Tansley insight

A new look at stress: abscisic acid patterns and dynamics at high-resolution

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Summary

Abscisic acid (ABA) is a key phytohormone promoting abiotic stress tolerance as well as developmental processes such as seed dormancy. A spatiotemporal map of ABA concentrations would greatly advance our understanding of the cell type and timing of ABA action. Organ and tissue-level ABA measurements, as well as indirect *in vivo* measurements such as cell-specific transcriptional analysis of ABA metabolic enzymes and ABA-responsive promoters, have all contributed to current views of the localization and timing of ABA accumulations. Recently developed Förster resonance energy transfer (FRET) biosensors for ABA that sense ABA levels directly promise to add unprecedented resolution to *in vivo* ABA spatiotemporal mapping and expand our knowledge of the mechanisms controlling ABA levels in space and time.

I. Introduction

In aerial plant tissues, maintaining proper water status requires the coordination of growth with the rate of water loss due to evapotranspiration and water availability in roots. Water stress in roots triggers a series of adjustments to plant physiology, including stomatal closure to limit evapotranspiration and production of compatible solutes to lower cellular solute potential (Yamaguchi-Shinozaki & Shinozaki, 2006). Many of these adjustments depend on the accumulation of abscisic acid (ABA), a sesquiterpenoid plant hormone that has long been recognized as a key player in plant abiotic stress responses (Yamaguchi-Shinozaki & Shinozaki, 2006). In addition to lowering turgor in guard cells for stomatal closure (Schroeder *et al.*, 2001) and inducing compatible solute accumulation (e.g. proline; Ober & Sharp, 1994; Verslues & Bray, 2006) in other cells, ABA accumulation activates diverse responses in a variety of cell types such as seed dormancy in developing embryos (Bentsink & Koornneef, 2008; Finkelstein, 2013), senescence-associated yellowing of mesophyll cells (Weaver *et al.*, 1998) and salt stress responses in endodermal cells adjacent to emerging lateral roots (Duan *et al.*, 2013). Because developmentally programmed and conditionally induced ABA accumulations control diverse responses

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in multiple cell types, the question of how cellular ABA concentrations are patterned across tissues and over time (Fig. 1) is of crucial importance to understanding how ABA promotes abiotic stress tolerance and regulates developmental transitions such as germination and senescence. Several complementary methodologies now exist for the measurement of ABA, both in plant extracts and *in vivo* (Table 1). Recently developed fluorescent biosensors, termed ABACUS and ABAleon, report ABA levels at high spatiotemporal resolution and promise to facilitate *in vivo* ABA quantification in a number of cell types and plant species.

II. ABA in the coordination of whole plant responses to water stress

A popular model of coordination of water relations across plant organs held that water stress in roots stimulated ABA biosynthesis for translocation, via the transpiration stream, to the apoplasm surrounding leaf guard cells. Upon import of the root-derived ABA signal, guard cells would then reduce stomatal aperture and decrease evapotranspiration (Sauter *et al.*, 2001; Wilkinson & Davies, 2002). This model had particular relevance to an agronomic technique for improving water use efficiency (i.e. partial root zone drying) in which parts of a plant root system are alternately allowed to dry, thereby stimulating shootward long-distance stress signals from drought-stressed roots while maintaining shootward water flow from wetted roots (Davies *et al.*, 2002). However, although roots can synthesize ABA and xylem ABA levels were observed to correlate with reduced stomatal conductance (Sauter *et al.*, 2001; Wilkinson & Davies, 2002), a series of experiments indicated that one or more mobile stress signals independent of ABA trigger *de novo* ABA biosynthesis in the leaves. In tomato grafting experiments, even ABA-deficient root stocks, when drought stressed, send a mobile signal that triggers ABA accumulation and stomatal closure in wild-type shoot scions (Holbrook *et al.*, 2002). Christmann *et al.* (2005) exposed Arabidopsis roots to water stress and found that an ABA-responsive luciferase reporter was induced earliest in the leaf vasculature and guard cells, but only later and to a lesser extent in the root. Indeed, when the roots and leaves of intact Arabidopsis seedlings were either exposed to water stress or kept well watered, only the seedlings whose leaves were exposed to water stress exhibited high ABA levels and ABA-responsive gene expression, suggesting that the leaf is the key site for stress-induced ABA biosynthesis (Christmann *et al.*, 2007; Ikegami *et al.*, 2009). It remains unclear precisely how roots communicate water stress to leaves in order to trigger foliar ABA biosynthesis and induce stomatal closure, although current models include chemical and hydraulic signals (Christmann *et al.*, 2013; Mittler & Blumwald, 2015).

