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REVIEW

Structural analyses of ABA transporters give new impetus to the study of ABA regulation

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Abstract

Abscisic acid (ABA) is a plant hormone that regulates key physiological processes, including stomatal closure, seed germination and development, and is central to the coordination of abiotic stress responses. In the six decades since it was first described, a huge amount of work has been conducted on ABA synthesis, breakdown and signalling mechanisms. ABA is synthesised mainly in vascular tissues and transported to distal sites to exert its physiological functions. This review presents an integrated overview of ABA metabolism and signalling. A major focus is placed on recent structural breakthroughs in the characterisation of adenosine triphosphate-binding cassette transporters, particularly ABCG25, which have revealed detailed molecular mechanisms of ABA recognition, binding and transmembrane transport. These discoveries, including transporter conformational dynamics and dimerisation with ABCG16, reveal how ABA movement is tightly controlled across cell membranes and intracellular compartments. As climate change intensifies, understanding ABA transport offers a promising avenue for future plant breeding and agricultural innovation.

KEYWORDS
ABA function, abiotic stress, adenosine triphosphate (ATP)-binding cassette (ABC) class transporters, climate change

1 | INTRODUCTION

Abscisic acid (ABA) is a plant hormone that was first identified and characterised in 1963 as a promoter of abscission (Ohkuma et al., 1963). At that time, it was called Abscisin-II, but the name ABA was adopted as it became clear that it was involved in many other plant physiological processes (Addicott et al., 1968). Indeed, it is now known that the regulation of abscission is not one of ABA's major functions. ABA is sometimes referred to as a stress hormone because of its role in plant adaptation to abiotic stress conditions, including heat, cold, drought and salinity. The response to drought stress, of course, involves stomatal closure, and ABA has a specific role in the control of stomatal closing and opening (see Assmann & Jegla, 2016, for review). However, ABA is also involved in the regulation of plant growth and key developmental phases, including seed dormancy (Karssen et al., 1983) and germination (Schopfer et al., 1979), as well as fruit ripening (Wu et al., 2023).

Extensive research over the past six decades has elucidated the biosynthetic pathways, signalling mechanisms and regulatory roles of ABA in plant development and stress responses. However, until recently, comparatively little was known about how ABA is transported between tissues and across cellular membranes, which is an essential component of its spatial and temporal regulation. This review summarises the current state of knowledge of ABA biosynthesis and signalling, while addressing this important knowledge gap by highlighting recent structural and mechanistic advances in the study of ABA transporters. In particular, we focus on recent cryo-electron microscopy groundbreaking work that has revealed the molecular architecture and function of key

ABC transporters such as ABCG25 and ABCG16, shedding new light on how ABA movement is controlled at the cellular level (Huang et al., 2023; Xin et al., 2024; Ying et al., 2023; Zhou et al., 2024).

2 | ABA BIOSYNTHESIS AND BREAKDOWN

ABA ($C_{15}H_{20}O_4$) (Figure 1) is a sesquiterpene; that is, a member of a class of terpenes made up of three isoprene ($CH_2{=}C(CH_3){-}CH{=}CH_2$) units. Recent evidence indicates that ABA biosynthesis occurs mainly in the leaves (Manzi et al., 2015; McAdam, Brodribb, & Ross, 2016; McAdam, Manzi, et al., 2016). The first steps in the pathway are plastidic and begin with the hydroxylation of β-carotene ($C_{40}H_{56}$) to produce zeaxanthin ($C_{40}H_{56}O_2$). Zeaxanthin is then converted to all-*trans*-violaxanthin ($C_{40}H_{56}O_4$) via the xanthophyll cycle, and all-*trans*-violaxanthin is either isomerised to 9-*cis*-violaxanthin or converted to all-*trans*-neoxanthin ($C_{40}H_{56}O_4$), the latter through the action of neoxanthin synthase. 9-*trans*-neoxanthin is isomerised to the 9-*cis* form and finally, as far as the plastid part of the pathway goes, both 9-*cis*-neoxanthin and 9-*cis*-violaxanthin undergo oxidative cleavage to form xanthoxin ($C_{15}H_{22}O_3$), catalysed by the enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED) (Figure 1). This is the first committed, non-reversible step in ABA biosynthesis and is the rate-limiting step in the process (Thompson et al., 2000). The xanthoxin that is formed moves to the cytosol, where it is converted to abscisic aldehyde ($C_{15}H_{20}O_3$) and then ABA.

Under abiotic stress conditions, the expression of ABA biosynthetic genes is up-regulated (Cheng et al., 2002; Iuchi et al., 2001; Seo et al., 2007; Xiong et al., 2001, 2002). When the stress conditions alleviate, ABA can be degraded by the cytochrome p450 enzyme, ABA 8′-hydroxylase (Krochko et al., 1998; Saito et al., 2004), which converts ABA to 8′hydroxy-ABA. ABA can also be converted to its glucose ester, β-D-glucopyranosyl abscisate (ABA-GE), through the action of ABA glucosyltransferase (Dong et al., 2014; Xu et al., 2002).

