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## **REVIEW ARTICLE**

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

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**Running title:** ABA transporters, ABA signalling and regulation of ABA function

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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 synthesized 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 characterization of adenosine triphosphate ATP-binding cassette (ABC) transporters, particularly ABCG25, which have revealed detailed molecular mechanisms of ABA recognition, binding, and transmembrane transport. These discoveries, including transporter conformational dynamics and dimerization 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; climate change; adenosine triphosphate (ATP)-binding cassette (ABC) class transporters

## **Introduction**

Absciscic acid (ABA) is a plant hormone that was first identified and characterized 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 absciscic acid (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-EM 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).

## **ABA biosynthesis and breakdown**

ABA ( $C_{15}H_{20}O_4$ ) (Figure 1) is a sesquiterpene; i.e. 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 et al., 2016a, b). The first steps in the pathway are plastidic and begin with the hydroxylation of  $\beta$ -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 upregulated (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,  $\beta$ -D-glucopyranosyl abscisate (ABA-GE), through the action of ABA glucosyltransferase (Xu et al., 2002; Dong et al., 2014). Under biotic stress, abscisic acid (ABA) enhances physical barrier defences. The PAMP-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; Xiong & Yang, 2003; Asselbergh et al., 2008).

### **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 (Kogayashi et al., 2005; Furihata et al., 2006). AREBPs (also known as ABFs) are a family of basic leucine zipper (bZIP) transcription factors that recognize 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, for example (Halford et al., 2003; Chen et al., 2021; 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 (Kobayashi et al., 2004, Boudsocq et al., 2004; 2007). 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 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 (Purcell et al., 1998; Kanegae et al., 2005; McKibbin et al., 2006; Jain et al., 2008). 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 (Halford et al., 2003; Chen et al., 2021; Halford & Hardie, 1998). They are unique to plants and, given their complete absence from animals and fungi, it is likely that they emerged only 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 (Halford et al., 2003; Chen et al., 2021).

This rapid evolution may have occurred as they took on new roles to enable plants to link metabolic and stress signalling (Halford and 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 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).

### **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 2 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  $\alpha$ -amylase genes in response to ABA (Gomez-Cadenas et al., 1999; Johnson et al., 2008). In contrast, an  $\alpha$ -amylase gene ( *$\alpha$ -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).

### **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 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<sup>-</sup>), 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 ~5.0-6.1) and diffuses into cells, but once it enters the cytosol (pH ~7.5) it dissociates into ABA<sup>-</sup>, 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 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 localized 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; Wilkinson & Davies, 1997; Dodd et al., 2003).

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 *Brachypodium distachyon* (Pawela et al., 2019; Kuromori et al., 2021).

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 stabilize ABA within the cavity. The binding cavity is selective, favouring ABA in its anionic form (ABA<sup>-</sup>) 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 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 stabilizing 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 stabilizing 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  $\beta$ -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 across the cellular plasma membrane ABA-GE across the endoplasmic reticulum membrane is shown in Figure 4.

## **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 has 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 optimize 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 (Redhead et al., 2025; Ffoulkes et al., 2023).

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 (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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## References

- Addicott, F.T., Lyon, J.L., Ohkuma, K., Thiessen, W.E., Carns, H.R., Smith O.E., Cornforth, J.W., Milborrow, B.V., Ryback, G., Wareing, P.F. (1968). Absciscic acid: a new name for abscisin II (dormin). *Science*, 159, 1493.
- Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert C., Maclean, D.J., Ebert, P.R., Kazan, K. (2004) Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in *Arabidopsis*. *The Plant Cell*, 16, 3460–3479.
- Anfang, M., Shani, E. (2021). Transport mechanisms of plant hormones. *Current Opinion in Plant Biology*, 63, 102055.
- Asselbergh, B., De, Vleeschauwer, D., Hofte, M. (2008) Global switches and fine-tuning- ABA modulates plant pathogen defense. *Molecular Plant-Microbe Interactions*, 21, 709–719.
- Assmann, S.M., Jegla, T. (2016). Guard cell sensory systems: recent insights on stomatal responses to light, abscisic acid, and CO<sub>2</sub>. *Current Opinion in Plant Biology*, 33, 157-167.
- Boudsocq, M., Barbier-Brygoo, H., Lauriere, C. (2004). Identification of nine sucrose nonfermenting 1-related protein kinases 2 activated by hyperosmotic and saline stresses



