Journal of Experimental Botany, Vol. 53, No. 366, pp. 27–32, January 2002

Journal of Experimental Botany

Abscisic acid in the xylem: where does it come from, where does it go to?

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Received 20 August 2001; Accepted 25 September 2001

Abstract

Abscisic acid is a hormonal stress signal that moves in the xylem from the root to the different parts of the shoot where it regulates transpirational water loss and leaf growth. The factors that modify the intensity of the ABA signal in the xylem are of particular interest because target cells recognize concentrations. ABA_{xyl}, will be decreased as radial water flow through the roots is increased, assuming that radial ABA transport occurs in the symplast only. Such dilutions of the plant hormone concentration can be compensated in different ways, which help to keep the ABA-concentrations in the xylem constant: (i) apoplastic bypass flows of ABA, (ii) ABA flows between the stem parenchyma and the xylem during transport and (iii) the action of β-D-glucosidases that release free ABA from its conjugates to the root cortex and the leaf apoplast. The significance of reflection coefficients (σ_{ABA}), permeability coefficients of membranes (P_S^{ABA}) and apoplastic barriers for ABA is discussed.

Key words: Abscisic acid, roots, shoots, stress signal, xylem.

Introduction

The role and the physiological significance of abscisic acid (ABA) that is transported in the xylem sap from the roots to the shoots as a stress signal during early stress is well established. Its significance as a stress signal has been discussed recently in a number of review articles (Davies and Zhang, 1991; Hartung *et al*., 1999; Sauter *et al*., 2001). These results strongly indicate that roots can sense several aspects of the soil water status. Stomatal behaviour and the development of the shoot can be regulated as a function of the strength of these signals.

Increased attention has been paid in the past to the factors that regulate the intensity of the hormonal signal in the xylem. Elegant and fundamental work of Michael Jackson's group (Else *et al*., 1994, 1995) has shown that large concentration changes of ABA_{xyl} may also occur under unstressed conditions, when lateral water transport in the root to the xylem is altered. However, under field conditions such fluctuations have not often been observed (Tardieu *et al*., 1992). Mechanisms have, therefore, to be postulated that maintain an ABA homeostasis in the xylem under unstressed conditions.

The events occurring during the transport of ABA from the site of formation to the target cells are presented in the schematic diagrams of Figs 1, 2 and 3 and are discussed in the following paragraphs using those schemes.

ABA in roots: where does it come from?

Abscisic acid in roots increases as a soil is drying and is derived to a significant extent from synthesis in the root tissues (Fig. 1A). Both tissue types of the root, stele and cortex possess an equal capacity to synthesize ABA even at water losses of 50% and more. The largest accumulation is often observed in the root tips. This is very likely a result of the low vacuolization of the root tip cells with a high percentage of cytosol; that compartment where ABA is formed (Hartung *et al*., 1999).

A second internal source of ABA_{xyl} originates in the leaves (Fig. 3A). Leaf synthesized ABA can be loaded to the phloem (Fig. 3B) and transported to the roots (Figs 1B, 2F, 3C). In the roots one part may be deposited in the tissue and another part recirculated to the xylem vessels (Figs 1C, 2G). Salt stress, phosphate deficiency and ammonium nutrition enhance the percentage of

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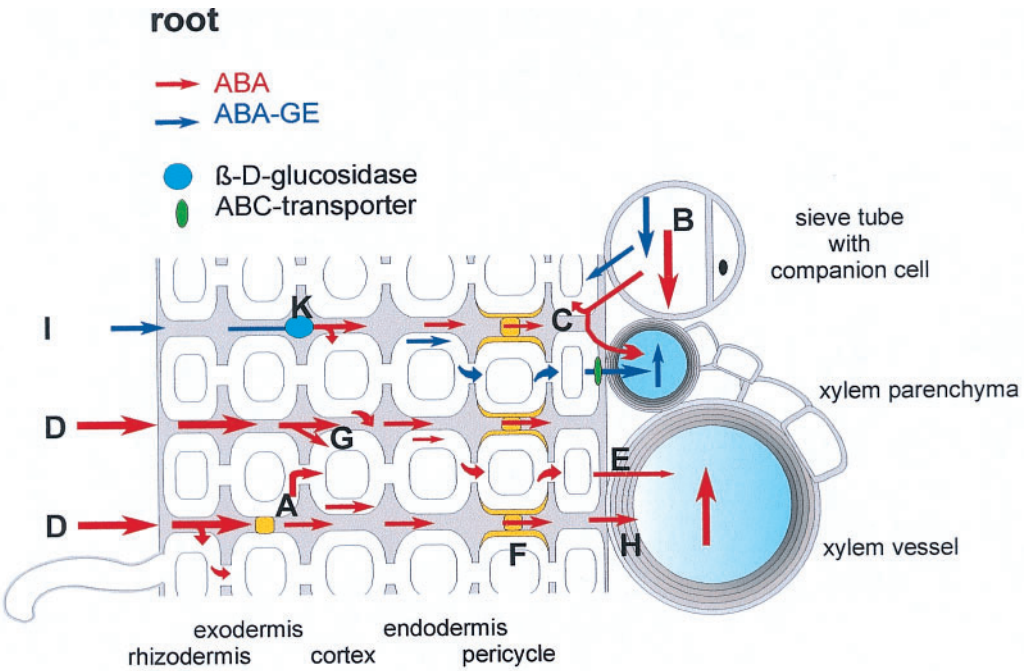