Under biotic stress, ABA enhances physical barrier defences. The pathogen-associated molecular pattern-triggered stomatal closure depends on an active ABA signalling pathway within guard cells, which is essential for restricting pathogen entry. However, ABA often acts antagonistically to other key hormones such as salicylic acid (SA), jasmonic acid (JA) and ethylene. It suppresses SA-dependent systemic acquired resistance (SAR) and reduces resistance to biotrophic pathogens, as shown by increased susceptibility in ABA-overproducing plants and enhanced resistance in ABA-deficient mutants (Fan et al., 2009; Yasuda et al., 2008). Likewise, ABA inhibits JA and ethylene signalling pathways, downregulating defence genes and ethylene responses, which leads to greater vulnerability to necrotrophic pathogens (Anderson et al., 2004; Asselbergh et al., 2008; Xiong & Yang, 2003).

3 | THE ABA SIGNALLING PATHWAY

The ABA signalling pathway was elucidated in the 2000s and type 2C protein phosphatases (PP2C) were identified as negative regulators, acting through the dephosphorylation and inactivation of protein kinases of the sucrose nonfermenting-1-related protein kinase 2 (SnRK2) class (Figure 2) (Gosti et al., 1999; Kuhn et al., 2006). Interactions between PP2Cs and SnRK2 were confirmed in yeast two-hybrid assays (Cutler et al., 2010) and PP2Cs were shown to inactivate SnRK2 in the absence of ABA (Figure 2a) by dephosphorylation of one of the serine residues in the activation loop (Umezawa et al., 2009). SnRK2 had already been shown to phosphorylate transcription factors of the ABA response element binding protein (AREBP) class at multiple sites (Furihata et al., 2006; Kobayashi et al., 2005). AREBPs (also known as ABFs) are a family of basic leucine zipper (bZIP) transcription factors that recognise G-box binding sites known as ABA response elements (ABREs) present in many ABA-regulated genes (Cutler et al., 2010). This was followed by the identification of the PYR/PYL/RCAR family of proteins as ABA receptors (Nishimura et al., 2010). In the presence of ABA, PYR/PYL/RCARs bind to and inhibit PP2Cs, allowing the accumulation of active SnRK2s and phosphorylation of AREBPs (Figure 2b) (Cutler et al., 2010).

This system looks quite simple, but there are layers of complexity to it. For example, there are multiple PP2Cs, and three of these appear to co-operate in ABA signalling in Arabidopsis (Rubio et al., 2009). Plants also contain multiple SnRK2s (*Arabidopsis thaliana*, barley and rice have 10, e.g., Chen et al. (2021), Halford et al. (2003), Kobayashi et al. (2004)), not all of which are regulated by ABA. They are all up-regulated and activated by osmotic stress, but this occurs via both ABA-dependent and -independent mechanisms in both *Arabidopsis* and rice (Boudsocq et al., 2004, 2007; Kobayashi et al., 2004). These mechanisms involve different patterns of phosphorylation at two serine residues in the activation loop. SnRK2.6, for example, which is induced by both ABA and osmotic stress, is phosphorylated independently on both of these residues, while for SnRK2.10, which is induced by osmotic stress but not ABA, phosphorylation of one site is dependent on phosphorylation at the other (Vlad et al., 2010).

The role of different SnRK-type protein kinases in ABA signalling is also complex. Plants contain three classes of SnRKs: SnRK1, SnRK2 and SnRK3 (Halford & Hardie, 1998). SnRK1 is a metabolic regulator that is activated during metabolic stress and acts to restore and maintain energy balance (Peixoto & Baena-González, 2022). Evidence from several studies also indicates the involvement of SnRK1 in starch biosynthesis in plants, a process that in storage organs such as seeds and tubers, at least, is sucrose-inducible and which in developing cereal seeds is a characteristic of grain filling (Jain et al., 2008; Kanegae et al., 2005; McKibbin et al., 2006; Purcell et al., 1998). SnRK1 is closely related to 5′AMP-activated protein kinase (AMPK) of mammals and sucrose nonfermenting-1 (SNF1) of budding yeast (*Saccharomyces cerevisiae*), sharing 47% amino acid sequence identity and showing similar substrate specificity; incredible, given that plants, animals and fungi diverged approximately 1.5 billion years ago.

