

Review

ABA Transport and Plant Water Stress Responses

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To understand the integrative networks of signaling molecules, the sites of their biosynthesis and action must be clarified, particularly for phytohormones such as abscisic acid (ABA). The relationship between the sites of ABA biosynthesis and transport has been discussed extensively in the context of guard cells and stomatal regulation. However, guard cells are not the only site of ABA action. Recent studies have reported multiple sites of ABA biosynthesis and multiple ABA transporters, indicating that ABA transport regulation is not unidirectional but rather forms complex networks. Therefore, it is important to determine how multiple ABA sources coordinately contribute to individual biological processes under various physiological conditions.

Plant Hormone Transport

Hormones are endogenous chemicals that induce various physiological responses at low concentrations. In animals, hormones are secreted from specific cell types (e.g., endocrine cells) and transported to distant target sites through the circulatory system within the body. Similar to hormones in animals, most plant hormones are mobile. However, the sites of their synthesis and actions are often not clearly defined, and it is not always clear whether movement or transport of plant hormones is a prerequisite of their biological function. This is possibly because cells in a relatively wide range of tissues and organs can synthesize hormones as well as respond to them.

The plant hormone ABA regulates various physiological processes throughout plant life cycles [1–4]. For example, in response to water deficit ABA induces stomatal closure and the expression of numerous stress-responsive genes. By contrast, ABA promotes the accumulation of seed storage compounds during seed development and is also required for the induction and maintenance of seed dormancy. It has been also reported that ABA is involved in plant pathogen responses. Here we try to determine how the biosynthesis and transport of ABA are coordinately regulated within a plant. Especially, we focus on plant water relations, since multiple sites of ABA biosynthesis, namely vascular cells and guard cells, have been suggested in this context. The recent identification of several classes of ABA transporters indicates that plants are equipped with a highly sophisticated system to sense and respond to water availability under diversely fluctuating environments.

Sites of ABA Biosynthesis and Transport between Roots and Shoots

Higher plants absorb water in soil from roots and transpire water from aboveground organs such as leaves and stems through stomata located on their epidermal tissue. In addition, stomata mediate the uptake and release of carbon dioxide and oxygen for photosynthesis and respiration. Therefore, plants must integrate multiple environmental signals to optimize the stomatal aperture under diverse environmental conditions. Since the discovery of ABA as a chemical that induces stomatal closure, many studies have examined its role in root-to-shoot

Highlights

Plants close stomata to limit water loss on water deficit. It is evident that ABA is required in this process based on the phenotypes of mutants defective in ABA biosynthesis or signal transduction.

ABA may be translocated from the sites of biosynthesis, such as roots and leaf vascular tissues, to the guard cells. Recent identification of multiple transmembrane ABA transporters indicates that the movement of this hormone within a plant is actively regulated in an intercellular network.

ABA regulates various molecular events in different organs and tissues as well as guard cells to tolerate water stress dependent on environmental conditions. However, the dynamics of ABA transport within a plant must be analyzed and discussed in a more spatiotemporally physiological context.