- in *Arabidopsis thaliana*. *Journal of Biological Chemistry*, 279, 41758-41766.
- Boudsocq, M., Droillard, M.J., Barbier-Brygoo, H., Lauriere, C. (2007). Different phosphorylation mechanisms are involved in the activation of sucrose non-fermenting-1 related protein kinases 2 by osmotic stresses and abscisic acid. *Plant Molecular Biology*, 63, 491-503.
- Boursiac, Y., L eran, S., Corrat  -Faillie, C., Gojon, A., Krouk, G., Lacombe, B. (2013). ABA transport and transporters. *Trends in Plant Science*, 18, 325-333.
- Chen, Z., Muttucumaru, N., Powers, S.J., Halford, N.G. (2013). Expression analysis of abscisic acid (ABA) and metabolic signalling factors in developing endosperm and embryo of barley. *Journal of Cereal Science*, 58, 255-262.
- Chen, Z., Zhou, L., Jiang, P., Lu, R., Halford, N.G., Liu, C. (2021). Genome-wide identification of sucrose nonfermenting-1-related protein kinase (*SnRK*) genes in barley and RNA-seq analyses of their expression in response to ABA treatment. *BMC Genomics*, 22, 300.
- Cheng, W.H., Endo, A., Zhou, L., Penney, J., Chen, H.C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T., Sheen, J. (2002). A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell*, 14, 2723-2743.
- Choi, H., Park, H.J., Park, J.H., Kim, S., Im, M.Y., Seo, H.H., Kim, Y.W., Kim, S.Y. (2005), *Arabidopsis* calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiology*, 139, 1750-1761.
- Coello, P., Hirano, E., Hey, S.J., Muttucumaru, N., Martinez-Barajas, E., Parry, M.A.J., Halford, N.G. (2012). Evidence that abscisic acid promotes degradation of SNF1-related protein kinase (*SnRK*) 1 in wheat and activation of a putative calcium-

- dependent SnRK2. *Journal of Experimental Botany*, 63, 913-924.
- Cutler, S.R., Rodriguez, P.L., Finklestein, R.R., Abrams, S.R. (2010). Absciscic acid: emergence of a core signaling network. *Annual Review of Plant Biology*, 61, 651-679.
- Do, T.H.T., Martinoia, E., Lee, Y., Hwang, J.U. (2021). 2021 update on ATP-binding cassette (ABC) transporters: how they meet the needs of plants. *Plant Physiology*, 187, 1876-1892.
- Dodd, I.C., Tan, L.P., He, J. (2003). Do increases in xylem sap pH and/or ABA concentration mediate stomatal closure following nitrate deprivation? *Journal of Experimental Botany*, 54, 1281-1288.
- Dong, T., Xu, Z.Y., Park, Y., Kim, D.H., Lee, Y., Hwang, I. (2014). Absciscic acid uridine diphosphate glucosyltransferases play a crucial role in absciscic acid homeostasis in Arabidopsis. *Plant Physiology*, 165, 277-289.
- Fan, J., Hill, L., Crooks, C., Doerner, P., Lamb, C. (2009) Absciscic acid has a key role in modulating diverse plant-pathogen interactions. *Plant Physiology*, 150, 1750–1761.
- Ffoulkes, C., Oliver, H., and Clarke, J. (2023) Research to assess resilience measures that support UK agriculture in adapting to drought, extreme heat, and wildfires. ADAS report for Defra.
- Furihata, T., Maruyama, K., Fujita, Y., Umezawa, T., Yoshida, R., Shinozaki, K., Yamaguchi-Shinozaki, K. (2006). Absciscic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proceedings of the National Academy of Sciences USA*, 103, 1988-1993.
- Gómez-Cadenas, A., Verhey, S.D., Holappa, L.D., Shen, Q., Ho, T.-H.D., Walker-Simmons, M.K. (1999). An absciscic acid-induced protein kinase, PKABA1, mediates absciscic acid-suppressed gene expression in barley aleurone layers. *Proceedings of the National Academy of Sciences USA*, 96, 1767-1772.