SnRK2s and SnRK3s have diverged further from SNF1 and AMPK than SnRK1 has (Chen et al., 2021; Halford et al., 2003; Halford & Hardie, 1998). They are unique to plants and, given their complete absence from animals and fungi, it is likely that they emerged only

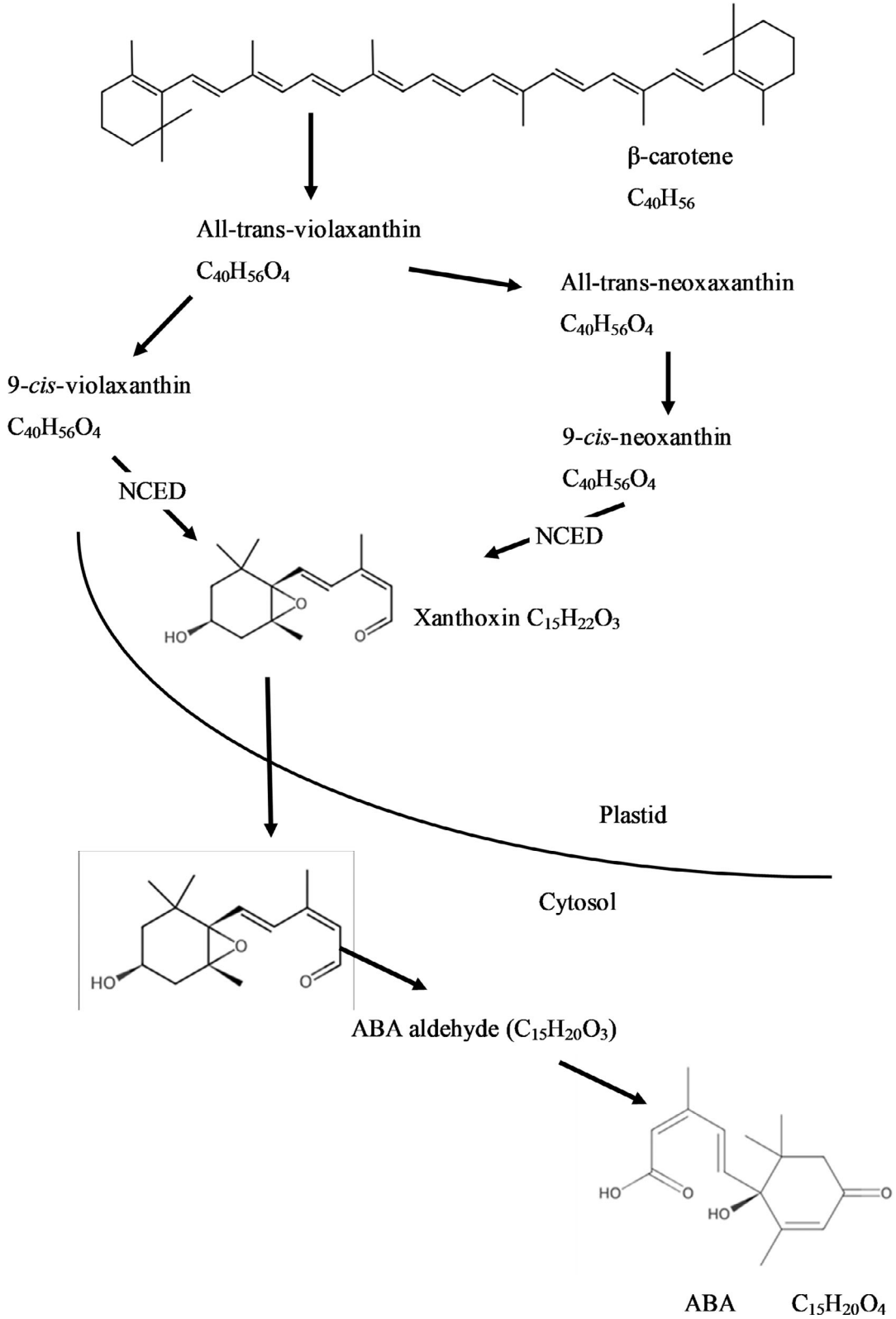

FIGURE 1 Biosynthesis of the sesquiterpene, abscisic acid (ABA). β-carotene in plastids is converted via zeaxanthin and all-*trans*-violaxanthin to 9-*cis*-violaxanthin and 9-*cis*-neoxanthin. These molecules then undergo oxidative cleavage to form xanthoxin, catalysed by the enzyme 9-*cis*-epoxycarotenoid dioxygenase (NCED). The xanthoxin that is formed moves to the cytosol, where it is converted to abscisic aldehyde and then ABA. Molecular structures obtained from the Royal Society of Chemistry ChemSpider website (https://www.chemspider.com; accessed 6 November 2024).

