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The pH gradients in the root system and the abscisic acid concentration in xylem and apoplastic saps

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SUMMARY

Abscisic acid (ABA) is a stress signal that is transported from the root system to leaves, and induces stomatal closure before water relations of the leaves are affected by soil drying. Xylem vessels are in direct contact with the leaf apoplasm, the only leaf compartment that is directly connected with the primary site of ABA action, the outer surface of the guard cell plasma membrane (Hartung 1983). ABA distributes among the leaf compartments according to the anion trap concept and the Henderson–Hasselbalch equation, with the free acid as the permeating and the anion as the nearly non-permeating molecular species. Applying this concept, a flattening of the intracellular pH gradients increases the apoplastic ABA concentration. Indeed, stress increases the apoplastic pH (Hartung *et al.* 1988) and decreases slightly the cytosolic pH. The validity of this concept has been shown repeatedly and was confirmed by a mathematical leaf model (Slovik *et al.* 1992).

It is appropriate to ask whether these mechanisms also contribute to ABA compartmentation and redistribution in the root system. Therefore, we have incorporated compartmental pH values of unstressed and stressed root cells, the permeability coefficients of root membranes for ABA and anatomical data into a mathematical model, similar to that of Slovik *et al.* (1992). The simulation shows that ABA redistribution in roots caused by changing pH gradients can account for up to a 2 to 3-fold accumulation of ABA in the xylem sap of stressed plants.

The model also predicts that the pH gradient across the cortical plasma membrane has the most distinct effects on redistribution of ABA into the xylem sap of stressed plants and, additionally, that the ABA concentration in the rhizospheric aqueous solution can play an important role in root-to-shoot signalling.

1. INTRODUCTION

Plant hormones that are synthesized in the root system can be released to the xylem vessels to be transported to the shoot where physiological processes may be influenced. A classic example is the synthesis in roots of cytokinins that are needed in leaf mesophyll cells to retard senescence. As early as 1964, Phillips pointed out that in flooded sunflowers, root-synthesized gibberellins and auxins serve as hormonal signals to influence growth and movement of leaves.

Important work on root-derived hormonal signals in drought stressed plants has been done by Lachno & Baker (1986) and by Davies and colleagues who showed that abscisic acid plays an important role in root-to-shoot communication of mildly stressed plants by affecting leaf conductance and elongation (Zhang & Davies 1990*a*,*b*, see also Gowing *et al.*, this volume).

Besides ABA, there are other plant growth regulators that may also act as root stress signals. Fußeder *et al.* (1992) have shown that in desert-grown *Prunus dulcis* plants, cytokinins, particularly those of the zeatin type, are transported to the leaves where they may modify the sensitivity of guard cells to ABA (Hartung & Heilmeier 1993). In the case of the annual desert plant, *Anastatica hierochuntica*, stomatal reactions and shoot development are not exclusively controlled by ABA. Depending on the available soil water, gibberellins and, to a lesser extent, auxins also seem to influence the rosette-type morphology of the plant (Hartung *et al.* 1990*a*).

As far as the roots are concerned, the exact sources and mechanisms that control the concentration of plant growth regulators in the xylem sap are obscure. It is well known, however, that stress-dependent ABA redistribution in the leaf is caused by flattening of the intracellular pH gradients. In this contribution we will consider whether similar mechanisms may cause hormone release from root tissues into xylem elements.

2. THE PHYSICO-CHEMICAL PROPERTIES OF PLANT GROWTH REGULATORS AND THE CONSEQUENCES FOR THEIR INTRACELLULAR DISTRIBUTION

Abscisic acid is a weak acid with a pK_a of 4.8. Depending on the proton concentration, ABA exists in two forms, the lipophilic free acid and the lipophobic

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anion. Whereas the free acid permeates biomembranes relatively easily, ABA^- is almost completely non-permeant (Heilmann *et al.* 1980; Kaiser & Hartung 1981). These physico-chemical properties result in an intracellular ABA distribution according to the anion trap concept, leading to high ABA concentrations in alkaline compartments, e.g. the cytosol and the stroma of illuminated chloroplasts, and ABA depletion in compartments of low pH such as the vacuole and apoplastic compartments (Hartung *et al.* 1982). Consequently, alterations of the pH gradients also cause changes in the intracellular distribution of ABA.

This distribution pattern does not apply to all plant growth regulators that are weak acids. In the case of auxin (IAA) and gibberellic acid (GA_3), the anions are also able to permeate biomembranes and so they do not accumulate in alkaline compartments because the negative membrane potential causes efflux of the permeant anions (Hartung & Slovik 1991).