- Gosti, F., Beaudoin, N., Serizet, C., Webb, A.A., Vartanian, N., Giraudat, J. (1999). ABI1 protein phosphatase 2C is a negative regulator of abscisic acid signaling. *Plant Cell*, 11, 1897-1910.
- Guo, Y., Xiong, L., Song, C.-P., Gong, D., Halfter, U., Zhu, J.-K. (2002). A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in Arabidopsis. *Developmental Cell*, 3, 233-244.
- Halford, N.G., Hardie, D.G. (1998). SNF1-related protein kinases: global regulators of carbon metabolism in plants? *Plant Molecular Biology*, 37, 735-748.
- Halford, N.G., Hey, S.J. (2009). Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signalling in plants. *Biochemical Journal*, 419, 247-259.
- Halford, N.G., Hey, S., Jhurrea, D., Laurie, S., McKibbin, R.S., Paul, M.J., Zhang, Y. (2003). Metabolic signalling and carbon partitioning: Role of Snf1-related (SnRK1) protein kinase. *Journal of Experimental Botany*, 54, 467-475.
- Huang, X., Zhang, X., An, N., Zhang, M., Ma, M., Yang, Y., Jing, L., Wang, Y., Chen, Z., Zhang, P. (2023). Cryo-EM structure and molecular mechanism of abscisic acid transporter ABCG25. *Nature Plants*, 9, 1709-1719.
- IPCC (2021). Climate Change 2021 – The physical science basis: working group I contribution to the sixth assessment report of the intergovernmental panel on climate change (eds Masson-Delmotte, V. et al.) (Cambridge University Press, Cambridge, United Kingdom and New York, 2021).
- Iuchi, S., Kobayashi, M., Taji, T., Naramoto, M., Seki, M., Kato, T., Tabata, S., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K. (2001). Regulation of drought tolerance by gene manipulation of 9-*cis*-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. *Plant Journal*, 27, 325-333.

- Jain, M., Lic, Q.-B., Chourey, P.S. (2008). Cloning and expression analyses of sucrose non-fermenting-1-related kinase 1 (*SnRK1b*) gene during development of sorghum and maize endosperm and its implicated role in sugar-to-starch metabolic transition. *Physiologia Plantarum*, 134, 161-173.
- Johnson, R.R., Shin, M., Shen, J.Q. (2008). The wheat PKABA1-interacting factor TaABF1 mediates both abscisic acid-suppressed and abscisic acid-induced gene expression in bombarded aleurone cells. *Plant Molecular Biology*, 68, 93-103.
- Jossier, M., Bouly, J.-P., Meimoun, P., Arjmand, A., Lessard, P., Hawley, S., Hardie, D.G., Thomas, M. (2009). SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in *Arabidopsis thaliana*. *Plant Journal*, 59, 316-328.
- Kaiser, W.M., Hartung, W. (1981). Uptake and release of abscisic acid by isolated photoautotrophic mesophyll cells, depending on pH gradients. *Plant Physiology*, 68, 202-206.
- Kanegae, H., Miyoshi, K., Hirose, T., Tsuchimoto, S., Mori, M., Nagato, Y., Takano M. (2005). Expressions of rice sucrose non-fermenting-1 related protein kinase 1 genes are differently regulated during the caryopsis development. *Plant Physiology and Biochemistry*, 43, 669-679.
- Kang, J., Hwang, J.U., Lee, M., Kim, Y.Y., Assmann, S.M., Martinoia, E., Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proceedings of the National Academy of Sciences USA*, 107, 2355-60.
- Kang, J., Yim, S., Choi, H., Kim, A., Lee, K.P., Lopez-Molina, L., Martinoia, E., Lee, Y. (2015). Abscisic acid transporters cooperate to control seed germination. *Nature Communications* 6, 8113.
- Karssen, C.M., Brinkhorstvanderswan, D.L.C., Breekland, A.E., Koornneef, M. (1983). Induction of dormancy during seed development by endogenous abscisic-acid - studies