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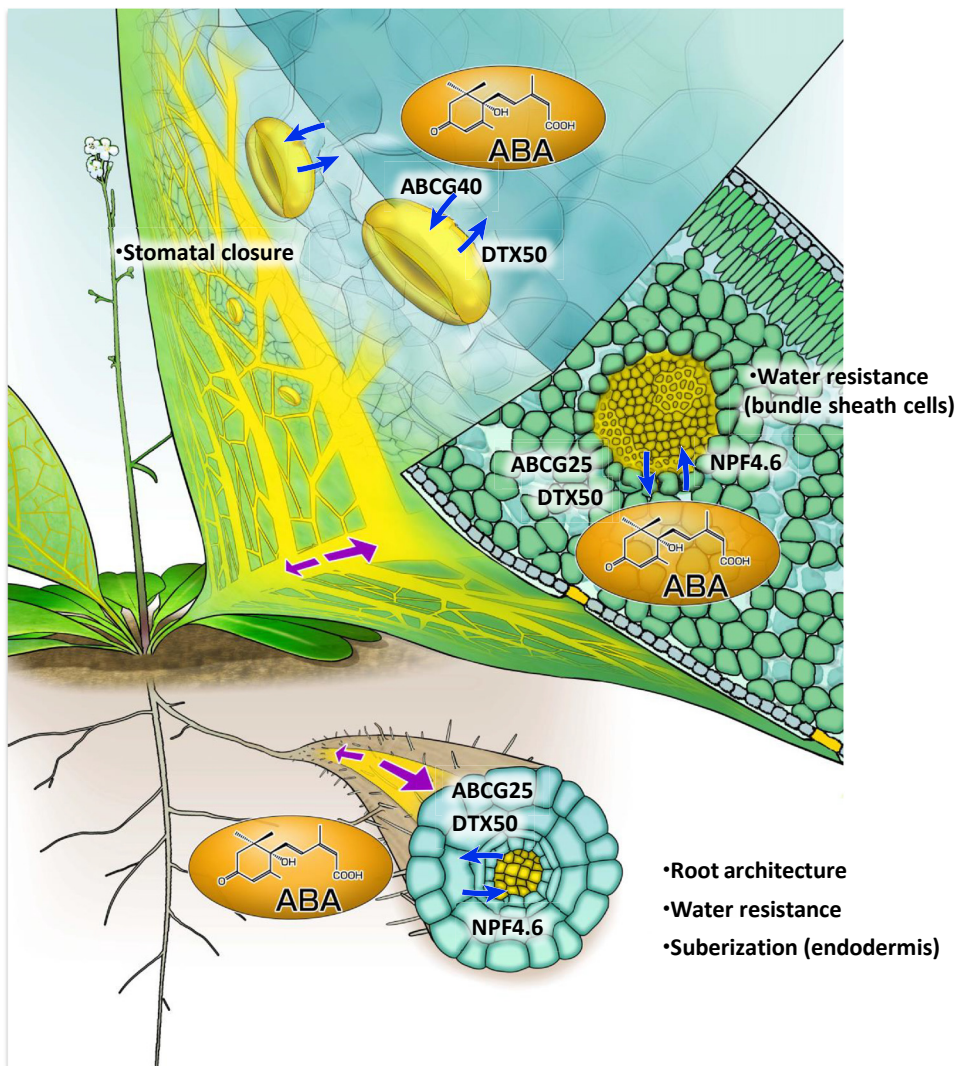
signaling under drought stress [5]. Under mild stress conditions when soil drying begins, ABA accumulation in root tissues is closely correlated with a decrease in leaf stomatal conductance [6]. In addition, several studies have found that drought stress can induce ABA accumulation in xylem sap [7,8]. Although the substance associated with root-to-shoot signaling under drought stress has not been fully elucidated, these observations suggest that ABA synthesized in root tissue is released to xylem vessels and transported to shoots. Because ABA is a weak acid containing a carboxyl group ($pK_a = 4.7$), it exists in solution in the protonated or deprotonated form, with the proportion of each determined by the pH. An increase in xylem sap pH in response to soil drying has been observed in many plant species, which could enable ABA to move more freely through extracellular space toward guard cells according to the transpiration stream, because a smaller amount of ABA would be trapped by surrounding cells during transport than under lower pH conditions [6,9–11].

Although the significance of root-derived ABA in stomatal closure has been widely accepted and documented in many textbooks, ABA is synthesized not only in roots but also in shoots (leaves). Moreover, several studies have shown that ABA accumulates at much higher concentrations in leaves than in roots on water deficit and that ABA accumulation in roots is sometimes dependent on basipetal ABA transport from aerial organs [12–17]. In addition, reciprocal grafting between ABA-deficient mutants and wild-type plants demonstrates that stomatal closure is affected by leaf genotype but not root genotype [18–22]. Several candidates involved in root-to-shoot signaling under drought stress have been proposed, such as hydraulic signals, pH, a small peptide, or some chemical agent other than ABA [23,24].