3. ABA REDISTRIBUTION IN LEAVES UNDER STRESS CONDITIONS

As a result of the properties described above, ABA distributes among leaf cell compartments predominantly according to the anion trap concept. Therefore, environmental influences that alter intracellular pH gradients are also able to change the ABA distribution pattern. This was shown to be true for drought stress that increases apoplastic pH (Hartung *et al.* 1988) and slightly decreases cytoplasmic pH resulting in flatter intracellular pH gradients (Daeter & Hartung 1990). Consequently, the apoplastic ABA concentration in drought stressed leaves rises rapidly by redistribution before the bulk leaf ABA content rises (Cornish & Zeevaart 1985*a*). Thus, anion trapping of ABA combined with stress-dependent pH alteration provides a mechanism to rapidly increase the ABA concentration of the apoplasm surrounding guard cells (Slovik & Hartung 1992*b*).

4. ABA TRANSPORT AND REDISTRIBUTION IN ROOTS

(*a*) *Anion trapping in roots and the action of carriers*

Compared with the situation in leaves, we have very little information on the ABA redistribution mechanisms which operate in roots. ABA transport across root membranes seems to occur mainly by diffusion (Hartung & Dierich 1983), except at the extreme root tip where ABA carriers have been detected (Astle & Rubery 1980). However, it is doubtful if these carriers play an important physiological role, because their pH optimum is extremely low (pH 3–4) and their kinetic parameters do not fit to endogenous ABA concentrations (Fleming *et al.* 1991).

(*b*) *The permeability of root membranes to ABA*

In all root zones other than the extreme tip, ABA seems to permeate both the plasma membrane and tonoplast by diffusion. The pH-dependence of ABA transport in these tissues strongly resembles that in green leaf tissue indicating that the permeability of the root membranes to ABA^- is also very low.

Stress-induced ABA biosynthesis in roots requires substantial water loss (Cornish & Zeevaart 1985*b*, Carandang, cited in Hartung & Davies 1993) but ABA is released to the xylem even when the water deficit is small. Thus, we have to postulate rapid and sensitive mechanisms to redistribute ABA into the xylem vessels under stress and in the absence of increased biosynthesis. Using the method of efflux compartmental analysis (described in detail by Behl & Hartung (1986)), Jovanović *et al.* (1992) have determined the ABA permeability coefficients (*Ps*) of maize and bean root cortical membranes. The results are summarized in table 1. For comparison, the permeability coefficients of some other plant membranes are also listed.

Obviously, the permeability of root cortical membranes to ABA is very low compared to that for other plant membranes. The permeability coefficient of the plasma membrane is lower by approximately one order of magnitude than that of the mesophyll cell plasma membrane. However, things might be different in the cells that release ABA directly to the xylem vessels, the xylem parenchyma cells in the stele. In these cells, a higher permeability coefficient of the plasma membrane for ABA may be necessary to enable a rapid ABA efflux to the xylem elements. Performing efflux compartmental analyses with isolated stelar tissue of maize seedlings, Lj. Jovanović *et al.* (unpublished data) obtained preliminary evidence for this but they could not determine the precise *Ps* value of the xylem parenchyma plasma membrane for ABA because there are different cell types present in the stele. However, the rate constants of ABA fluxes at the membranes of the fast- and the slow-exchanging compartment of stelar tissue (e.g. fluxes at the plasma membrane and tonoplast) were higher than at root cortical membranes but similar to those of mesophyll cells (Daeter & Hartung 1990). It was concluded that the overall permeability of stelar membranes to ABA is higher than that of cortical membranes and thus could be comparable to that of leaf cell membranes.

(*c*) *The role of changing proton concentration*

(i) *In the soil*

Under conditions where the soil water is slightly acidic, the uptake of rhizospheric ABA into the root system would be favoured. It is well known that roots of many plant species may acidify their rhizosphere, especially under conditions of nutrient deficiency (Hedley *et al.* 1983). Accordingly, this should result in an increased ABA uptake into the root system. However, some soils, e.g. desert soils with high salt concentrations usually have very alkaline pH values (Hartung *et al.* 1990*a*) and, under these conditions, the anion trap concept predicts a release of ABA into the rhizosphere.