III. Mapping developmental and stress-induced ABA biosynthesis

In plants, ABA is a carotenoid-derived compound with early biosynthetic steps catalyzed by zeaxanthin epoxidase (ZEP) and

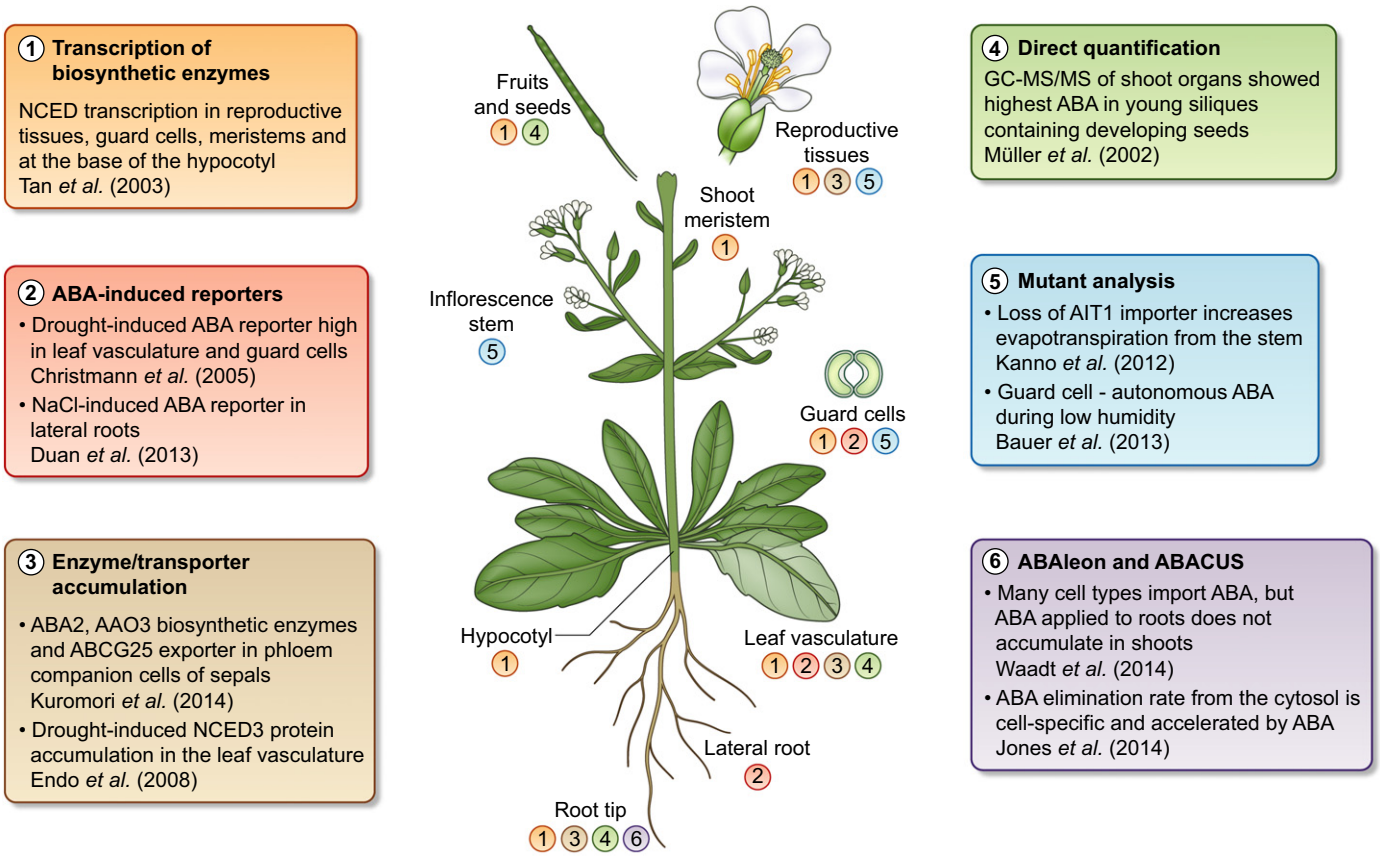

Fig. 1 A partial view of the spatiotemporal map of abscisic acid (ABA) accumulation in *Arabidopsis thaliana* highlighting examples of contributions from six distinct methodologies including the recently developed Förster resonance energy transfer (FRET) biosensors termed ABAleon and ABACUS.