Site of ABA Biosynthesis and Transport between Vascular Cells and Guard Cells

In addition to ABA biosynthesis in roots and leaves, tissue-specific expression of ABA biosynthesis genes and enzymes in *Arabidopsis thaliana* offers another level of complexity. Experiments using antisense RNAs, antibodies, or promoter-driven expression of fluorescent proteins have suggested that ABA biosynthesis enzymes (e.g., NCED3, ABA2, AAO3) are preferentially localized in vascular bundles in leaves [25–27]. Guard cells, the main ABA target sites in terms of stomatal closure, are located on the surface or epidermal layer of plants, whereas vascular tissue, which is possibly the primary site of ABA biosynthesis in leaves, is located inside the plant body. This suggests that ABA is transmitted between distant tissues within leaves. Moreover, a study showed that leaf surface temperature increased when NCED3 was expressed under the control of a promoter specific to phloem companion cells in a wild-type background, possibly due to the induction of stomatal closure [27].

Guard cells do not have direct connections with surrounding mesophyll or epidermal cells through plasmodesmata. The major ABA receptors responsible for stomatal closure (PYR/PYL/RCAR) are soluble proteins. Therefore, if ABA synthesized in vascular tissues were transported to guard cells, at least two transmembrane ABA transport steps would be required for stomatal closure. Supporting this hypothesis, several ABA transporters have been identified [11,28] (Figure 1, Box 1, and Table 1). Two *Arabidopsis* ATP-binding cassette (ABC) transporters, AtABCG25 and AtABCG40, have been identified as ABA transporters involved in ABA sensitivity or stomatal closure based on the phenotypes of mutants defective in the respective proteins [29,30]. Biochemical characterization using heterologous expression systems has shown that AtABCG25 and AtABCG40 exhibit ABA export and import activity, respectively [27,29,30]. For example, the K_m values of AtABCG25 and AtABCG40 for ABA were relatively low (260 nM and 1 μ M, respectively) compared with ABA levels in water-stressed guard cells of



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Figure 1. Abscisic Acid (ABA) Transport and ABA Regional Functions in Plant Water Stress Responses. This schematic diagram shows root and leaf cross-sections, including three possible sites of ABA biosynthesis: root (vascular tissue), leaf vascular tissue, and guard cells. Tissues and cells expressing ABA transporters are shaded yellow (Box 1). The blue arrows indicate transmembrane ABA transport mediated by transporters. Purple indicates the possible movement of ABA through xylem and phloem. The physiological functions of ABA in roots, leaf vascular tissues, and guard cells discussed in the text are also shown.

Box 1. ABA Transporters

The designation of the four membrane factors AtABCG25, AtABCG40, AtNPF4.6, and AtDTX50 as typical ABA transporters is supported by several lines of evidence (summarized in Table 1). All four proteins are localized to plasma membranes, implying the involvement of ABA transfer between the inside and outside of cells. All gene-disruption mutants showed aberrant sensitivity to ABA accompanied by biochemical evidence of ABA transport activity. Interestingly, these transporter genes are predominantly expressed in vascular tissues or guard cells, strongly indicating that ABA transport is regulated in a network spanning distant tissues related to the site of biosynthesis and the site of physiological actions (see main text). Although this review focuses on ABA transporters related to water stress response, additional ABA transporters have been reported (e.g., those related to seed germination) [66].

Table 1. Characteristics of the Four Typical ABA Transporters Reported to Date

| Gene name | Gene family | Gene identification | Tissue expression | Subcellular localization | Transport assay | KO phenotype of germinative growth | KO phenotype of adult stage | OE phenotype of germinative growth | OE phenotype of adult stage | Suggested function | Refs |
|------------------------------|---------------------------|--|----------------------------------|--------------------------|---|------------------------------------|--|------------------------------------|---|--------------------|------|
| AtABCG25 ^a | ABCG half-size type (WBC) | Mutant screen based on ABA sensitivity during seed germination/early seedling growth stages from transposon-tagged lines | Vascular tissues | Plasma membrane | Vesicles and insect cells | ABA hypersensitive | (N.D.) | ABA insensitive | Higher surface temperature of leaves, less water loss | Exporter | [30] |
| AtABCG40 | ABCG full-size type (PDR) | Characterization of mutants defective in PDR members | Broad, highest in guard cells | Plasma membrane | Yeast and BY2 cells | ABA insensitive | Drought sensitive | (N.D.) | (N.D.) | Importer | [29] |
| AtNPF4.6/ NRT1.2/ AIT1 | NPF | Screening of proteins capable of transporting ABA in yeast | Vascular tissues | Plasma membrane | Yeast and insect cells | ABA insensitive | Lower surface temperature of inflorescence stems | ABA hypersensitive | Lower surface temperature of leaves and inflorescence stems | Importer | [34] |
| AtDTX50 | MATE | Characterization of mutants defective in MATE members | Guard cells and vascular tissues | Plasma membrane | <i>Escherichia coli</i> and <i>Xenopus</i> oocyte | ABA hypersensitive | Growth arrest, drought tolerant | (N.D.) | Rapid wilting under drought stress | Exporter | [35] |