Fig. 1. Schematic presentation of the origin and the transport of ABA and ABA-GE in plant roots. The arrows indicate the flows of free ABA (red) and ABA-GE (blue). The lettering refers to the text. Extracellular enzyme activity is coloured light green, the putative transporter of the xylem parenchyma plasma membrane light blue. Cell walls are stained grey, the thickenings of the endo- and exodermis are yellow. The width of the arrows symbolize the intensity of the flows of free and conjugated ABA.

recirculated ABA (Jeschke *et al.*, 1997*a*, *b*; Wolf *et al.*, 1990; Peuke *et al.*, 1994).

Both free ABA and ABA-glucose ester (ABA-GE) have been detected in the soil solution under a range of crop plants at concentrations up to 10 nM or 30 nM, respectively (Hartung *et al.*, 1996; Sauter and Hartung, 2000). External free ABA can be taken up by the roots (Fig. 1D). It participates in maintaining an ABA equilibrium between roots and the external medium (Hartung *et al.*, 1996). The uptake of external conjugated ABA is strongly dependent on the existence and properties of apoplastic barriers as discussed later in this article.

How does root ABA reach the xylem?

Xylem ABA concentrations are strongly affected by changes of radial water flows in the roots caused by transpiration (Else *et al.*, 1994, 1995). When ABA is transported in the symplast exclusively, efflux from the xylem parenchyma cells across the plasma membrane to the apoplast and the xylem vessels is a rate-limiting step (Fig. 1E). Lateral water flows caused by transpiration will dilute the ABA_{xyl} dramatically and changes in stomatal opening as a result of light-induced stomatal oscillations will cause huge concentration changes of ABA_{xyl} under unstressed conditions.

Freundl *et al.* have shown that an apoplastic bypass flow of ABA across the endodermis is possible (Fig. 1F) (Freundl *et al.*, 1998), participating in an ABA_{xyl} homeostasis. They have determined the reflection coefficient σ of ABA for maize and sunflower roots under different conditions. When $\sigma_{ABA} = 1$, all ABA molecules are reflected at the endodermis. They are forced to enter the symplast. Table 1 shows that σ_{ABA} is always below 1 indicating that substantial amounts of ABA can be dragged with the water across the endodermis directly into the xylem, buffering ABA fluctuations caused by increased transpirational water transport. An ABA_{xyl} homeostasis has been observed in field-grown plants subjected to abrupt changes of evaporative demand (Tardieu *et al.*, 1992).

The exodermis: an apoplastic barrier that retards ABA loss

Most of the roots growing in a well-aerated soil form a Casparian band in the hypodermis—the exodermis. Only roots of a few species, predominantly legumes, lack an exodermis (Perumalla *et al.*, 1990). It has been demonstrated (Freundl *et al.*, 2000; Hose *et al.*, 2001, 2002) that efflux of ABA from the apoplast to the surrounding medium or rhizosphere can be retarded significantly when Casparian bands are present. Thus a high apoplastic ABA concentration can build up in the cortex. Redistribution to the symplast (Fig. 1G) can enforce the hydraulic conductance of roots (Hose *et al.*, 2000). So, under