Table 1 Measuring abscisic acid (ABA)

Method	Example	Description	Advantages	Disadvantages
Direct assays – *in vitro*				
Immunoassay	Weiler (1986)	Mono- and polyclonal antibody detection of ABA	High sensitivity Quantitative Ease of use	Typically low spatial resolution Cost
Mass-spectrometry	Rock & Zeevaart (1991); Muller *et al.* (2002)	Detection of ABA along with biosynthetic intermediates, catabolic/modification products, and other hormones	High sensitivity Quantitative Multiplexing	Typically low spatial resolution Cost
Indirect assays – *in vivo*				
Radiolabeled ABA	Kaiser & Hartung (1981); Daie & Wyse (1983); Creelman & Zeevaart (1984)	Tracking radioactivity after treatment with radiolabelled ABA	Transport analysis Metabolic analysis	Radioactivity required Cost
Expression of biosynthetic enzymes	Tan *et al.* (2003); Endo *et al.* (2008)	Quantification of transcript or protein levels for key biosynthetic enzymes	Ease of use (for transcriptional analysis) Cellular spatial resolution	Reporting can be confounded by post-translational regulation and/or ABA inactivation or transport
Transcriptional reporters	Ishitani *et al.* (1997); Christmann *et al.* (2005); Kim *et al.* (2011); Duan *et al.* (2013)	Tracking luminescence or fluorescence in transgenic plants expressing ABA transcriptional reporters (e.g. proATHB:LUC, proRAB18:GFP).	High-throughput Track ABA activity Cellular spatial resolution Genetically encoded	Susceptible to artifacts from crosstalk Low temporal resolution Transgenic required
Direct assay – *in vivo*				
FRET biosensors for ABA	Jones *et al.* (2014); Waadt *et al.* (2014)	Tracking a fluorescence emission ratio in transgenic plants expressing a FRET biosensor (e.g. ABACUS or ABAleon)	Direct sensing of ABA Quantitative Sub-cellular spatial resolution Real-time temporal resolution Genetically encoded	Low-throughput Ratio imaging required Transgenic required Buffering of ABA possible

FRET, Förster resonance energy transfer.

9-cis-epoxycarotenoid dioxygenase (NCED) enzymes in the chloroplast and later biosynthetic steps catalyzed by short-chain alcohol dehydrogenase/reductase (SDR) and abscisic aldehyde oxidase (AO) enzymes in the cytosol (Rock & Zeevaart, 1991; Nambara & Marion-Poll, 2005; Schwartz & Zeevaart, 2010). The NCED enzymes catalyze the first committed step in ABA biosynthesis and are the key regulatory step as evidenced by the tight correlation between NCED expression and ABA accumulation (Iuchi *et al.*, 2001; Tan *et al.*, 2003; Endo *et al.*, 2008; Schwartz & Zeevaart, 2010). Therefore, elucidating the developmental patterning and stress-induced dynamics of NCED enzymes contributed indirectly to a spatiotemporal map of ABA (Fig. 1). Arabidopsis has five NCED genes, each with unique developmental expression patterning suggestive of localized ABA action in a variety of cell types (Tan *et al.*, 2003). For example, ProAtNCED2:GUS and ProAtNCED3:GUS reporters show punctate expression patterns in root pericycle cells that predict the sites of lateral root initiation (Tan *et al.*, 2003), consistent with a role for ABA in regulating lateral root growth and initiation (De Smet *et al.*, 2003). Additional ProAtNCED:GUS expression domains in reproductive (e.g. floral organs and pollen) and vegetative tissues (e.g. root tips and the base of the hypocotyl) suggest broader roles for ABA in development than are presently understood (Tan *et al.*, 2003). By contrast, the strong induction of NCED3 expression and subsequent ABA accumulation during water stress has a clear role in promoting drought tolerance (Iuchi *et al.*, 2001). The leaf vasculature has been proposed as the key site of stress-induced ABA biosynthesis because NCED3 protein levels are induced exclusively in xylem and phloem parenchyma cells (Endo *et al.*, 2008). As ABA-responsive gene expression is nevertheless induced in mesophyll and guard cells during water stress (Christmann *et al.*, 2005; Endo *et al.*, 2008), it was proposed that ABA is primarily made in the vasculature for subsequent translocation to other tissues within the leaf (Endo *et al.*, 2008; Seo & Koshiba, 2011). However, Bauer *et al.* (2013) complemented a low-humidity leaf wilting phenotype of the Arabidopsis *aba3-1* ABA biosynthetic mutant by expressing ABA3 under control of a guard cell-specific promoter, suggesting guard cells can autonomously biosynthesize ABA and thereby respond directly to reduced water availability. Kuromori *et al.* (2014) also used cell-specific genetic intervention into ABA biosynthesis – by expressing NCED3 under a phloem companion cell promoter – to demonstrate that vascular

