

Review

Abscisic Acid Biosynthesis and Signaling in Plants: Key Targets to Improve Water Use Efficiency and Drought Tolerance

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Abstract: The observation of a much-improved fitness of wild-type plants over abscisic acid (ABA)-deficient mutants during drought has led researchers from all over the world to perform experiments aiming at a better understanding of how this hormone modulates the physiology of plants under water-limited conditions. More recently, several promising approaches manipulating ABA biosynthesis and signaling have been explored to improve water use efficiency and confer drought tolerance to major crop species. Here, we review recent progress made in the last decade on (i) ABA biosynthesis, (ii) the roles of ABA on plant-water relations and on primary and secondary metabolisms during drought, and (iii) the regulation of ABA levels and perception to improve water use efficiency and drought tolerance in crop species.

Keywords: ABA receptors; ABA receptor antagonists; plant metabolism; plant-water relations; stomatal closure; water deficit; xylem embolism

1. Introduction

The phytohormone abscisic acid (ABA) is a fundamental regulator of the morpho-physiological response of plants during drought stress. The central and best-known plant response to ABA during drought is the closure of stomata in seed plants [1,2]. However, this hormone regulates many other aspects of plant-water relations (Figure 1). For instance, many studies have demonstrated a pronounced inhibitory effect of increased levels of endogenous ABA on shoot growth in plants experiencing drought [3,4]. At the same time, high levels of ABA promote root elongation [5], while strongly inhibiting lateral root formation [6] as an adaptive response to increase the effectiveness of root water uptake under water deficit conditions. The effects of ABA on shoot and root growth, however, can be positive or negative depending on its concentration and the plant species [7]. Increased levels of endogenous ABA also play key roles in down-regulating leaf hydraulic conductance (which is proposed to further induce stomatal closure) [7–9] and mesophyll conductances [10–12], as well as up-regulating the cuticular wax formation (which results in a thicker, less permeable cuticle) [13,14]. Finally, ABA is a critical messenger produced in response to water deficit that promotes a variety of biochemical responses in different plant tissues [15]. Due to the ABA's key roles in the physiology of plants during drought,

several promising approaches to manipulate ABA biosynthesis and signaling (i.e., either genetically or through chemical intervention with agonists) have been explored aiming at conferring drought tolerance to major crop species and improving water use efficiency (WUE, i.e., the ratio of photosynthesis to water loss through foliar transpiration). In this review, we cover the progress made in the last decade in our understanding of how ABA signaling regulates plant physiology and biochemistry during drought. Special attention has been paid to (i) the ABA biosynthesis within the plant, (ii) the impacts of ABA on stomatal aperture and xylem embolism, (iii) the regulation of primary and secondary metabolisms by ABA, and (iv) the potential applications of approaches modulating ABA levels and perception to improve WUE and drought tolerance in crop species.

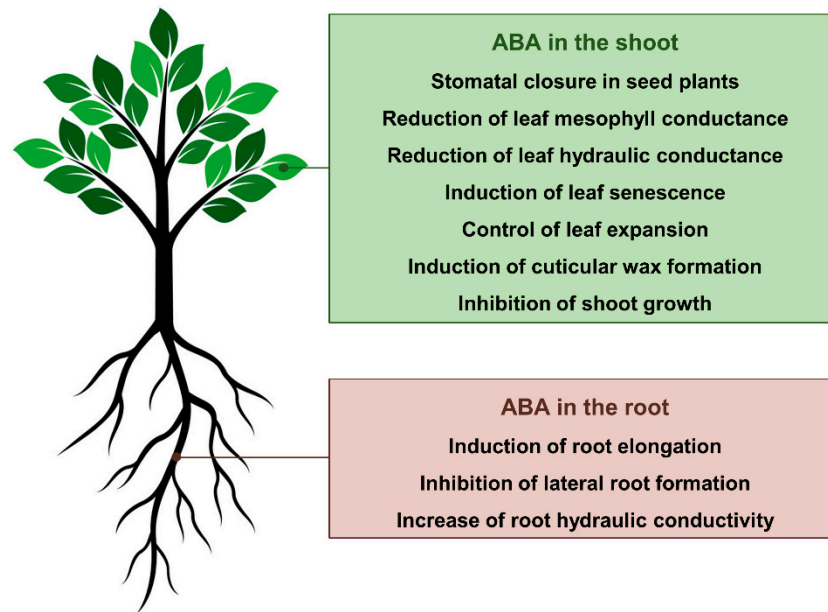


Figure 1. Current understanding of how abscisic acid (ABA) regulates plant form and function during drought.