- on abscisic-acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta*, 157, 158-165.
- Kaya, C., Uğurlar, F., Adamakis, I.S. (2024). Molecular mechanisms of CBL-CIPK signaling pathway in plant abiotic stress tolerance and hormone crosstalk. *International Journal of Molecular Sciences*, 25, 5043.
- Kobayashi, Y., Murata, M., Minami, H., Yamamoto, S., Kagaya, Y., Hobo, T., Yamamoto, A., Hattori, T. (2005). Abscisic acid-activated SnRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. *Plant Journal*, 44, 939-949.
- Kobayashi, Y., Yamamoto, S., Minami, H., Kagaya, Y., Hattori, H. (2004). Differential activation of the rice sucrose nonfermenting1-related protein kinase2 family by hyperosmotic stress and abscisic acid. *Plant Cell*, 16, 1163-1177.
- Krochko, J.E., Abrams, G.D., Loewen, M.K., Abrams, S.R., Cutler, A.J. (1998). (+)-Abscisic acid 8'-hydroxylase is a cytochrome P450 monooxygenase. *Plant Physiology*, 118, 849-60.
- Kuhn, J.M., Boisson-Dernier, A., Dizon, M.B., Maktabi, M.H., Schroeder, J.I. (2006). The protein phosphatase AtPP2CA negatively regulates abscisic acid signal transduction in *Arabidopsis*, and effects of *abh1* on *AtPP2CA* mRNA. *Plant Physiology*, 140, 127-139.
- Kuromori, T., Fujita, M., Urano, K., Tanabata, T., Sugimoto, E., Shinozaki, K. (2016). Overexpression of *AtABCG25* enhances the abscisic acid signal in guard cells and improves plant water use efficiency. *Plant Science*, 251, 75-81.
- Kuromori, T., Miyaji, T., Yabuuchi, H., Shimizu, H., Sugimoto, E., Kamiya, A., Moriyama, Y., Shinozaki, K. (2010). ABC transporter *AtABCG25* is involved in abscisic acid transport and responses. *Proceedings of the National Academy of Sciences USA*, 107,

2361-2366.

- Kuromori, T., Sugimoto, E., Shinozaki, K. (2021). Brachypodium BdABCG25 is a homolog of Arabidopsis AtABCG25 involved in the transport of abscisic acid. *FEBS Letters*, 595, 954-959.
- Laurie, S., McKibbin, R.S., Halford, N.G. (2003). Antisense SNF1-related (SnRK1) protein kinase gene represses transient activity of an  $\alpha$ -amylase ( $\alpha$ -Amy2) gene promoter in cultured wheat embryos. *Journal of Experimental Botany*, 54, 739-747.
- Li, J., Zhao, C., Hu, S., Song, X., Lv, M., Yao, D., Song, Q., Zuo, K. (2020). Arabidopsis NRT1. 2 interacts with the PHOSPHOLIPASE D $\alpha$ 1 (PLD $\alpha$ 1) to positively regulate seed germination and seedling development in response to ABA treatment. *Biochemical and Biophysical Research Communications*, 533, 104-109.
- Manzi, M., Lado, J., Rodrigo, M.J., Zacarías, L., Arbona, V., Gómez-Cadenas, A. (2015). Root ABA accumulation in long-term water-stressed plants is sustained by hormone transport from aerial organs. *Plant and Cell Physiology*, 56, 2457-2466.
- McAdam, S.A.M., Brodribb, T.J., Ross, J.J. (2016a). Shoot-derived abscisic acid promotes root growth. *Plant Cell & Environment*, 39, 652-659.
- McAdam, S.A.M., Manzi, M., Ross, J.J., Brodribb, T.J., Gómez-Cadenas, A. (2016b). Uprooting an abscisic acid paradigm: Shoots are the primary source. *Plant Signaling & Behavior*, 11, 11-12.
- McKibbin, R.S., Muttucumaru, N., Paul, M.J., Powers, S.J., Burrell, M.M., Coates, S., Purcell, P.C., Tiessen, A., Geigenberger, P., Halford, N.G. (2006). Production of high starch, low glucose potatoes through over-expression of the metabolic regulator, SnRK1. *Plant Biotechnology Journal*, 4, 409-418.
- Nishimura, N., Sarkeshik, A., Nito, K., Park, S.-Y., Wang, A., Carvalho, C., Lee, S., Caddell, D.F., Cutler, S.R., Chory, J., Yates, J.R., Schroeder, J.I. (2010). PYR/PYL/RCAR