accumulation of ABA is sufficient to alter unstressed leaf transpiration rates. ABA biosynthesis in plants is clearly spatially and temporally dynamic, and the balance of evidence supports the view that active pools of ABA can be generated both through local biosynthesis and translocation from other cells, tissues and organs.

IV. ABA on the move

The study of mechanisms of ABA transmembrane transport and long-distance translocation has entered a new era with the recent discovery of a series of transporter proteins capable of importing (ABCG40, AIT1) or exporting (ABCG25) ABA (reviewed by Boursiac *et al.*, 2013; Seo, 2014). These discoveries provide additional ABA transport mechanisms to complement previous models of ABA redistribution in which stress-induced variation of apoplastic pH around the pKa of ABA (*c.* 4.8) alters the rate of ion-trapping in the cytosol of extracellular ABA (e.g. Slovik & Hartung, 1992). The drought-sensitive phenotype of loss-of-function mutations in ABCG40 indicated that transmembrane transporter-dependent movement of ABA contributes to drought-induced stomatal closure (Kang *et al.*, 2010). AIT1 mutant plants have increased stomatal aperture and decreased surface temperature in stems, indicating that stems import ABA synthesized in other tissues (Kanno *et al.*, 2012). However, many plant cell types are competent to import ABA (Christmann *et al.*, 2005) and it is likely that additional transporters remain undiscovered. This is particularly true for ABA exporter proteins as the promoter activity of ABCG25 (Kuromori *et al.*, 2010) is largely confined to phloem companion cells (Kuromori *et al.*, 2014) and ion-trapping mechanisms do not account for ABA export from the cytosol. Future discoveries could identify the mechanisms controlling ABA transport activities first observed decades ago (e.g. from maternal to zygotic tissues in developing seeds; Karssen *et al.*, 1983).

V. ABA inactivation

Although more attention is focused on the regulation of ABA accumulation, depletion of ABA is also crucial to plant development and stress responses. For example, ABA accumulated during water stress is rapidly eliminated once the stress is relieved and ABA levels in seeds fall during germination (Zeevaart & Creelman, 1988). All four AtCYP707A enzymes that perform 8′hydroxylation of ABA to inactive phaseic acid are transcriptionally induced by water stress and ABA (Kushiro *et al.*, 2004; Saito *et al.*, 2004), but show differential cell-specific expression patterns during development, for example during seed maturation and germination (Kushiro *et al.*, 2004; Okamoto *et al.*, 2006; Umezawa *et al.*, 2006). Cytosolic ABA glucosyltransferases reduce pools of free ABA through conjugation to ABA-glucose ester (Dong *et al.*, 2014; Liu *et al.*, 2015), and endoplasmic reticulum (ER) and vacuolar beta-glucosidase (BG) enzymes perform the reverse reaction to increase active ABA pools (Lee *et al.*, 2006; Xu *et al.*, 2012). Although it is clear that the transcriptional regulation of CYP707A, ABA glucosyltransferase and BG enzymes, along with biosynthetic enzymes and transmembrane transporters, is spatially and temporally regulated, it is not yet clear how these patterns integrate into ensemble cellular or sub-cellular ABA accumulation rates, elimination rates and steady states. This is particularly true for enzymatic activities that are regulated post-translationally, for example NCED inactivation by removal from thylakoid membranes (Endo *et al.*, 2008) and activation of BG through stress-induced polymerization (Lee *et al.*, 2006). Recently, genetically encoded fluorescent biosensors have been developed that detect ABA levels directly and promise to advance our understanding of spatiotemporal ABA maps.