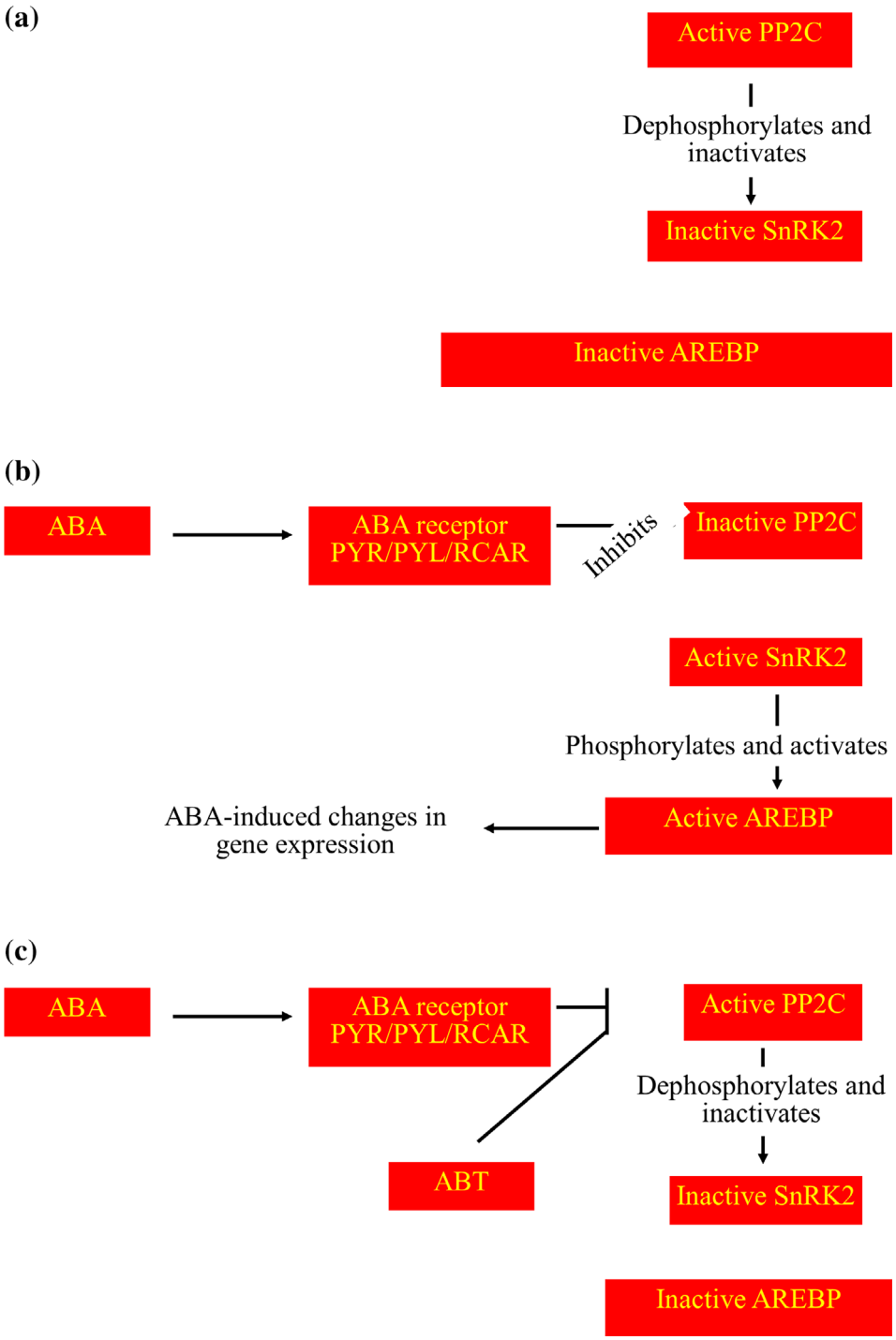

FIGURE 2 Abscisic acid (ABA) signalling pathways. (a) In the absence of ABA, protein kinases of the SNF1-related protein kinase 2 (SnRK2) class are kept in a dephosphorylated, inactive state through the action of type 2C protein phosphatases (PP2C). (b) ABA interacts with receptor proteins (PYR/PYL/RCAR), which then bind to and inhibit PP2Cs, allowing the accumulation of active SnRK2s and phosphorylation of ABA response element binding proteins (AREBPs). (c) Termination of ABA signalling can be brought about by the action of an ABA signalling terminator (ABT), which interferes with the interaction between the ABA receptors and PP2C protein phosphatases.

during plant evolution. The SnRK2 and SnRK3 families are much larger and more diverse than SnRK1. In *Arabidopsis*, there are 10 SnRK2 and 26 SnRK3 members, while barley has 10 SnRK2 and 34 SnRK3 members. In contrast, SnRK1 has only three members in Arabidopsis and six in barley (Chen et al., 2021; Halford et al., 2003).

This rapid evolution may have occurred as they took on new roles to enable plants to link metabolic and stress signalling (Halford & Hey, 2009). SnRK3-type protein kinases are calcium-dependent through their interaction with calcineurin B-like (CBL) calcium-binding proteins (Guo et al., 2002). For this reason, they are also known as