2. Drought-Induced Biosynthesis of ABA

Given the key role played by ABA in enhancing plant survival during drought, a comprehensive understanding of how the biosynthesis of ABA occurs, how it is regulated within the plant, and where it mostly takes place is of major importance. In recent years, the pathway by which ABA is synthesized in plants has been extensively investigated through the use of mutants deficient in the synthesis and perception of ABA [16–20]. It has been demonstrated that the biosynthetic pathway for ABA accumulation begins in the chloroplasts with the hydroxylation of β -carotene to zeaxanthin, which is then converted to violaxanthin through the xanthophyll cycle. Next, neoxanthin synthase converts violaxanthin to neoxanthin, which is then isomerized from 9-trans to 9-cis (neoxanthin). The last step of the biosynthetic pathway that occurs in the chloroplasts is the oxidative cleavage of 9-cis-neoxanthin and/or 9-cis-violaxanthin to xanthoxin, which is further catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED). NCED is known to be the key rate-limiting enzyme in the ABA biosynthesis and it is responsible for catalyzing the first non-reversible step in the pathway [21]. The formed xanthoxin then moves from the chloroplasts to the cytosol, where it is converted to abscisic aldehyde, and finally to ABA. ABA accumulation in plants occurs not only through activation of ABA biosynthesis but also by the hydrolysis of its glycosyl ester (i.e., ABA-GE) [22,23], which can be utilized to maintain the high levels of ABA in plants during drought [24–26].

Since the first studies linking ABA with stomatal closure under drought, a number of results have led to the critical assumption that the pool of ABA resulting in stomatal closure in leaves is mostly synthesized in the root tips [27,28]. However, this conventional view of root-to-shoot ABA signaling

has recently been challenged by studies using a number of experimental approaches, including the reciprocal grafting between ABA biosynthetic mutant and wild-type plants and exogenous labeled ABA [29–33]. In fact, these studies clearly demonstrate that although multiple organs and tissues can accumulate small amounts of ABA, leaves are the predominant site for ABA biosynthesis, and they are even responsible to export ABA to the roots, maintaining normal root levels of ABA levels and determining root architecture and growth [30,32,33]. Inside leaves, phloem companion cells, guard cells, and mesophyll cells have been demonstrated to be capable of synthesizing ABA [34–37]. In addition, ABA biosynthesis has been recently observed to occur in parenchyma cells around the vascular tissues [38]. Regarding the ABA transport within plants, the recent identification of a number of transmembrane ABA transporters strongly suggests that the movement of this hormone is actively regulated in an intercellular network [15]. However, the fine regulation of ABA biosynthesis and transport among different plant tissues remains a matter of debate [15].

The major production of ABA in leaves, not roots, during drought seems considerably more advantageous for plants to avoid excessive declines in water potential as leaves represent the main organ exchanging (and mostly losing) water with the atmosphere. For instance, a *de novo* biosynthesis of ABA over an extremely short time-frame following leaf exposure to high vapor pressure deficit (VPD) has been demonstrated to allow an efficient stomatal closure in angiosperm species [39,40]. Declines in leaf water potential to a threshold leaf water potential results in major increases in foliar ABA [41,42] and such threshold water potential strongly coincides with bulk leaf turgor loss point [20,40,43,44]. Although the water potential at turgor loss is broadly accepted as the threshold for the major increment in foliar ABA biosynthesis, it is still uncertain whether changes in turgor itself or changes in cell volume are the main signal up-regulating the enzymes responsible for ABA production [20,45]. In addition, given the lower water potential in the guard cells and the mesophyll cells nearby stomata in transpiring leaves [46], small levels of ABA are expected to be produced in these cells as transpirational demand increases, resulting in stomatal closure even prior to leaf turgor loss point [40].