- family members are major in-vivo ABI1 protein phosphatase 2C-interacting proteins in Arabidopsis. *Plant Journal*, 61, 290-299.
- Ohkuma, K., Lyon, J.L., Addicott, F.T., Smith, O.E. (1963). Abscisin II, an abscission-accelerating substance from young cotton fruit. *Science*, 142, 1592-1593.
- Pawela, A., Banasiak, J., Biala, W., Martinoia, E., Jasinski, M. (2019). MtABCG20 is an ABA exporter influencing root morphology and seed germination of *Medicago truncatula*. *Plant Journal*, 98, 511-523.
- Peixoto, B., Baena-González, E. (2022). Management of plant central metabolism by SnRK1 protein kinases. *Journal of Experimental Botany*, 73, 7068-7082.
- Purcell, P.C., Smith, A.M., Halford, N.G. (1998). Antisense expression of a sucrose nonfermenting-1-related protein kinase sequence in potato results in decreased expression of sucrose synthase in tubers and loss of sucrose-inducibility of sucrose synthase transcripts in leaves. *Plant Journal*, 14, 195-202.
- Redhead, J.W., Brown, M., Price, J., Robinson, E., Nicholls, R.J., Warren, R. and Pywell, R.F. (2025). National horizon scanning for future crops under a changing UK climate. *Climate Resilience and Sustainability*, 4, 70007.
- Rubio, S., Rodrigues, A., Saez, A., Dizon, M.B., Galle, A., Kim, T.-H., Santiago, J., Flexas, J., Schroeder, J.I., Rodriguez, P.L. (2009). Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to endogenous abscisic acid. *Plant Physiology*, 150, 1345-1355.
- Saito, S., Hirai, N., Matsumoto, C., Ohigashi, H., Ohta, D., Sakata, K., Mizutani, M. (2004). Arabidopsis CYP707As encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiology*, 134, 1439-1449.
- Schopfer, P., Bajracharya, D., Plachy, C. (1979). Control of seed germination by abscisic acid: I. Time course of action in *Sinapis alba* L. *Plant Physiology*, 64, 822-827.

- Seiler, C., Harshavardhan, V.T., Rajesh, K., Reddy, P.S., Strickert, M., Rolletschek, H., Scholz, U., Wobus, U., Sreenivasulu, N. (2011). ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *Journal of Experimental Botany*, 62, 2615-2632.
- Seo, M., Peeters, A.J., Koiwai, H., Oritani, T., Marion-Poll, A., Zeevaart, J.A., Koornneef, M., Kamiya, Y., Koshiba, T. (2007). The *Arabidopsis* aldehyde oxidase 3 (AAO3) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. *Proceedings of the National Academy of Sciences USA*, 97, 12908-12913.
- Thompson, A.J., Jackson, A.C., Symonds, R.C., Mulholland, B.J., Dadswell, A.R., Blake, P.S., Burbidge, A., Taylor, I.B. (2000). Ectopic expression of a tomato 9-*cis*-epoxycarotenoid dioxygenase gene causes overproduction of abscisic acid. *Plant Journal*, 23, 363-374.
- Umezawa, T., Sugiyama, N., Mizoguchi, M., Hayashi, S., Myouga, F., Yamaguchi-Shinozaki, K., Ishihama, Y., Hirayama, T., Shinozaki, K. (2009). Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in *Arabidopsis*. *Proceedings of the National Academy of Sciences USA*, 106, 17588-17593.
- Vlad, F., Droillard, M.J., Valot, B., Khafif, M., Rodrigues, A., Brault, M., Zivy, M., Rodriguez, P.L., Merlot, S., Laurière, C. (2010). Phospho-site mapping, genetic and in planta activation studies reveal key aspects of the different phosphorylation mechanisms involved in activation of SnRK2s. *Plant Journal*, 63, 778-790.
- Wang, Z., Ren, Z., Cheng, C., Wang, T., Ji, H., Zhao, Y., Deng, Z., Zhi, L., Lu, J., Wu, X., Xu, S., Cao, M., Zhao, H., Liu, L., Zhu, J., Li, X. (2020). Counteraction of ABA-mediated inhibition of seed germination and seedling establishment by ABA signaling terminator in *Arabidopsis*. *Molecular Plant*, 13, 1284-1297.
- Wilkinson, S., Davies, W.J. (1997). Xylem sap pH increase: a drought signal received at the



- apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. *Plant Physiology*, 113, 559-573.
- Wu, W., Cao, S.-F., Shi, L.-Y., Chen, W., Yin, X.-R., Yang, Z.-F. (2023). Abscisic acid biosynthesis, metabolism and signaling in ripening fruit. *Frontiers in Plant Science*, 14, 1279031.
- Xin, J., Zhou, Y., Qiu, Y., Geng, H., Wang, Y., Song, Y., Liang, J., Yan, K. (2024). Structural insights into AtABCG25, an angiosperm-specific abscisic acid exporter. *Plant Communications*, 5, 100776.
- Xiong, L., Ishitani, M., Lee, H., Zhu, J.K. (2001). The Arabidopsis *LOS5/ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. *Plant Cell*, 13, 2063-2083
- Xiong, L., Lee, H., Ishitani, M., Zhu, J.K. (2002). Regulation of osmotic stress-responsive gene expression by the *LOS6/ABAI* locus in *Arabidopsis*. *Journal of Biological Chemistry*, 277, 8588-8596.
- Xiong, L., Yang, Y. (2003) Disease resistance and abiotic stress tolerance in rice are inversely modulated by an abscisic acid-inducible mitogen-activated protein kinase. *The Plant Cell*, 15, 745–759.
- Xu, Z.J., Nakajima, M., Suzuki, Y., Yamaguchi, I. (2002). Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. *Plant Physiology*, 129, 1285-1295.
- Yasuda, M., Ishikawa, A., Jikumaru, Y., Seki, M., Umezawa, T., Asami, T., Maruyama-Nakashita, A., Kudo, T., Shinozaki, K., Yoshida, S., Nakashita, H. (2008). Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. *The Plant Cell*, 20, 1678-1692.
- Ying, W., Liao, L., Wei, H., Gao, Y., Liu, X., Sun, L. (2023). Structural basis for abscisic acid efflux mediated

- by ABCG25 in *Arabidopsis thaliana*. *Nature Plants*, 9, 1697-1708.
- Zhang, L., Yu, Z., Xu, Y., Yu, M., Ren, Y., Zhang, S., Yang, G., Huang, J., Yan, K., Zheng, C., Wu, C. (2021). Regulation of the stability and ABA import activity of NRT1.2/NPF4.6 by CEPR2-mediated phosphorylation in *Arabidopsis*. *Molecular Plant*, 14, 633-646.
- Zhang, Y., Amichai, B., Eilon, S. (2023). Plant hormone transport and localization: signaling molecules on the move. *Annual Review of Plant Biology*, 74, 453-479.
- Zhang, Y., Andralojc, P.J., Hey, S.J., Primavesi, L.F., Specht, M., Koehler, J., Parry, M.A.J., Halford, N.G. (2008). Arabidopsis SNF1-related protein kinase-1 and calcium-dependent protein kinase phosphorylate conserved target sites in ABA response element binding proteins. *Annals of Applied Biology*, 153, 401-409.
- Zhang, Y., Kilambi, H.V., Liu, J., Bar, H., Lazary, S., Egbaria, A., Ripper, D., Charrier, L., Belew, Z.M., Wulff, N., Damodaran, S., Nour-Eldin, H.H., Aharoni, A., Ragni, L., Strader, L., Sade, N., Weinstain, R., Geisler, M., Shani, E. (2021). ABA homeostasis and long-distance translocation are redundantly regulated by ABCG ABA importers. *Science Advances*, 7, eabf6069.
- Zhou, Y., Wang, Y., Zhang, D., Liang, J. (2024). Endomembrane-biased dimerization of ABCG16 and ABCG25 transporters determines their substrate selectivity in ABA-regulated plant growth and stress responses. *Molecular Plant*, 17, 478-495.
- Zhu, S.Y., Yu, X.C., Wang, X.J., Zhao, R., Li, Y., Fan, R.C., Shang, Y., Du, S.Y., Wang, X.F., Wu, F.Q., Xu, Y.H., Zhang, X.Y., Zhang, D.P. (2007). Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in *Arabidopsis*. *Plant Cell*, 19, 3019-3036.



## Figure legends

**Figure 1.** Biosynthesis of the sesquiterpene, abscisic acid (ABA).  $\beta$ -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<sup>th</sup> November 2024)).

**Figure 2.** 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 PP2C protein phosphatases. **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.