VI. Direct biosensing for improved quantitation of ABA

ABA is perceived by a family of ABA receptors (PYR/PYL/RCAR proteins; Ma *et al.*, 2009; Park *et al.*, 2009) that then form a ternary complex with ABA co-receptor proteins (protein phosphatase 2CAs (PP2CA; Umezawa *et al.*, 2009; Vlad *et al.*, 2009). Recently, Jones *et al.* (2014) and Waadt *et al.* (2014) co-opted this ABA perception machinery to engineer fluorescent biosensors for ABA based on Förster Resonance Energy Transfer (i.e. FRET sensors; Okumoto *et al.*, 2012). These FRET sensors are fusion proteins that harbor an ABA sensory domain (a PYR/PYL/RCAR receptor linked to a PP2CA co-receptor) flanked by fluorescent proteins of a FRET pair. ABA induces an intramolecular interaction that alters the efficiency of energy transfer from the FRET donor to the FRET acceptor. During excitation of the donor, the ratio of acceptor emission over donor emission will vary in proportion to the FRET efficiency that, in turn, varies according to ABA binding. Thus, fluorescent emission ratio reports ABA concentrations in the solution, cell-type or sub-cellular compartment containing the FRET biosensor.

Waadt *et al.* (2014) generated ABAleon2.1 by fusing an ABA sensory domain (PYR1 linked to the catalytic domain of the ABI1 PP2CA) to two fluorescent proteins of a FRET pair (mTurquoise as FRET donor and cpVenus173 as FRET acceptor). ABAleon2.1 and variants have high-affinity for ABA (100–600 nM) that correspond well with the affinities of their intrinsic PYR1 domains in the presence of ABI1 and an ABA-dependent ratio change of *c.* 10% (Waadt *et al.*, 2014). Jones *et al.* (2014) created an accelerated FRET biosensor engineering platform that allows for screening libraries of constructs after expression in bacteria or yeast. FRET biosensors for ABA were identified from a combinatorial screen of ABA sensory domain variants and FRET pair variants expressed in a protease-deficient strain of yeast. ABACUS1 (abscisic acid concentration and uptake sensor) harbors an ABA sensory domain (PYL1 linked to a highly truncated – and likely noncatalytic – ABA interaction domain of the ABI1 PP2CA) fused to two fluorescent proteins of a FRET pair (enhanced dimerization Cerulean as FRET donor and enhanced dimerization Citrine as FRET acceptor) (Jones *et al.*, 2014). ABACUS1 and variants have medium affinities for ABA (2–80 μM) that correspond well with the affinities of their intrinsic PYL1 domains in the absence of ABI1 and an ABA-dependent ratio change of *c.* 60% (Jones *et al.*, 2014). The first-generation biosensors have complementary affinity ranges and relative strengths with ABAleons exhibiting high-affinity and

superior expression stability *in planta* and ABACUSs exhibiting medium-affinity and superior ABA-induced ratio change. Re-engineering both sensors has the potential to combine the best attributes of ABAleon and ABACUS while eliminating ABA-related phenotypes resulting from ABAleon and ABACUS expression *in planta*. At present, these ABA-related phenotypes raise interesting questions that warrant further investigation in their own right. For example, the ABA hypersensitivity of ABACUS-expressing plants is consistent with overexpression of the intrinsic PYL1 domain (Jones *et al.*, 2014), but does not match ABA hyposensitivity of ABAleon-expressing plants potentially associated with overexpression of the intrinsic ABI1 domain (Waadt *et al.*, 2014). Although the different phenotypic outcomes could be attributed to the different ABI1 truncations used – ABACUS harbors a likely inactive truncation of ABI1 – further experimentation is required to distinguish between this and other explanations for the observed contrast in phenotypes. Despite potential for reengineering these and other parameters, the first experiments using ABAleon and ABACUS clearly demonstrate their utility for elucidating spatiotemporal ABA maps and the mechanisms that control them.