3. ABA and Drought Tolerance: From Stomata Closure to the Modulation of Plant Metabolism

As previously mentioned, guard cells are not the only site of ABA signaling and action. Instead, ABA plays crucial roles in transducing the water stress signal to a variety of cell-types and tissues [47]. For instance, ABA accumulation activates seed dormancy in developing embryos [48–50], leaf osmotic adjustment inducing compatible solute accumulation (e.g., proline and sucrose [51–53]), and leaf senescence [54–56]. In addition, ABA has a protective effect against oxidative damage by enhancing the activity and expression of antioxidant enzymes and the levels of secondary metabolites in different plant organs [38,57–59]. Amongst the multiple processes controlled by ABA under drought, we describe here the regulation of gas exchange through the stomata and how it affects the water transport within the xylem. In addition, potential roles of ABA on the modulation of primary and secondary metabolites are also discussed.

3.1. Linking ABA with Stomatal Aperture and Xylem Embolism

Stomatal closure during drought is one of the first attempts of vascular plants to reduce transpiration, preventing tissue desiccation and damage [60,61]. The signaling pathway for ABA-induced stomatal closure in angiosperm species has been the subject of detailed investigations in recent years and it is now reasonably well characterized [62–65]. As angiosperms often exhibit stomatal conductances higher than the minimum amount sufficient to allow maximum rates of photosynthesis, declines in stomatal conductances during drought driven by increased foliar ABA levels often result in optimized WUE [66,67]. Aiming to increase WUE in economically important crop species, genetic and molecular tools have been applied to generate plants with increased ABA biosynthesis and signaling in recent years [66,68,69]. See topic 4.0 for further details.

Although increasing levels of foliar ABA has long been associated with stomatal closure and increases in WUE in angiosperm species during drought [1,38,67,70,71], it has recently been discovered

that the stomata of ferns and lycophytes do not close in response to both endogenous and exogenous ABA [72–74]. Instead, in these early-diverging species, changes in leaf water status regulate stomatal aperture by an ABA-independent, hydropassive mechanism [72,75,76]. In a similar way, recent findings demonstrate a number of hornwort species that lack stomatal responses to ABA [77]. These findings indicate a gradualistic model of stomatal control, which has been the focus of considerable debate in the last decade [76,78–83]. For a recent and comprehensive review on ABA signaling pathway and function across the evolution of land plants, see McAdam and Susmilch [84].

Early closure of stomata has been demonstrated to occur prior to leaf and stem xylem embolism in a number of species from ferns to angiosperms, supporting the idea of the water-saving function of stomata and xylem protection during drought [61,76,85–87]. This protective mechanism is of paramount importance given that leaf xylem embolism has been associated with foliar mortality across the canopy [88,89], potentially affecting plant growth and productivity. In addition, extensive embolism in the stem xylem and consequent hydraulic failure have been largely associated with drought-induced mortality of plants [90–93]. Curiously, ABA signaling has very recently been associated with the construction of conduits of stem xylem more prone to suffer embolism in a study comparing a transgenic line of tomato overproducing ABA with its wild-type [94]. The specific ABA over-expressing transgenic line utilized in this study, however, exhibits several differences from the wild-type plants regarding stem xylem anatomy traits that are closely linked to xylem resistance to embolism (i.e., xylem conduits are much less numerous and narrower in the transgenic line compared with the wild-type). Whether this represents that ABA signaling modulates xylem anatomy, or if this arises from pleiotropic effects is of major importance to confirm the association between ABA and xylem vulnerability to embolism. Besides, the transgenic line utilized in this study presents a very similar hydraulic resistance to other cultivars of tomato that do not overproduce ABA [95] (and personal observations), which might suggest that the wild-type utilized, rather than the transgenic line itself, presents a different xylem vulnerability from other cultivars potentially due to differences in the xylem anatomy. In any case, the idea of hydraulic vulnerable plants when ABA biosynthesis is increased might challenge the use of transgenic plants with increased ABA biosynthesis and signaling to improve crop production under drought and thus should be further investigated.