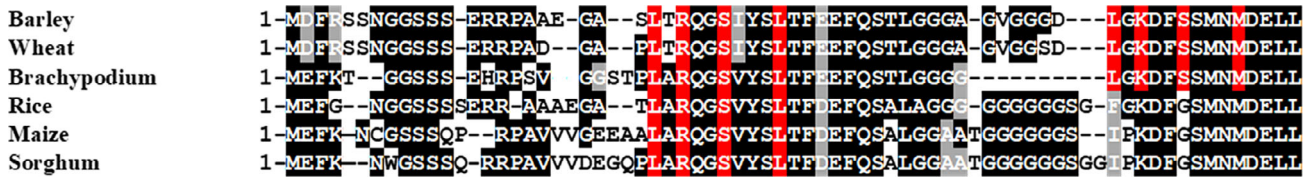

FIGURE 3 Alignment of the N-terminal region of representative abscisic acid (ABA)-response element binding proteins (AREBPs) of the ABI5 type from barley (*Hordeum vulgare*) (GenBank: AAO06115.1); wheat (*Triticum aestivum*) (GenBank: BAD97366.1); Brachypodium (*Brachypodium distachyon*) (National Center for Biotechnology Information [NCBI] Reference Sequence: XP_003578228.1); rice (*Oryza sativa*) (GenBank: EEC84700.1); maize (*Zea mays*) (NCBI Reference Sequence: NP_001132507.1); and sorghum (*Sorghum bicolor*) (NCBI Reference Sequence: XP_002460329.1), showing a SnRK1/2 target site. SNF1-related protein kinase 2 (SnRK2) requires the serine residue to be preceded by a basic residue at position −3 (arginine in this case). SnRK1 has the additional requirement of a hydrophobic residue (leucine in this case) at position −5 and +4. These residues are highlighted in red. Other residues in the region that are conserved across all of the proteins are highlighted in black, while conservative substitutions are highlighted in grey. *Source*: Adapted from Chen et al. (2013).

CBL-interacting protein kinases (CIPKs). SnRK3s/CIPKs are involved in multiple ABA and other hormone-mediated signalling pathways that regulate plant responses to drought, cold, salinity and osmotic stresses (Kaya et al., 2024).

SnRK2 phosphorylates AREBPs preferentially at serine residues with the relatively simple requirement of having a basic residue (usually arginine) at position −3 with respect to the serine (Arg-Xxx-Xxx-Ser). SnRK1 will also phosphorylate this target site but only if there is a hydrophobic residue at positions −5 and +4 with respect to the serine residue (Hyd-Xxx-Arg-Xxx-Xxx-Ser-Xxx-Xxx-Xxx-Hyd, where Hyd indicates hydrophobic). Figure 3 shows a phosphorylation site towards the N-terminal end of cereal ABA-insensitive-5 (ABI5) AREBPS of barley, wheat, rice, *Brachypodium distachyon*, maize and sorghum. These are just representative examples because this phosphorylation site is present in all AREBPs (Zhang et al., 2008). Mutations of the serine at this site in an *Arabidopsis* AREBP, AREB1, resulted in a complete loss of ABA-dependent phosphorylation by a SnRK2 protein kinase activity (Furihata et al., 2006). As shown in Figure 3, this site also fits the requirements for SnRK1 phosphorylation, and a peptide based on this sequence has been shown to be an excellent substrate for purified SnRK1 in vitro and a SnRK1 activity present in soluble protein extracted from *Arabidopsis* seedlings grown in liquid culture (Zhang et al., 2008). This is consistent with transgenic *Arabidopsis* plants over-expressing SnRK1 having an ABA-hypersensitive phenotype (Jossier et al., 2009). The same peptide was also phosphorylated by a calcium-dependent activity in the *Arabidopsis* seedlings. This could be attributable to SnRK3/CIPK or to the closely related calcium-dependent protein kinases (CDPKs). CDPKs, themselves, are positive regulators of ABA signalling and have been shown to phosphorylate some AREBPs to stimulate gene expression (Choi et al., 2005; Zhu et al., 2007). These results suggest that this highly conserved phosphorylation site in AREBPs could be convergence points for multiple signalling pathways involving all three SnRK subfamilies and CDPKs. They also add to the evidence showing calcium to be an important second messenger during ABA signalling.

ABA signalling can be terminated by ABA degradation, of course, but recent evidence has revealed that ABA-induced dormancy in *Arabidopsis* seeds is released through the action of an ABA signalling terminator (ABT) (Wang et al., 2020). ABT is a WD40 protein that interferes with the interaction between the ABA receptors and PP2C protein phosphatases, preventing inhibition of the protein phosphatases and terminating ABA signalling (Figure 2c).