^aAbbreviations: ABCG, ABC G subfamily; AIT, ABA-importing transporter; DTX, detoxification efflux carrier; KO, knockout mutant; MATE, Multidrug and toxin efflux; NPF, nitrate transporter 1/peptide transporter family; NRT, nitrate transporter; OE, overexpressed plant; PDR, pleiotropic drug resistance; WBC, white-brown complex; Y2H, yeast two-hybrid system.

Vicia faba (~15 μM) [31]. Both AtABCG25 and AtABCG40 are localized to the plasma membrane when expressed as fluorescent fusion proteins in plant cells [29,30]. Interestingly, one study showed that plasma membrane localization of AtABCG25 was regulated by abiotic stress and ABA [32]. In another remark, the AtABCG25 promoter was active in vascular tissue, where ABA biosynthesis enzymes are preferentially expressed, whereas higher AtABCG40 expression determined by a promoter–reporter system was observed in guard cells. In addition, AtABCG25 expression under the control of the CaMV 35S promoter enhanced stomatal closure on activation of the ABA-inducible *RD29B* promoter [33]. These findings are consistent with the hypothesis that vascular tissue is the primary site of ABA synthesis, from which ABA is transported to guard cells to elicit stomatal response, possibly mediated by these ABA transporters (Figure 1). However, mutants defective in either AtABCG25 or AtABCG40 exhibit relatively mild phenotypes compared with typical ABA-deficient mutants, suggesting that passive-diffusion ABA transport mechanisms could compensate for the functions of AtABCG25 and AtABCG40 or that active ABA transport mediated by transporters is highly redundant. Subsequent studies have identified additional ABA transporters and support the latter scenario, as described below.

Functional screening using a yeast system identified an arabidopsis NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER FAMILY (NPF) member, NPF4.6 (originally named AIT1), as a protein that could mediate ABA uptake into cells [34]. NPF4.6 was previously characterized as a low-affinity nitrate transporter NRT1.2 ($K_m = 5.9 \text{ mM}$); however, the same protein exhibited much higher affinity for ABA ($K_m = 5 \mu\text{M}$). Fluorescent protein-fused NPF4.6 is predominantly localized to the plasma membrane, suggesting that this protein could also have a role in intercellular ABA transport. Expression of NPF4.6 under the control of the CaMV 35S promoter results in a reduction in leaf surface temperature, possibly due to defective stomatal closure. Constitutive ABA import activity could inhibit ABA movement within plant tissues. This suggests that ABA transport is necessary for proper stomatal function. Conversely, loss-of-function *npf4.6* mutants have shown only limited phenotypes in terms of stomatal closure, as observed for *abcg25* and *abcg40*, suggesting that ABA transport systems are highly redundant. Interestingly, while both NPF4.6 and AtABCG40 mediate cellular ABA uptake in heterologous systems, their spatial expression patterns differ; NPF4.6 is expressed in vascular cells whereas AtABCG40 is expressed in guard cells. NPF4.6 could regulate the amount of ABA transported from vascular tissues toward guard cells in association with AtABCG25 (Figure 1). Moreover, NPF4.6-mediated ABA uptake into vascular tissues could be related to ABA distribution within plants via xylem and phloem (Figure 1).