In order to investigate long-standing questions regarding the role of ABA in coordination of development and stress responses between plant organs, Waadt *et al.* (2014) supplied exogenous ABA to plant organs in order to track the translocation of ABA to other tissues. This approach offers improvements over tracking radiolabeled ABA in the ease of use, fidelity to ABA itself and not catabolic products, and the ability to resolve cellular differences in near real-time. ABAleon imaging revealed that ABA applied to root tips did not result in ABA accumulation in leaves, whereas ABA application to the shoot did result in ABA accumulation in root tips (Waadt *et al.*, 2014). These results are consistent with several lines of evidence indicating that the direction of long-distance ABA translocation in Arabidopsis is more leaf-to-root rather than root-to-leaf (Christmann *et al.*, 2005, 2007; Ikegami *et al.*, 2009). Waadt *et al.* (2014) also revealed evidence of ABA accumulation in several tissues and cell types during stress by comparing ABAleon ratios in samples with and without low-humidity or high-NaCl treatment. New imaging modalities will be needed to accomplish time-course imaging of transpiring leaves in order to address the key question of the source of ABA, whether vascular and/or guard cell-autonomous, that serves to drive stomatal closure during water stress. In light of recent work highlighting a role for nonguard cell ABA signaling in controlling stomatal conductance (Pantin *et al.*, 2013), biosensor imaging could also reveal the location of active ABA pools beyond the stomata. Indeed, although ABAleon imaging of epidermal peels demonstrated that guard cells are competent to import exogenous ABA (Waadt *et al.*, 2014), many other cell types also accumulated ABA in response to exogenous treatment (Waadt *et al.*, 2014), consistent with results using ABA transcriptional reporters (Christmann *et al.*, 2005).

Direct biosensing using ABAleon and ABACUS now allows for higher spatiotemporal resolution of ABA accumulation and elimination rates. ABACUS sensors were used in the cytosol and nucleus of Arabidopsis roots to detect reversible and dose-dependent accumulation of exogenous ABA delivered to roots growing in the RootChip (a microfluidic device for continuous measurement of living roots in tightly controlled environmental conditions; Grossmann *et al.*, 2011; Jones *et al.*, 2014). An analysis of ABACUS ratios following a pulsed ABA treatment revealed contrasting ABA elimination rates in different regions of the Arabidopsis root tip (Jones *et al.*, 2014), suggesting a patterning of ABA export and/or inactivation activities. Furthermore, these cell-specific elimination rates were dynamic: after repeated ABA pulses, the elimination rate accelerated in all regions of the root tip, recapitulating ABA induction of ABA elimination rates observed previously (Zeevaart & Creelman, 1988) at high spatiotemporal resolution (Jones *et al.*, 2014).

VII. Conclusions

In plants, ABA is a master regulator of water use efficiency and other mechanisms of tolerance to drought and osmotic stress, as well as seed dormancy and germination (Finkelstein, 2013). Understanding the diverse roles of ABA in multiple tissues requires quantitative knowledge of ABA levels and dynamics in individual cells over time (Fig. 1). Recent work with ABAleon and ABACUS biosensors in Arabidopsis demonstrated the utility of high-resolution ABA detection (Jones *et al.*, 2014; Waadt *et al.*, 2014). More broadly, the accelerated biosensor engineering platform created by Jones *et al.* (2014) can be used for the generation of new FRET biosensors – a largely empirical process that can require screening of numerous constructs – for spatiotemporal mapping of other compounds. There are at least five other phytohormones whose perception mechanism involves a hormone-dependent protein–protein interaction analogous to that for ABA (Dharmasiri *et al.*, 2013). In the future, current and next generations of ABACUS and ABAleon can be used to dissect out the quantitative contributions of different ABA metabolic components to ABA spatiotemporal maps in order to arrive at a systems-level understanding of the regulation of ABA in space and time. Such an understanding could guide targeted interventions into plant physiology for improved outcomes during abiotic stresses that cause significant crop losses.

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