## Figure 3.

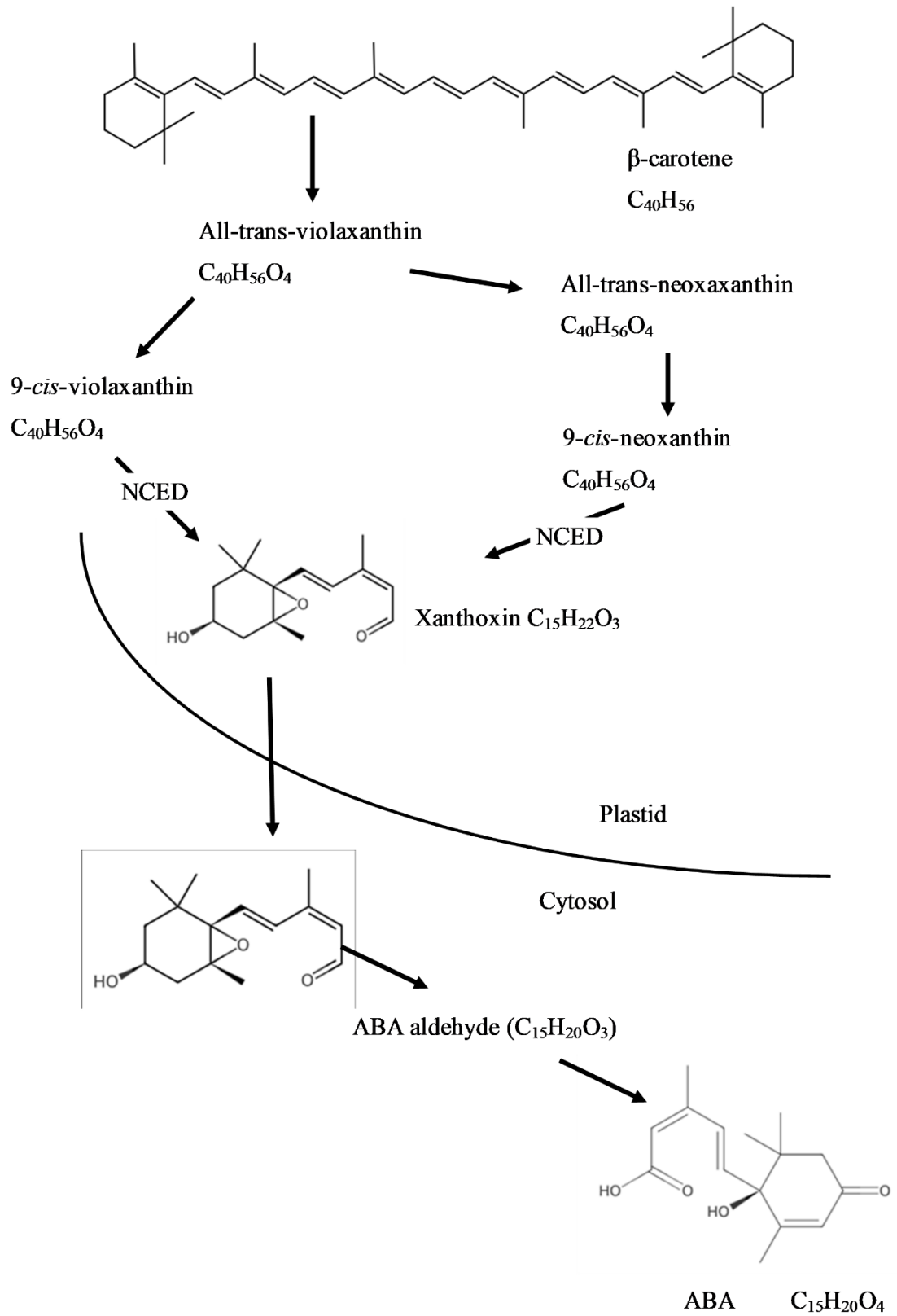
Alignment of the N-terminal region of representative ABA-response element binding proteins (AREBPs) of the ABI5 type from barley (*Hordeum vulgare*) (GenBank: AAO06115.1); wheat (*Triticum aestivum*) (GenBank: BAD97366.1); Brachypodium (*Brachypodium distachyon*) (NCBI Reference Sequence: XP\_003578228.1); 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.

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. Adapted from (Chen et al., 2013).

**Figure 4.**

A proposed model of ABA transport involving 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 ABA-GE across the endoplasmic reticulum membrane.

Figure 1.