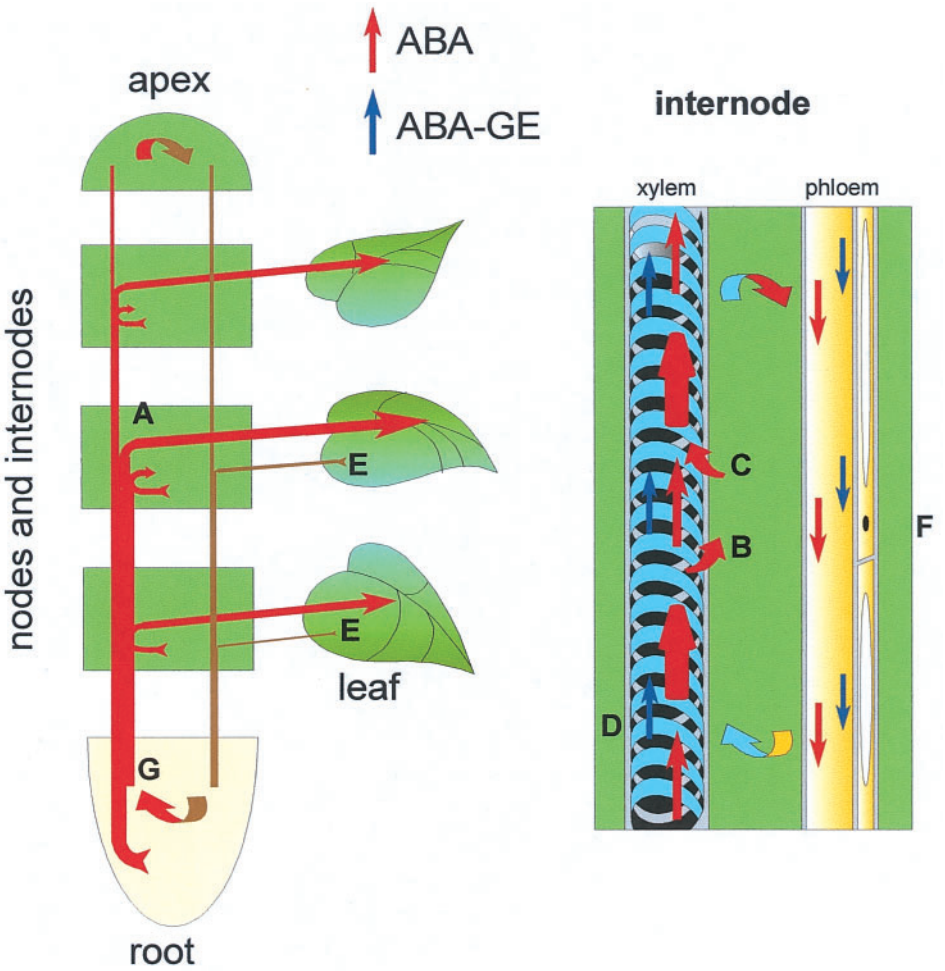

Fig. 2. Schematic presentation of the transport, recirculation and redistribution of ABA and ABA-GE in plant stems. The arrows indicate the flows of free ABA (red) and ABA-GE (blue). Transport of ABA in the phloem from the leaves to the roots is brown. The width of the arrows symbolize the intensity of the flows of free and conjugated ABA. The lettering refers to the text (modified after Jeschke *et al.*, 1997*a*).

transpiring conditions more ABA can be dragged with the enhanced water flow directly into the xylem (Fig. 1F, H).

The velocity of ABA loss to the rhizosphere depends strongly on the permeability coefficients of root cortex membranes for ABA. Root membranes of maize and runner bean exhibit the lowest permeability coefficients for ABA known (Table 2). ABA loss directly from the symplast to the apoplast is up to 4000 times lower than from mesophyll cells, phloem elements and stem parenchyma cells.

The contribution of conjugated ABA to ABA_{xyl}

ABA_{xyl} may also originate from ABA-glucose ester (ABA-GE) taken up by the roots into the cortex apoplast. Sauter and Hartung have shown that, besides free ABA, ABA-GE can also be present in the soil solution, often at higher concentrations than free ABA (Fig. 1I) (Sauter and Hartung, 2000). Both the exodermis and the endodermis are perfect barriers for ABA-GE. Sauter *et al.* concluded from a series of transport experiments with ABA-GE that the $\sigma_{ABA\text{-}GE} = 1$ (Sauter *et al.*, 2002). When Casparian bands are not formed in the hypodermis (hydroponic culture, root systems of legumes, seminal root of barley) external ABA-GE can be dragged with the water into the root cortex. β-D-glucosidases have been shown to be present in the root cortex of maize. They can release free ABA from the conjugate which again can be dragged directly to the xylem (Fig. 1K).