A member of the multidrug and toxin efflux (MATE) transporter family, AtDTX50, has also been identified as an ABA exporter [35]. A comprehensive reverse genetic approach in MATE transporters found that a mutant defective in AtDTX50 (*dtx50*) exhibited growth defects, possibly due to its higher sensitivity to ABA or higher endogenous ABA levels. Fluorescence-tagged AtDTX50 proteins were localized predominantly to the plasma membrane and AtDTX50 facilitated ABA efflux in both *Escherichia coli* and *Xenopus* oocyte cells. DTX50 was preferentially expressed in vascular tissues, indicating that it regulates ABA export from vascular tissues in association with AtABCG25 and NPF4.6 (Figure 1). AtDTX50 expression in guard cells suggests that ABA levels in these cells, and hence stomatal aperture, are regulated not only by ABA influx but also by ABA extrusion (Figure 1).

Leaf vascular tissue appears to largely contribute to ABA accumulation in response to drought under many experimental conditions. However, this does not mean that this tissue is the only source of ABA. For example, guard cells themselves autonomously support the

ABA synthesis required for the stomatal response to leaf water status [36,37]. This system would be beneficial for plants to respond to rapid changes in aerial humidity before the root-derived root-to-shoot signals on soil drying induce ABA biosynthesis in vascular tissues, whereas a large quantity of ABA produced in vascular tissues might be required for plants to more comprehensively respond to severe water deficit (discussed more in detail below). In addition, it is possible that ABA synthesized in guard cells could be exported outside by AtDTX50 and affects some ABA responses in the surrounding cells. Interestingly, it has been shown that ABA catabolism is differentially regulated in vascular tissues and guard cells by distinct CYP707As. CYP707A3 is predominantly expressed in vascular tissues and regulates the total amount of ABA accumulated in leaves. Conversely, CYP707A1, which is preferentially expressed in guard cells, makes a minor contribution to the bulk ABA content in leaves. However, CYP707A1 could regulate the stomatal aperture similarly to CYP707A3 [38]. These results indicate that site-specific ABA levels, for example in guard cells, are determined based on a combination of biosynthesis, catabolism, and transport in vascular cells and guard cells [39].

ABA Action in Guard Cells for Stomatal Aperture Regulation

One of the major causes of stomatal closure is the activation of outward K^+ channels, which leads to a reduction in the water potential inside guard cells. As a result, water is exported from guard cells according to the potential, resulting in a reduction in turgor and finally stomatal closure [40]. ABA-induced stomatal closure is generally considered to be a relatively rapid response that occurs within a few seconds or minutes [41] and does not require *de novo* transcription. Nevertheless, microarray analysis using isolated guard cell protoplasts has identified genes that are up- or downregulated by exogenous ABA treatment [36,42,43]. Interestingly, three basic helix-loop-helix (bHLH) transcription factors, AKS1, 2, and 3, have been identified as possible SnRK2 kinase substrates [44,45]. Unphosphorylated AKSs form homomultimers that transactivate the expression of shaker-type inward K^+ channel genes, including *KAT1*, to promote stomatal opening. Meanwhile, phosphorylation results in the monomerization of AKSs, which inhibits DNA binding and transcription of target genes. This suggests that ABA-mediated transcriptional regulation inside guard cells is important for stomatal aperture regulation. It is important to further understand how the rapid and long-term stomatal responses are regulated by multiple ABA sources and ABA transporters.

Involvement of guard cell aquaporins in stomatal aperture regulation has also been suggested. Plasma membrane-intrinsic proteins (PIPs) are the major aquaporins that facilitate diffusion of water across the plasma membrane [46]. It was recently shown that ABA regulates transmembrane water transport in guard cells [47]. Mutants defective in PIP2;1 (*pip2;1*) were less sensitive to exogenously applied ABA than the wild type in terms of stomatal closure, while mutants exhibited high carbon dioxide-induced stomatal closure, similar to the wild type. In guard cell protoplasts prepared from *pip2;1*, ABA-induced increases in water permeability and reactive oxygen species, which normally occur in wild-type guard cell protoplasts, were not observed. OST1/SRK2E/SnRK2.6 enhanced PIP2;1-mediated water transport when coexpressed in *Xenopus* oocytes and phosphorylation of PIP2;1 by OST1/SRK2E/SnRK2.6 was required for the response. These results suggest that ABA-mediated activation of PIP2;1 in guard cells could help induce stomatal closure by facilitating water transport, although the stomatal aperture of *pip2;1* is similar to that of the wild type in the absence of exogenously applied ABA. The lack of a stomatal phenotype in *pip2;1* in the absence of exogenous ABA might be due to the complex roles of PIP2;1 in different tissues and organs.