4 | ROLE OF ABA IN SEED MATURATION AND DORMANCY

ABA plays a crucial role in seed development, maturation and the establishment and maintenance of dormancy (Karssen et al., 1983). In early seed development, ABA may be derived from maternal tissue, but accumulation after that is dependent on biosynthesis in the developing seed. The genes involved in ABA biosynthesis in developing barley seeds have all been described and many have been shown to be highly responsive to drought stress, resulting in increased ABA accumulation under drought conditions (Seiler et al., 2011). A study of the interactions between ABA, SnRK1 and SnRK2 in wheat showed contrasting effects of ABA on SnRK1 and SnRK2 protein levels and phosphorylation state (Coello et al., 2012). Application of ABA to wheat roots brought about a dramatic decrease in SnRK1 protein, and phosphorylation/activation of a 42 kDa SnRK2, implying differential roles for SnRK1 and SnRK2 in ABA signalling and antagonistic effects of SnRK1 and SnRK2 on gene expression. The latter may arise through phosphorylation of the same transcription factors at different sites or the phosphorylation of different transcription factors that have antagonistic roles (Coello et al., 2012). We propose that the transition from grain filling to maturation involves an ABA-induced change from a SnRK1 dominated scenario to a SnRK2-dominated one (Chen et al., 2013). The maintenance of dormancy when maturation is complete involves tight repression of the genes encoding enzymes associated with germination, and a SnRK2, PKABA1, has been shown to down-regulate α-amylase genes in response to ABA (Gómez-Cadenas et al., 1999; Johnson et al., 2008). In contrast, an α-amylase gene (*α-Amy2*) promoter is repressed by transient down-regulation of SnRK1 in cultured wheat embryos (Laurie et al., 2003), again indicating antagonistic roles of SnRK1 and SnRK2 protein kinases and different interactions with ABA.

The ending of dormancy and the transition into germination involves multiple signals, hormones and processes, but the cessation of ABA action is an absolute requirement (Schopfer et al., 1979). Recent evidence shows that this is brought about in Arabidopsis seeds through the action of the ABT protein described in the previous section (Wang et al., 2020) (Figure 2c).

5 | ABA TRANSPORTATION AND RECENT ADVANCES

Transport plays an important role in ABA function by facilitating the transfer of ABA between its synthesis sites and target tissues. Indeed, efficient ABA transport is essential to regulate plant responses to abiotic stresses, as well as seed dormancy/germination and other developmental processes. There are two main mechanisms by which ABA is transported in plants, the first diffusion-based and the second mediated through specific transporters (Boursiac et al., 2013; Zhang, Kilambi, et al., 2021; Zhang, Yu, et al., 2021). Diffusion-based transport occurs because ABA is a weak acid and its protonated form (ABA-H), which has no charge, can diffuse freely across lipid membranes. Its anionic form (ABA^-), on the other hand, is charged and requires the action of specific transporters in order to cross membranes. The 'ionic trap model' well explains ABA's flux between organs based on pH (Boursiac et al., 2013; Kaiser & Hartung, 1981). According to this model, ABA stays in its protonated (ABA-H) form in apoplasts (pH approximately 5.0–6.1) and diffuses into cells, but once it enters the cytosol (pH approximately 7.5) it dissociates into ABA^-, creating a unidirectional transport mechanism favouring its movement into cells.

Specialised membrane transporters regulate the influx, efflux and distribution of ABA across plant tissues, and these transporters are crucial for maintaining ABA homeostasis (Zhang et al., 2023). Key ABA transporters include adenosine triphosphate (ATP)-binding cassette (ABC) transporters and nitrate transporter, NRT1.2, belonging to the NRT1/PTR family. In Arabidopsis, AtABCG25, AtABCG31 and AtDTX50 act as ABA exporters, while AtABCG30, AtABCG40, AtABCG17/18 and NRT1.2/NPF4.6 act as importers (Anfang & Shani, 2021; Do et al., 2021; Kang et al., 2010). The first exporter to be identified was AtABCG25 (Kuromori et al., 2010) and it has been the subject of intensive study. AtABCG25 is localised in hypocotyls, roots and vascular bundles and plays a key role in exporting ABA from cells into the xylem, facilitating long-distance transport from roots to shoots. AtABCG25 has been shown to be involved the regulation of late seed development and germination (Kuromori et al., 2010), and its overexpression induces an ABA response in guard cells (Kuromori et al., 2016). In contrast, AtABCG40, an influx transporter found in roots, mesophyll tissues and guard cells, mediates ABA uptake to regulate stomatal closure and drought tolerance (Kang et al., 2015). Mutations in the genes encoding these transporters result in altered ABA sensitivity and impaired drought responses. NRT1.2 is also an influx transporter found in guard cells, but as well as having a role in the uptake of ABA for stomatal closure, it is involved in ABA import in roots and stems, linking ABA signalling to nitrogen regulation and influencing lateral root development and seed dormancy (Li et al., 2020).

ABA transporters are important for maintaining ABA distribution under changing physiological conditions. For example, under drought stress or nitrogen deprivation, xylem pH increases, leading to a reduction in the protonated ABA-H pool. This slows passive diffusion, but transporters like AtABCG25 and AtABCG40 compensate by transporting anionic ABA (Boursiac et al., 2013; Dodd et al., 2003; Wilkinson & Davies, 1997).

Very recently, three groundbreaking studies have elucidated the molecular structure of Arabidopsis ABCG25 and the mechanism of ABCG25-mediated ABA transmembrane transport (Huang et al., 2023; Xin et al., 2024; Ying et al., 2023). All three used cryogenic electron microscopy to reveal the molecular mechanisms of ATP binding, substrate recognition and cross-membrane transport of ABA. They describe ABCG25 functioning as a homodimer, with each monomer composed of a nucleotide-binding domain (NBD) and a transmembrane domain (TMD) containing six helices (TM1–TM6). Both monomers participate in forming a central, hydrophobic binding cavity, structured primarily by TM1, TM2 and TM5a, where ABA binds. These helices are highly conserved among ABCG25 homologues across diverse plant species, including, for example, *Medicago truncatula* and *B. distachyon* (Kuromori et al., 2021; Pawela et al., 2019).

