriptionally and the efficiency of cotranscriptional splicing depends mainly on gene expression level and the number and position of introns (Jia et al., 2020; Kindgren et al., 2020; Li et al., 2020; Zhang et al., 2020c; Zhu et al., 2018, 2020). Splicing of multi-intron genes largely follows the order of transcription, with approximately 70% of the upstream introns spliced first and a significant part of upstream introns spliced before the transcribing Pol II passes the downstream introns (Jia et al., 2020). Moreover, the speed of splicing of the downstream intron is generally faster than that of the upstream intron, which can aid efficient splicing of multi-intron genes.

Cotranscriptional splicing and AS are coupled to transcription. It has been postulated that this coupling is provided by interactions between transcription and splicing machineries (recruitment model) or by the rate of polymerase elongation, which determines the occurrence of cotranscriptional processes and the extent of AS events (kinetic model) (Dujardin et al., 2013). Because the speed of transcription is largely dictated by chromatin accessibility and nucleosome occupancy, which in turn are modulated by DNA methylation, histone variants and modifications, and chromatin remodelling, cotranscriptional processes are also regulated by epigenetic factors. There are several studies describing transcriptional reprogramming by modulation of epigenetic marks during both abiotic and biotic stresses (Alonso et al., 2019; Deleris et al., 2016; Espinas et al., 2016; Jabre et al., 2019; Kim, 2021; Reddy et al., 2020; Ueda & Seki, 2020). It turned out that several DNA and histone modifying enzymes and factors are involved in a large variety of stress response pathways, including drought, salinity, and heat as well as infection by viral, bacterial, and fungal pathogens. For example, in the case of Pst-infected Arabidopsis plants, expression of genes encoding major defence factors, PR1 (PATHOGENESIS-RELATED GENE 1), PR2, PR5, NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1), and PAD4 (PHYTOALEXIN DEFICIENT 4), is induced by histone acetylation and DNA methylation mediated by the Elongator complex.
(DeFraia et al., 2013; Wang et al., 2013b). Likewise, several other histone deacetylases (e.g., HD2B and HDA6), methyltransferases (e.g., SDG8, SDG25, and OS97), demethylases (e.g., IBM1, JMJD7, LDL1, and LDL2), and ubiquitin ligases (e.g., HUB1 and HUB2) as well as DNA methylases and demethylases (e.g., ROS1 or RNA polymerase V subunits that mediate siRNA-dependent transcriptional gene silencing) regulate the expression of immune-responsive genes, including transcription factors, resistance genes, and abscisic acid, salicylic acid, and jasmonic acid hormone signalling genes, in response to pathogen attack (Deleris et al., 2016; Kim, 2021; Noh et al., 2021).

AS is also associated with the state of chromatin and dynamic changes in nucleosome occupancy. These features strongly affect the type and level of AS events, which undergo distinct and more complex regulation than constitutive splicing. In particular, chromatin is more open in retained introns and consequently the rate of transcription in these regions is higher, in line with reduced DNA methylation level compared to constitutively spliced introns. Along with weaker splice sites of retained introns, a faster elongation rate may prevent recognition and correct cotranscriptional excision of these introns by the spliceosome (Jabre et al., 2021; Ullah et al., 2018). Despite obvious connections between AS and chromatin, and the observations that epigenetic regulation contributes to the biotic stress response, there are no compelling examples demonstrating that modulation of plant immunity by AS is directly mediated by epigenetic factors.

On the other hand, although AS events are often determined at the chromatin level, alternative introns appear to be less efficiently spliced than constitutive introns and they are more prone to be removed postranscriptionally (Jia et al., 2020; Li et al., 2020; Zhu et al., 2020). Incompletely spliced and polyadenylated transcripts that have been detected on chromatin do not become released and exported to the cytoplasm and are resistant to NMD, so they probably undergo further processing to mature mRNAs. Although such postranscriptionally spliced introns constitute only 28% of all introns, the majority of IR events belong to this group (Jia et al., 2020). This suggests that widespread IR in plants may be associated with a relatively high level of postranscriptional splicing. Interestingly, postranscriptionally spliced introns constitute the main part of retained introns that are increased under various abiotic stress conditions. It has been proposed that splicing of this class of introns is regulated in response to various environmental signals and, consequently, it represents an additional layer of reprogramming of gene expression in response to various environmental signals. In fact, there are some examples supporting this notion in the case of abiotic stress, namely that splicing of such introns is inhibited following cold and heat treatments (Jia et al., 2020). While no research has yet been done on the relationship between co- and postranscriptional splicing and biotic stress, we can speculate that response to bacterial and viral attacks is also regulated in a similar manner. This is all the more likely as biotic and abiotic stresses are interconnected and share many regulatory principles, and some immune signalling components are also involved in abiotic stress tolerance (Saijo & Luo, 2020; Zhang & Sonnewald, 2017). Also, transcriptional responses to both types of stress overlap to some extent, suggesting that changes in gene expression to abiotic and biotic cues are a part of the general stress response. Notably, processing of posttranscriptionally spliced introns is more dependent on certain splicing regulators, such as PRMT5 and SKIP, than of constitutive introns. As already mentioned, these proteins regulate AS in response to developmental and stress cues, including pathogen infection (Huang et al., 2016; Li et al., 2019; Sanchez et al., 2010; Wang et al., 2012; Zhang et al., 2020a). Thus, the distinct features of co- and postranscriptional splicing can be used to dynamically modulate the expression of defence-related factors by a group of specific splicing regulators to fine-tune the plant immune response.

6 | CONCLUSION

The widespread character of AS in plants and its key role in response to both abiotic and biotic stresses has already been firmly established. This regulation is mainly achieved by modulating the level of defence factors or their alternative isoforms. The repertoire of AS events that contribute to the scope of these mechanisms has recently been expanded by exitrons and postranscriptionally spliced introns, which, while much less frequent than cotranscriptional events, are functionally relevant. It is increasingly apparent that the coupling between transcription, AS and translation plays an important role in plant resistance, development, and growth. New high-throughput technologies, including RNA-Seq-based approaches and chromatin mapping and proteomic profiling, are likely to provide new insights into the tremendous regulatory potential of AS.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed.

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