3.2. Primary and Secondary Metabolic Pathways Regulated by ABA

ABA signaling is involved in transcriptional and post-transcriptional regulation of genes encoded for enzymes related to carbohydrate metabolism (Table 1) [96–98]. ABA can regulate sucrose metabolism through the induction of vacuolar invertase activity and the up-regulation of vacuolar invertase gene expression [97,99]. Furthermore, Thalmann et al. [100] showed that ABA promotes leaf starch degradation under osmotic stress by the synergistic action of β -AMYLASE1 (BAM1) and α -AMYLASE3 (AMY3). Both mechanisms lead to the release of hexoses (i.e., fructose, glucose, and maltose) that function as compatible solutes, preserving membrane integrity and maintaining leaf turgor [101]. The accumulation of soluble carbohydrates concomitantly with the up-regulation of ABA biosynthesis in stem of *Populus nigra* exposed to drought has been recently observed [38,102]. Stem soluble carbohydrates may help to preserve xylem hydraulic integrity and act as a buffer when photoassimilation decreases under drought, thus ensuring an adequate energy source for stress defense and viable seed production [103,104]. In addition, ABA has been demonstrated to regulate foliar proline accumulation under osmotic stress (Table 1), although a direct action of ABA on the transcriptional regulation of the key genes and enzymes involved in proline biosynthesis is still unclear [59,105–109]. Similar to other compatible osmolytes, significant levels of proline can accumulate in the cytoplasm and organelles allowing osmotic adjustment, stabilizing protein structure and cell membranes, and acting as a free radical scavenger [110]. Furthermore, proline may provide regulation of cytosolic acidity and act as a signaling molecule able to activate further stress responses [111].

Table 1. Representative metabolites and enzymes associated with primary and secondary metabolisms that are affected by ABA.

Metabolite/Enzyme	Alteration	Organ	Plant Species	Reference
IVR2 acid vacuolar invertase activity and expression	Increase	Leaf	<i>Zea mays</i>	[99]
Starch levels	Decrease	Shoot	<i>Arabidopsis thaliana</i>	[97]
Starch levels	Decrease	Leaves	<i>Arabidopsis thaliana</i>	[100]
β -AMYLASE1 and α -AMYLASE3 activity	Increase	Leaves	<i>Arabidopsis thaliana</i>	[100]
Δ 1-pyrroline-5-carboxylate synthase expression	Increase	Whole seedling	<i>Arabidopsis thaliana</i>	[106,107]
Flavonoid biosynthesis and gene expression	Increase	Leaves	<i>Camellia sinensis</i>	[112]
Proline levels	Increase	Leaves and Fruits	<i>Vitis vinifera</i>	[59]

Regarding the regulation of secondary metabolites under water deficit, several studies have shown the impact of exogenous application of ABA on flavonoid biosynthetic pathway (Table 1) [59,112–114]. In addition, many key genes of the flavonoid biosynthetic pathway were demonstrated to be up-regulated both in fruits and leaves in parallel to increases in the ABA content during drought [115–117]. However, whether these genes are activated by ABA or by the stress conditions remains under debate. The possible relationship between ABA and flavonoid could be important due to the ability of flavonoids to modulate the reactive oxygen species-signaling cascade and to buffer stress-induced alterations in metabolic homeostasis [118]. Future studies focusing on ABA-flavonoid interaction utilizing ABA-deficient mutants or any additional tools to manipulate the endogenous levels of ABA are necessary in order to confirm the role of ABA on flavonoid biosynthesis both at the transcriptional and post-transcriptional levels.