Conserved residues within the TMD cavity are critical for ABA binding and specificity, forming a binding environment tailored to ABA's structure and creating hydrophobic and electrostatic interactions that stabilise ABA within the cavity. The binding cavity is selective, favouring ABA in its anionic form (ABA^-) and displaying minimal conformational changes upon ABA binding, indicating a design with high specificity for ABA optimised for efficient ABA export across the membrane.

The three studies also described multiple conformational states adopted by ABCG25: an inward-facing (apo) state, an ABA-bound state and an outward-facing ATP-bound state. The cycle of conformational changes between these states is essential for ABA recognition and transport. ATP binding triggers significant structural shifts: the NBDs close, resulting in a compact configuration that facilitates ABA export, then ATP hydrolysis powers a shift from an inward-facing conformation, exposing the binding cavity to the cytosol, to an outward-facing conformation, releasing ABA extracellularly. All three studies found ATPase activity to be essential for ABA transport, with mutations in the ATP-binding site abolishing ATPase activity and thus inhibiting ABA transport.

Huang et al. (2023) also proposed a 'gate-flipper' mechanism, where TM2 and TM5a undergo rotational shifts that alternately open and close access to the substrate-binding cavity, helping drive ABA across the membrane, with ATP hydrolysis prompting the outward conformational change. They also provided a detailed look at how ABA binds symmetrically within the cavity, adopting a 2-*cis*, 4-*trans* configuration, which matches the configuration observed in ABA receptors. They noted that the binding site architecture of ABCG25 shares some commonality with human ATP-binding cassette