(ii) *In the cortex tissue*

To increase the cytoplasmic ABA concentration of

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Table 1. *The permeability coefficients (*Ps*) of plant membranes for ABA*

(The data for root cortical cells were determined by performing a series of efflux compartmental analyses with root cortical tissue of maize and bean seedlings. *Ps* values of the other cell types are from the literature.)

	permeability coefficient [10^{-9} m s^{-1}]	
cell type	plasma membrane	tonoplast
root cortical cell	2.6[a]	1.4
	7.8[b]	3.0
	5.0[c]	1.5
mesophyll cell[d]	30	5
guard cell[e]	222	13
epidermal cell[f]	70	—
sieve tube[g]	90	—

[a] *Zea mays*, line F2.
[b] *Zea mays*, line Polj 17.
[c] *Phaseolus vulgaris*.
[d] *Valerianella locusta*; Daeter & Hartung (1990).
[e] *Valerianella locusta*; Baier *et al.* (1990).
[f] *Hordeum vulgare*; Daeter & Hartung (1993).
[g] *Plantago major*; Baier & Hartung (1991).

cortical cells under stress conditions, the pH gradients between vacuole, apoplasm and cytoplasm should become steeper. This could be achieved by alkalinizing the cytoplasm or acidifying one or both of the adjacent compartments. Then, ABA might be transported symplastically via the passage cells of the endodermis to the stele. Having arrived there, ABA should be released easily from the xylem parenchyma cells into the xylem vessels.

A prerequisite for this putative pathway of ABA transport to the xylem vessels is the demonstrated (table 1) relatively low permeability of the cortical plasma membrane that minimizes ABA leaching to the rhizosphere.

(*d*) *The role of drought, osmotic and salt stress*

Spickett *et al.* (1992) have performed ^{31}P-NMR studies with intact maize root tips. Under osmotic stress, caused by PEG or mannitol, the cytosolic and vacuolar pH values are increased by approximately 0.2 units. Under salt stress (300 mM NaCl), however, the vacuolar pH is increased more distinctly by at least 0.6 units (Spickett *et al.* 1993). In some preliminary ^{31}P-NMR studies we could confirm these findings (table 2), but in contrast to the experiments of Spickett *et al.* (1992), bean roots were allowed to lose water by drying in air. This treatment should mimic the situation in drying soils more realistically than it does the treatment with osmotica.

(*e*) *The role of the nutritional state*

When plants are grown with ammonium as the only or predominant nitrogen source, intracellular pH gradients may be affected significantly. This was shown for unicellular algae (Müller *et al.* 1990) and also for maize root tips, in which cytosolic and vacuolar pH is increased (Fox *et al.* 1992). The pH gradient becomes even steeper by ammonium-induced acidification of the rhizosphere (Marschner & Römheld 1983). In contrast, nitrate nutrition alkalinizes the rhizosphere (Marschner & Römheld 1983). This results in flatter intracellular pH gradients and ABA should leach from the cortex cytosol via the apoplasm to the surrounding soil.

Table 2. *The pH values of root cell compartments and the xylem sap under various experimental conditions*

	pH			
species and treatment	cytoplasm	vacuole	rhizosphere	xylem sap
Zea mays				
unstressed	7.40[a]	5.05[a]		
0.5 M mannitol	7.55[a]	5.35[a]		
0.43 M PEG-300	7.50[a]	5.15[a]		
300 mM NaCl	7.60[a]	5.70[a]		
NO_3^--nutrition			7.5[b]	
NH_4^+-nutrition			4.0[b]	
Phaseolus coccineus				
NO_3^--nutrition	7.27[c]	5.74[c]		6.8[d]
NO_3^--nutrition and dried in air to 14% waterloss	7.48[c]	5.94[c]		
NH_4^+-nutrition				6.1[d]
Phaseolus vulgaris				
1.5 mM NO_3^-	7.3[e]	6.3[e]		
Hordeum vulgare				
10 mM NO_3^-	7.24[f]	5.09[f]		

[a] ^{31}P-NMR studies; Spickett *et al.* (1992, 1993).
[b] Marschner & Römheld (1983).
[c] Preliminary results of ^{31}P-NMR studies; W. Daeter, J. Syha & W. Hartung (unpublished data).
[d] Kruse, cited by Hartung & Radin (1989).
[e] Efflux compartmental analyses; W. Daeter & W. Hartung (unpublished data).
[f] Measured with microelectrode; Miller & Smith (1992).