4. Regulation of ABA Levels and Perception in Crop Species

Ongoing climate change coupled with the projected increase in human population by over 2.4 billion until 2050 [119,120] represents a major challenge for the agricultural sector [121]. Over the next decades, climate change is expected to result in considerable rise in the intensity and frequency of drought events. In this water-limited scenario, achieving ‘more crop per drop’ (i.e., crop water productivity that is defined as the mass of agricultural produce per unit of water consumed) is a critical target for food production. Especially when considering that 70% of the available fresh water is used for crop production and that drought is expected to cause the largest yield reductions relative to any other abiotic and biotic stresses [122]. Therefore, enhancing crop WUE is still foreseen as a good means for saving water in agriculture [123] and has proven to be an accurate target in both genotype selection [124–126] and engineering [127].

The WUE can be determined both at the plant and leaf levels. At the leaf level, the photosynthesis-to-stomatal conductance ratio is defined as the intrinsic water-use efficiency (WUE_i). This parameter is typically determined in studies aimed at improving crop yield while saving soil water and mitigating yield limitations by water deficit [127,128]. In this context, the importance of ABA for WUE_i improvement is well-known due to its central role in regulating plant-water relations, specially by modulating stomatal conductance in several crop species [66,129–131]. As outlined above, the direct influence of ABA on WUE_i in angiosperms has already been demonstrated and several studies have concluded that plant responses driven by ABA depend on the severity and the duration of the drought events [67]. Particularly under short-term water deficit, ABA induces stomatal closure through its direct action on guard cells and modulates mesophyll conductance without restraining CO₂ fixation [8,12,38]. During severe and prolonged drought conditions, ABA can trigger further alterations at the transcriptome level, including genes encoding for LEA (late embryogenesis abundant) proteins [132]. Besides, it can alter the levels of osmoprotectants [100] and cause morphological changes in stomatal size and density [133],

leaf size, and shoot and root development [7,134]. All these alterations strongly affect plant water balance and WUE in the long-term [135]. In addition, ABA have been observed to regulate WUE through a tissue-specific action on hydraulic conductivity. Particularly in leaves, ABA has been found to decrease the hydraulic conductance by reducing the permeability of bundle sheath aquaporins [8,9,136] and also indirectly controlling mesophyll hydraulic conductances [11,137]. In the roots, ABA has been observed to increase hydraulic conductivity, facilitating water uptake under non-transpiring conditions [7,94]. Therefore, ABA signaling network can be modulated to reduce transpiration and increase crop water productivity under stressful conditions [67,138].

The ABA signal transduction pathway has recently been well documented, consisting of pyrabactin resistance/pyrabactin resistance-like/regulatory component of ABA receptors (PYR/PYL/RCAR), clade A type 2C protein phosphatases (PP2Cs), and SNF1 related protein kinase 2 (SnRK2s) [139,140]. The first attempts to use ABA in large scale agriculture were constrained by the chemical instability of ABA (e.g., under UV-light), its relatively expensive production, and its rapid cellular catabolism [141]. The modulation of ABA signaling, however, can be efficiently achieved through genetically engineered plants with an overexpression of ABA receptors [54,69,127,142] and signal transduction components [143,144], as well as through chemical intervention with ABA agonists [145–149].

Over the last years, studies on ABA overexpression receptors have led to promising results both in the field [150,151] and under controlled conditions [127,142]. The overexpression of PYL/RCAR receptors induces a higher plant sensitivity to ABA, leading to a globally enhanced WUE and drought resistance, as previously demonstrated in rice, poplar, and wheat [69,142,152,153]. The actual challenge, however, is to balance the improvements in WUE against the negative effects on growth due to ABA oversensitivity. Recent studies on plants overexpressing PYL receptors have shown an opposite effect on growth and stress adaptation and this may be due to the specific receptor choice. Indeed, the overexpression of some specific subfamilies result in plants with less transpiration for a similar leaf area and biomass compared to the wild-types, thus leading to an increased WUE [138]. In particular, PYL12/RCAR6 and PYL4/RCAR10 (subfamily I and II) overexpressing lines in *Arabidopsis* possessed a balance of reduced water use with negligible effects on growth and a “water-saving” phenotype that resulted in a significant improvement in total biomass relative to water use gains [127]. Similar observations have been made in transgenic wheat and in rice, in which the overexpression of specific PYL receptors has led to a reduced transpiration and a concomitant increase in photosynthetic activity compared to the wild-type, improving grain production per liter of water and protecting productivity during water deficit [69,142,151].