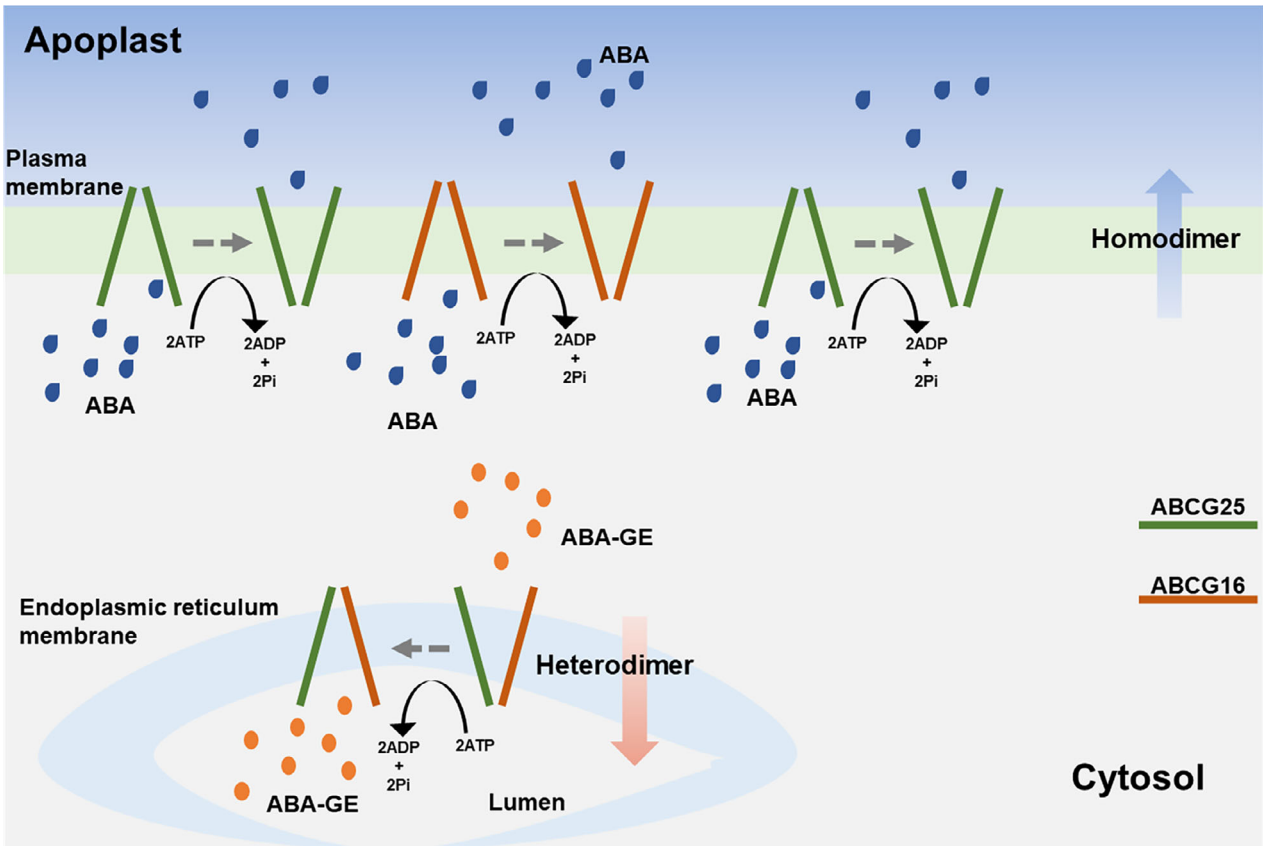

FIGURE 4 A proposed model of abscisic acid (ABA) transport involving adenosine triphosphate (ATP)-binding cassette (ABC) transporters, ABCG16 and ABCG25. These transporters form homodimers to export ABA across the cellular plasma membrane but interact to form heterodimers with each other to export conjugated β-D-glucopyranosyl abscisate (ABA-GE) across the endoplasmic reticulum membrane.

subfamily G transporter (ABCG) transporters but exhibits unique substrate-specific modifications that enable efficient ABA transport, providing a broader context for understanding ABA transporter evolution and functional specificity within the ABCG family.

Xin et al. (2024) considered the evolutionary context of ABCG25 in flowering plants, suggesting that it evolved to fulfil an essential role in ABA transport, particularly in endosperm tissue to support seed development. They suggested that this angiosperm-specific adaptation allows ABCG25 to regulate ABA levels in a way that supports ecological fitness and stress resilience, concluding that its function was highly relevant to modern agriculture. Xin et al. (2024) also identified auxiliary lipid binding sites in ABCG25, suggesting that lipid binding could be a modulating factor for ABCG25 stability and functionality, possibly with a regulatory role, affecting transporter activity under certain conditions. They showed that the cholesterol analogue, cholesteryl hemisuccinate, which was added during purification, bound within the TMD, possibly stabilising the structure without directly participating in ABA transport.

Ying et al. (2023) highlighted the role of the binding site's electrostatic potential, showing that the cavity possesses a mild positive charge at the bottom, which is essential for stabilising ABA's negatively charged form. This electrostatic feature potentially contributes to the high selectivity of the transporter for ABA, facilitating efficient efflux and distinguishing ABCG25's functionality from other members of the ABCG transporter family. The roles of key residues in ABA transport were validated by detailed mutational analysis, with mutations of Phe453 and Thr552, for example, greatly reducing or abolishing transport, underscoring the functional necessity of the binding site configuration.

Another very recent study (Zhou et al., 2024) used fluorescence resonance energy transfer (FRET) to analyse molecular interactions involving ABCG25 and another Arabidopsis ABA exporter, ABCG16. It showed that, like ABCG25, ABCG16 forms homodimers, responds specifically to ABA and undergoes major conformational changes as it exports ABA. ABA can be stored in its glucose conjugated form, ABA-GE, which can be converted back to active ABA by β-glucosidases, particularly during stress. Despite the advancements in the understanding of how ABA transporters work, the mechanisms of transport for ABA-GE had remained elusive. However, this study showed that ABCG16 and ABCG25 form heterodimers that facilitate the entry of ABA-GE into the endoplasmic reticulum. A proposed model of the interactions between ABCG16 and ABCG25 in the export of ABA-GE across the cellular plasma membrane and ABA-GE across the endoplasmic reticulum membrane is shown in Figure 4.

6 | CONCLUDING REMARKS

After 60 years of study, it might be expected that unexplored aspects of ABA functionality no longer existed, but recent advances in the

analysis of ABA transporters, including exquisite detail of their structure and the conformational changes that they undergo as they interact with ABA and move it across membranes, have opened up new areas of research. Ying et al. (2023), in particular, emphasise the potential for genetic interventions to be targeted at ABA transporters to improve abiotic stress tolerance, suggesting that precise manipulation of transporter structure could optimise ABA transport in crops.

Global climate change is altering the nature and increasing the severity of stresses that crops are exposed to. Extreme weather events are becoming more frequent and more severe with climate change. These extreme events have the severest consequences for the most vulnerable people, increasing levels of poverty, hunger and malnutrition. Plant breeders also have to face the uncertainty of which abiotic stresses will be the most severe in a particular region. In the UK, for example, there has been a lot of work on understanding plant responses to heat and drought stress in anticipation of warmer and drier growing conditions in the years ahead (Ffoulkes et al., 2023; Redhead et al., 2025).

However, the country suffered record rainfall in both the spring and autumn of 2024, with flooding causing crop damage and wet conditions in the autumn of 2024 delaying the sowing of winter wheat for the 2024–2025 season. In some ways, this should not be a surprise because it reflects the large uncertainties in the climate projections of Global Climate Models (Intergovernmental Panel on Climate Change [IPCC], 2021). What we can be sure of is that abiotic stress resilience will become increasingly important in plant breeding, and we will need a broad and deep understanding of all the aspects of ABA synthesis, breakdown, transport and signalling mechanisms to develop successful strategies for this crucial aspect of crop improvement.

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