Abscisic acid: where does it go? The significance of the stem

Very little is known about the fate of ABA during its transport in the xylem through the stem. Studies of Jeschke and co-workers (Jeschke and Hartung, 2000) have shown that ABA_{xyl} becomes significantly lower when ABA transport is diverted to leaves (Fig. 2A). Sauter and Hartung perfused bean internodes with ABA and ABA-GE in the concentrations that occur in the

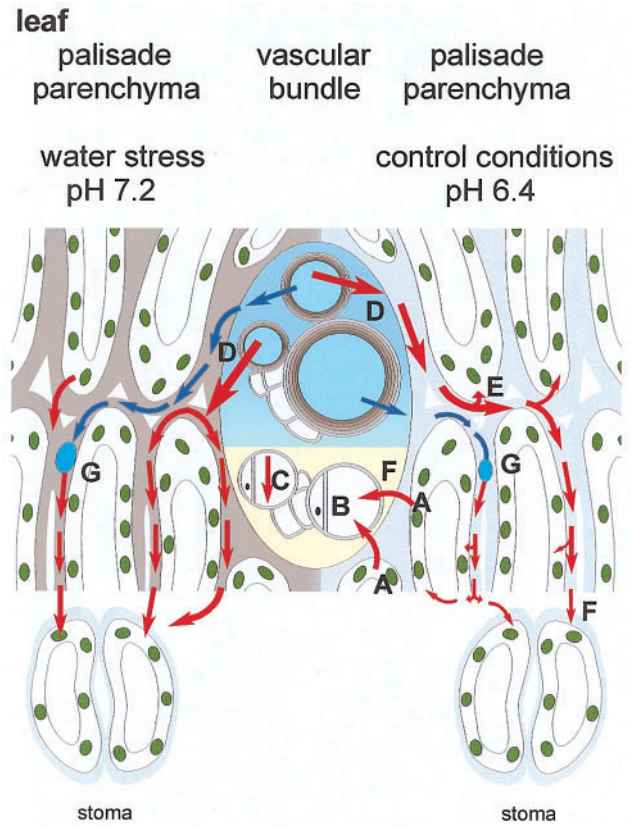

Fig. 3. Schematic presentation of the origin and the transport of ABA and ABA-GE in plant leaves. The arrows indicate the flows of free ABA (red) and ABA-GE (blue). The width of the arrows symbolize the intensity of the flows of free and conjugated ABA. The lettering refers to the text. Extracellular enzyme activity is coloured light green. Cell walls are grey, chloroplasts in the cytosol are green. In the leaf a schematic, cross-sectioned vascular bundle, parenchymatic tissue and a stoma are presented.

Table 1. *Apparent reflection coefficent of abscisic acid (σ_{ABA}) for young root systems of maize and sunflower*

Water flow was induced by application of a pressure difference of 0.06 MPa to the cut surface of decapitated plants. (For further explanation see Freundl *et al.*, 1998.)

ABA concentration of the nutrient solution	pH of the nutrient solution	Apparent reflection coefficient σ_{ABA}	
		Zea mays L. ($n = 6$)	*Helianthus annuus* L. ($n = 6$)
100 nM	4.8	0.26 ± 0.07	0.73 ± 0.05
100 nM	5.5	0.54 ± 0.04	0.97 ± 0.02
100 nM	8.0	0.84 ± 0.05	0.96 ± 0.01

xylem under natural conditions (Sauter and Hartung, 2002). They demonstrated that free ABA can be fed into the xylem from stem parenchyma when ABA_{xyl} is low (Fig. 2B), when ABA in the stem parenchyma is high and when pH_{xyl} increases (as shown by Wilkinson and Davies, 1997, for stressed plants). On the other hand ABA_{xyl} can also be redistributed to the stem parenchyma,

Table 2. *Permeability coefficient of ABAH for the plasmalemma of different cell types; the coefficients were determined by efflux compartmental analyses*

The calculation of P_S^{ABA} of stem parenchyma plasma membranes is based on the following assumptions: internodes are perfused with ABA free buffer (pH 5.8), ABA flow into the xylem: 1.1 pmol m^{-2} s^{-1}, cytosolic ABAH concentration: 0.89 µmol m^{-3}, pH_{cyt} 7.2.

	Permeability coefficient (P_S^{ABA}) of the plasmalemma (10^{-9} ms^{-1})
Guard cell[a]	12.9
Mesophyll cell[b]	3.30
Root cortical cell	0.26[c]
	0.78[d]
	0.50[e]
	0.70[f]
	2.17[g]
Sieve tubes	90[h]
Stem parenchyma	1200[i]

[a] *Valerianella locusta*; Baier *et al.*, 1988.
[b] *Valerianella locusta*; Daeter and Hartung, 1990.
[c,d] *Zea mays*; Jovanovic *et al.*, 1992.
[e] *Phaseolus vulgaris*; Jovanovic *et al.*, 1992.
[f] *Zea mays*; Gratzer and Hartung, unpublished data.
[g] *Phaseolus coccineus*; Gratzer and Hartung, unpublished data.
[h] *Plantago maior*, Baier and Hartung, 1991.
[i] Sauter and Hartung, unpublished data.

especially when ABA_{xyl} is high (Fig. 2C). Perfusion of an ABA free buffer through stems resulted in an ABA flow of 1.1 pmol m^{-2} s^{-1}. This flow rate can be used to estimate the permeability coefficient of the stem parenchyma plasma membranes for undisocciated ABAH (assumptions that are necessary to perform this calculation are given in the legend of Table 2). It was found to be the highest P_{ABAH} of all cell types, indicating that ABA redistribution between stem parenchyma and the xylem is the fastest within an intact plant, contributing significantly to ABA homeostasis in the xylem.