**Figure 2.**

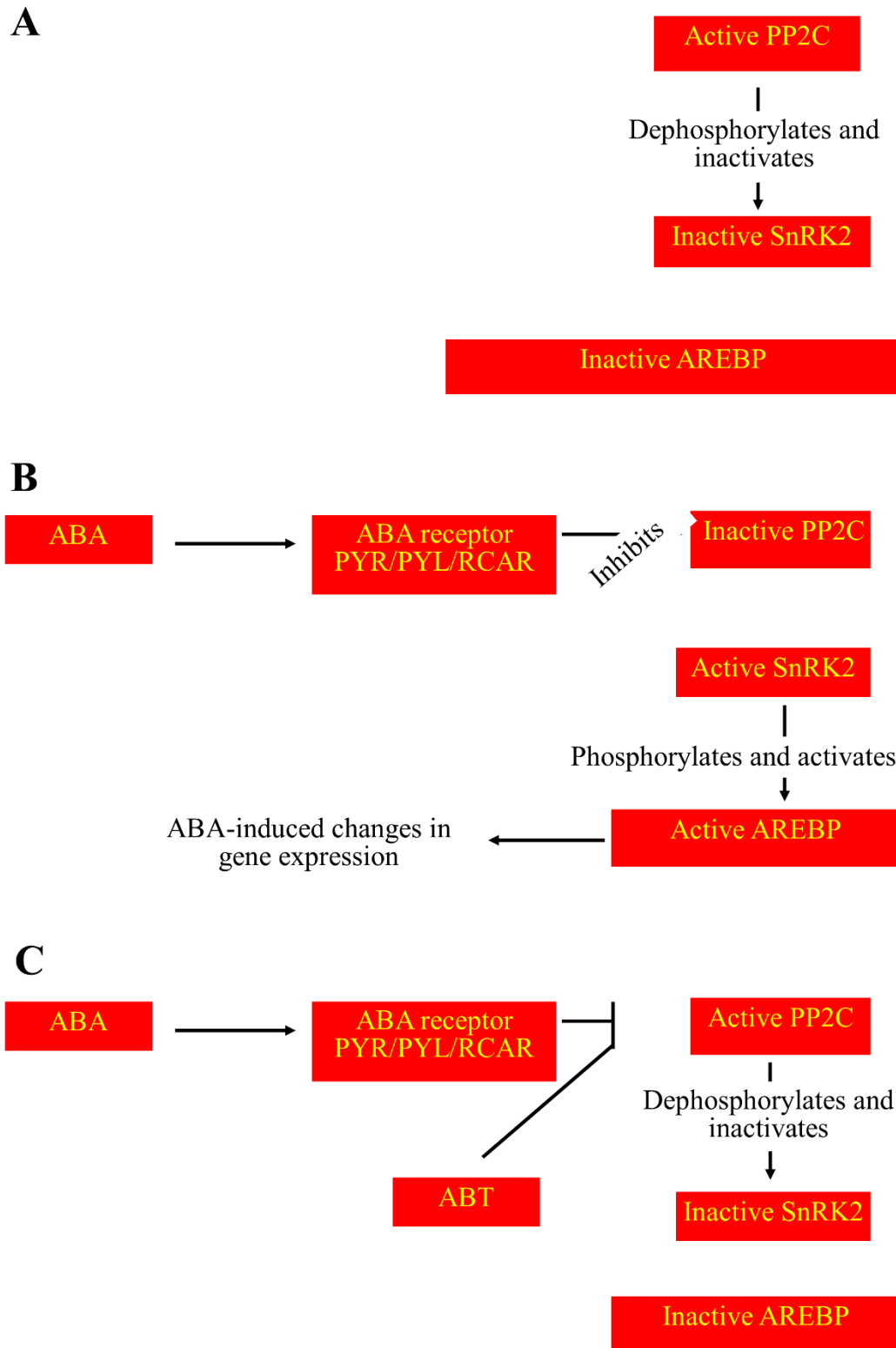


Figure 3.

Barley	1-MDFRSSNGGSSS-ERRPAE-GA--SLTRQGSIIYSLTFEEFQSTLGGGA-GVGGD--LGKDFSSMNDLL
Wheat	1-MDFRSSNGGSSS-ERRPAD-GA--PLTRQGSIIYSLTFEEFQSTLGGGA-GVGGSD--LGKDFSSMNDLL
Brachypodium	1-MEFT--GGSSS-EHRESV-GGSTPLARQGSVYSLTFEEFQSTLGGG--LGKDFSSMNDLL
Rice	1-MEFG--NGSSSSERR-AAE-GA--TLARQGSVYSLTFEEFQSAAGCC-GGGGGSG-FGKDFCSMNDLL
Maize	1-MEFK-NCGSSSQP--RPAVVVEEAALARQGSVYSLTFEEFQSAAGAAATGGGGGS--IPKDFCSMNDLL
Sorghum	1-MEFK--NWGSSSQ-RRPAVVDEGQPLARQGSVYSLTFEEFQSAAGAAATGGGGGGSGGIPKDFCSMNDLL



Figure 4.