A faster and a more manageable alternative to the expression of genes for increasing crop’s WUE and conferring drought resistance is the use of ABA agonists [147,154], which are small synthetic molecules binding ABA receptors and activating ABA signaling pathway [155]. Among the ABA agonists, the first synthesized molecule Pyrabactin has been identified in a chemical genetic screen for seed germination inhibitors and it has been instrumental in showing how ABA binds the PYR/PYL receptor family [140]. The direct application of this molecule is not practical for agricultural purposes since its major effects are in seeds rather than in vegetative tissues. However, the examination of the structure of Pyrobactin and its interaction with ABA receptors has provided a framework for the design of novel ABA agonists [154]. As a consequence, several ABA agonists have been developed during the last ten years, with promising results in increasing crop water productivity [68]. An example is represented by Quinabactin, which appeared to be an overall better ABA agonist than Pyrabactin, inducing ABA responses both in seeds and vegetative tissues. Application of Quinabactin in plants resulted in improved water use upon drought stress [145]. This molecule targets all of the dimeric subfamily III ABA receptors, affecting guard cell closure to prevent water loss from detached leaves and conferring drought tolerance in both *Arabidopsis* and soybean during drought [145]. In addition, Quinabactin has shown effect in rapeseed and tomato, inhibiting germination and eliciting drought stress responses [156–158]. Another example concerns a tetrafluoro derivative of Quinabactin, AMF4, which seems to be more effective than Quinabactin due to its higher stability and bioavailability,

even targeting only a subset of ABA receptors [145,159]. Finally, a recent example is represented by the agonist B2, which was shown to improve drought tolerance in wheat by increasing root biomass and preventing leaf dehydration as well as amplifying antioxidant responses and enhancing the photosynthetic performances [149].

A recent innovative approach aims at combining the application of agrochemicals on genetically modified crops such as AMF4 on PYL2 overexpressed plants or mandipropamid on engineered PYR1 receptor (PYR1MANDI) [146]. The mandipropamid-PYR1MANDI system resulted in increased seedling survival during drought and transcriptional responses similar to those induced by ABA. Recently, Cao et al. [159] optimized the synthesis of new molecules based on the Quinabactin backbone and applied them to transgenic Arabidopsis and soybean plants with an abiotic stress-inducible AtPYL2 overexpression. This combined approach dramatically increased drought resistance in these plants, making this system a compelling alternative strategy to manipulate plant water use. Although the utilization of agrochemicals acting as ABA receptor agonists combined with transgenic approaches to increase ABA signaling holds great promise for the production of plants with enhanced WUE, the translation of these applications at field level into practical improvements in crop yield can be challenging [160,161]. For instance, as previously stated, a recent study conducted on tomato has highlighted a higher vulnerability to embolism of the vascular system of the transgenic line sp12 which overproduces ABA [94]. A reduced hydraulic safety margin can compromise the maintenance of hydraulic conductivity during periods of soil water deficit, with negative consequences not only for photosynthesis and productivity, but also for plant survival [162]. Given the importance of the potential use of plants with increased ABA biosynthesis and signaling to the future of agriculture in a world facing an ongoing climate change, it is of critical importance to confirm whether increased levels of ABA result in more vulnerable xylem and to understand the underlying mechanisms linking ABA signaling and xylem functioning. Important questions remain unanswered, especially those related to the extent of variation in embolism resistance among single crop species and their different varieties [163,164], including the relationship with genotypic variations in endogenous ABA content. Therefore, to optimize WUE strategies in the field, a complete understanding of the changes at the hydraulic and metabolic levels induced by the manipulation of ABA levels is needed. Timely investments in research at different levels will likely allow to improve crop resilience and yields in a water-limited scenario.

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