Physiological Roles of ABA in Vascular Tissues

If the total amount of ABA synthesized in a plant predominantly depends on vascular tissues, why is ABA biosynthesized in vascular tissues far from guard cells located on epidermal tissue? The simplest answer is that vascular tissues have an important role in distributing ABA throughout the plant body to tolerate water stress by regulating physiological processes beyond direct induction of stomatal closure (Figure 1).

Leaf transpiration rates are regulated not only by stomatal aperture (stomatal conductance) but also by several factors such as hydraulic resistance and water flow rates within plant tissues. In principle, water transported to leaves via xylem can move through cells (via cell-to-cell transport across the biological membrane or plasmodesmata) and the apoplastic space toward stomata [48]. The density of cells in mesophyll tissues is often low and these tissues often contain a large proportion of air space, suggesting that water travels via apoplastic movement in such tissues in leaves. Conversely, vascular bundles comprise tightly associated cell layers. Bundle sheath cells (BSCs), which form a layer surrounding vascular tissues [49], are connected to neighboring mesophyll cells via plasmodesmata and the cells do not share symplastic connections with xylem and phloem [50]. When a water-soluble cell-wall-specific dye was loaded via the cut surface of a leaf petiole, staining was observed along veins but not in mesophyll cells [51], indicative of a tight apoplastic barrier surrounding the vascular bundle. In addition, in some plant species, outer tangential cell walls of BSCs are preferentially suberized [52]. These results suggest the importance of water uptake into BSCs and subsequent transcellular water flow.

In one study, feeding ABA to detached *Arabidopsis* leaves via xylem reduced leaf hydraulic conductance, whereas ABA application to the leaf surface did not affect leaf hydraulic conductance [51]. The water permeability of BSC protoplasts was reduced by ABA treatment; however, the same treatment did not affect water permeability in protoplasts prepared from mesophyll cells. Meanwhile, BSC-specific silencing of the PIP1 aquaporin subfamily by miRNA resulted in reduced water permeability of mesophyll cells and BSCs and decreased leaf hydraulic conductance [53]. These data suggest that ABA-mediated inhibition of aquaporin in BSCs has a role in reducing leaf hydraulic conductance and transpiration independent of the stomatal aperture. In addition, the stomata of an *Arabidopsis* *ost2* mutant, which is defective in an H⁺-ATPase, were insensitive to ABA; however, ABA application to *ost2* via xylem induced stomatal closure [54]. It is possible that ABA synthesized in vascular tissue induces stomatal closure indirectly by reducing the water permeability of BSCs and leaf hydraulic conductance.

Physiological Role of ABA in Roots

Soil drying can result in enhanced or reduced hydraulic conductivity in root cells depending on plant species, variety, and natural variations [55]. ABA can influence root hydraulic conductance, possibly by regulating aquaporin activity, although the effects can differ depending on the plant materials and experimental conditions. Such complex regulation might be required for plant adaptation to different environmental conditions. For example, increased water permeability is required for efficient water uptake during the early phase of water deficit. However, after prolonged water deficit, higher water permeability could result in the loss of water from root tissue to soil. Inhibiting root hydraulic conductance by 50% resulted in a greater than 50% reduction in leaf hydraulic conductance. This suggests that root hydraulic conductivity could affect leaf-water relationships [56]. However, it is unknown which cells in roots are responsible for ABA-mediated responses.

In roots, ABA regulates not only hydraulic conductance but also growth. Salt stress regulates the expression of numerous ABA-dependent genes in roots, some of which are regulated

globally in root tissues and others that are regulated in specific cell types [57]. It has been reported that ABA signaling in endodermis promotes lateral root quiescence during salt stress [58]. Arabidopsis root growth is also inhibited by osmotic stress, which is dependent on endogenous ABA because ABA-deficient mutants are less sensitive to osmotic stress [59]. Similarly, in most cases ABA inhibits root growth when applied exogenously. However, endogenous ABA has also been observed to promote root growth [21,60]. The effects of ABA on root growth could differ depending on the strength and duration of stress, as discussed with respect to the role of aquaporin.