Besides free ABA, conjugated ABA (predominantly ABA-GE) is also transported in the xylem. Because of its hydrophilic properties this molecule is, different from free ABA, transported without any losses or enrichments through the stem (Fig. 2D). The significance of ABA-GE as a stress signal is discussed in detail by Sauter *et al.* (Sauter *et al.*, 2002).

Abscisic acid: where does it go? The significance of the leaves

Biosynthesis of ABA in plant leaves is increased only when leaf turgor approaches zero. Stomatal closure, however, occurs as the soil starts drying when Ψ_{leaf} is still unaffected. It is therefore suggested that ABA import via the xylem is necessary to regulate leaf conductance under conditions of mild stress (Fig. 3D).

ABA_{xyl} imported into the leaf tissue does not always accumulate there. ABA will be degraded rapidly after

having acted on the stomata, especially under phosphate deficiency. Consequently, ABA is not deposited in the leaves despite an increased import of ABA via the xylem.

After its arrival in the leaf apoplast ABA may be redistributed to the leaf tissues (Figs 2E, 3E), in particular to alkaline compartments according to the anion trap concept (Slovik *et al*., 1995). The velocity of the redistribution again depends strongly on the permeability coefficients of the plasma membranes of the different cell types. It is highest in the case of the plasma membranes of the sieve tubes and the guard cells (Fig. 3F) (Table 2). Epidermal cells are also effective cells for ABA deposition, first because in many leaves they occupy a large percentage of the leaf volume and, second, because ABA transporters support ABA accumulation there (Daeter and Hartung, 1995; Dietz and Hartung, 1996).

Wilkinson *et al*. observed an alkalization of the xylem sap in plants growing in drying soil (Wilkinson *et al*., 1998). An alkalization of less than 1 pH unit is enough to close stomata significantly without extra ABA (Wilkinson and Davies, 1997). The authors have shown that under these conditions ABA redistribution to the leaf tissues is strongly reduced. Thus the intensity of the ABA signal is not weakened during its transport in the leaf apoplast to the guard cells.

ABA glucose ester in the leaf apoplast

Sauter *et al*. have pointed out that ABA-GE can only play a role as a hormonal stress signal when ABA is released from the extremely hydrophilic and physiologically inactive conjugate (Sauter *et al*., 2002). The different ratios of free ABA/conjugated ABA in xylem sap (4–7) and apoplastic washing fluids from barley leaves (22–32) permit the conclusion that fluids of the leaf apoplast contain glucosidases that may release the free hormone from the conjugate (Fig. 3G). Their activity increased 7-fold when plants were salt-stressed (Sauter *et al*., 2002), contributing to an increase of the concentration of free ABA in stressed barley leaves. Holbrook *et al*. performed experiments with grafted plants constructed from the ABA-deficient tomato mutants *sitiens* and *flacca* and their near isogenic wild-type parent (Holbrook *et al*., 2002). They concluded that stomata of stressed plants respond to ABA that is made *in situ* rather than transported from the root to the leaves. ABA-GE is a good candidate to act as a root-to-shoot signal that releases free ABA to the target cells *in situ* (Sauter *et al*., 2002).

Conclusion

Abscisic acid fluxes in the xylem can be regulated by several factors on their way from the roots to the target cells in the shoot. These factors include anatomical, physiological and biochemical features in the tissues of roots, stems and leaves. More research is required about the link between a change of soil conditions and the generation of the ABA signal, the putative transporters that may release ABA-glucose ester from the xylem parenchyma to the xylem vessels and the apoplastic glucosidases that liberate ABA from its conjugate at the site of action.

Acknowledgements

We are grateful to Deutsche Forschungsgemeinschaft [Ha 963/11–1; Graduiertenkolleg, SFB 251; (WH)] for generous financial support. The expert technical assistance of B Dierich (Lehrstuhl Botanik I, Universität Würzburg) and of Burkhard Stumpf (Lehrstuhl für Pflanzenökologie) Universität Bayreuth, is gratefully acknowledged.

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