The Casparian strip is a ring-like cell-wall modification in root endodermis that surrounds vascular tissues (e.g., BSCs in leaves). It is primarily composed of lignin and serves as a barrier against apoplastic diffusion of minerals and nutrients from vascular bundles [61]. A recent report indicated that endodermis is reversibly coated with hydrophobic suberin in response to various stresses such as nutrients and salinity, which might restrict apoplastic movement of water, minerals, and nutrients [62]. Interestingly, suberization was induced by the enhanced expression of a suberin synthesis gene in response to exogenous ABA treatment. Furthermore, in several mutants defective for ABA biosynthesis and signaling (*aba2*, *abi3*, *abi4*, and *abi5*), suberin deposition in endodermis was significantly delayed. ABA-mediated suberization requires ABA signaling in endodermis, because the expression of the dominant-negative *aba1-1* protein in endodermis under the control of CASP1 and ELTP1 inhibits suberization under non-stressed conditions and in response to exogenous ABA treatment. ABA synthesized in aboveground tissues is reportedly translocated to roots, possibly via the vasculature [14,16,17]. In addition, some ABA biosynthesis and transporter genes are expressed in root vascular tissues [25,30,34,35,63]. This suggests that ABA synthesized in or translocated to root vascular tissues might be associated with endodermis suberization.

Concluding Remarks

This review examined the relationship between ABA biosynthesis and transport, with a focus on water stress. First, we discussed the possible sites of ABA biosynthesis in relation to stomatal regulation. Leaf vascular tissue is an active site of ABA biosynthesis in response to water stress; however, guard cells also serve as a source of ABA. ABA synthesized in roots could also induce stomatal closure. Overall, ABA biosynthesis is not restricted to one site; however, it should be noted that the contribution of ABA synthesized at different sites to stomatal closure sometimes differs among reports, possibly due to differences in plant materials and/or experimental conditions. To understand how ABA derived from multiple sources coordinately regulates stomatal closure, the molecular mechanisms by which plants sense water deficit at different sites under various conditions (e.g., rapid, gradual, temporal, or successive water deficit in soil and/or atmosphere) must be clarified and the largely unknown signal transduction pathways upstream of ABA biosynthesis must be identified. At least four ABA transporters involved in stomatal regulation have been identified. Interestingly, both ABA exporters and importers are expressed in vascular tissues and guard cells, indicating that ABA can be transported bidirectionally across the plasma membrane and that ABA transport does not follow a simple one-way route. Since plants tolerate water deficit under the control of complex regulatory networks, understanding the physiological processes regulated by ABA in different organs, tissues, and cells will help create an overview of plant water stress responses. ABA is likely to regulate stomatal closure within and outside guard cells through combinational mechanisms. Furthermore, ABA functions are related not only to stomatal closure but more completely to water penetration and hydraulic control, to systemically cope with water stress. More detailed analyses of the spatiotemporal expression/localization of ABA transporters, as well as ABA biosynthesis and catabolism enzymes are required to determine the dynamics of ABA transport

Outstanding Questions

How do plants sense water deficit at different sites?

How does ABA synthesized at different sites contribute to a given physiological response?

How do transporters regulate the multidirectional movement of ABA within a plant?

How do ABA synthesis and transport differ between the initial and later stages of water stress?

How do various types of cells in shoots or roots orchestrate ABA synthesis and transport?

within plants. For example, we do not know the exact route of ABA transport in vascular tissue and guard cells. Some transporters of the plant hormone auxin and nutrients are distributed unevenly within cells. Thus, it would be interesting to determine whether polar distribution of ABA transporters within specific cell types can be observed. In addition, the visualization of ABA by recently developed systems [64,65] could be used to contribute to monitoring ABA movement within plants. Given that the phenotypes of mutants defective in the ABA transporters identified to date are relatively mild compared with typical severely ABA-deficient mutants, the identification of additional transporters is required to better understand the highly redundant ABA transport system (see Outstanding Questions).

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