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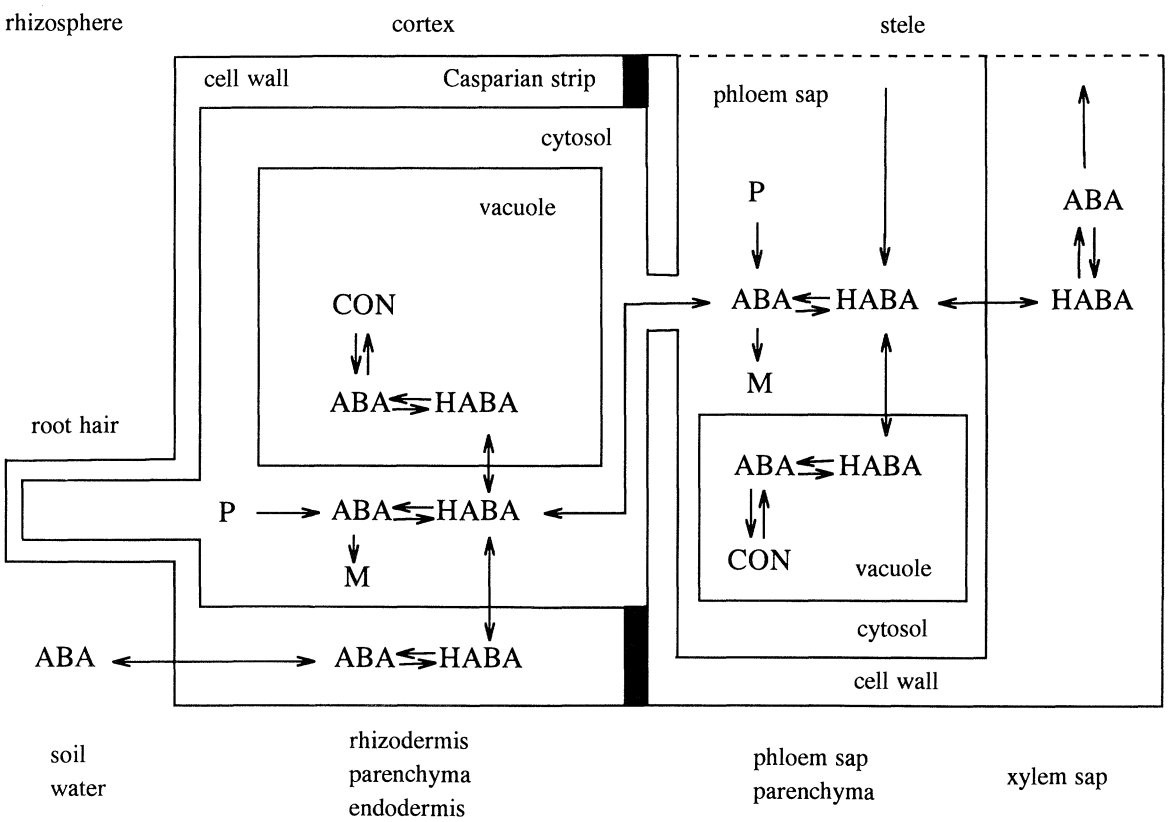

Figure 1. Overview of compartments and metabolic pathways considered by model analysis. P = ABA precursor; M = metabolites of ABA degradation; CON = ABA conjugates.

In NH_4^+-treated root systems the xylem sap pH is decreased by approximately 0.5 units (table 2). Thus, according to the anion trap concept, ammonium treatment should result in ABA accumulation in the root tissues rather than in ABA release to the xylem sap.

When exposed to conditions of iron or phosphate deficiency, some plants can acidify the rhizosphere (Marschner *et al.* 1982; Hedley *et al.* 1983). Under these conditions, ABA distribution in roots should be affected, too.

(*f*) *The role of anoxia*

When root systems of many plants are flooded, anoxic conditions can result in many important physiological changes. From many ^{31}P-NMR studies we know that anaerobiosis acidifies the cytosol of root cells (Roberts 1984). Armstrong (1993) investigated the lateral O_2 gradients in maize roots that were embedded in agar. Anoxic conditions could be observed only in stelar tissues where we consequently have to expect slightly reduced cytosolic pH values. In severely anoxic roots, the cortical tissues also suffer from O_2 depletion and should therefore exhibit lower cytosolic pH values leading to ABA leaching to the rhizosphere. Thus, no significant ABA export from the root system into the xylem sap is to be expected under these conditions. This agrees well with findings of Jackson (1991) who concluded that the increase in xylem sap ABA in shoots of flooded tomato plants does not originate from the root system but from a phloem to xylem transfer in the stem.

5. ABA RELEASE FROM THE STEM PARENCHYMA AND PHLOEM INTO THE XYLEM SAP

We know nothing about stress-dependent changes of pH gradients in the stem, but for green stem tissues we assume similar mechanisms to those occuring in leaves: a stress-dependent flattening of pH gradients causing ABA flux from the parenchyma cells into the xylem vessels that are in direct contact with the parenchyma apoplasm. From experiments with salt-stressed *Lupinus* plants, Wolf *et al.* (1990) concluded that such an ABA transfer does occur in the stem.

6. INCORPORATION OF AVAILABLE DATA INTO A MATHEMATICAL ROOT MODEL

As described in detail for leaves (Hartung *et al.* 1990*b*; Slovik *et al.* 1992; Slovik & Hartung 1992*a*,*b*), we have established a mathematical root model that simulates the redistribution of ABA from root tissues into the xylem elements depending on changes of either pH gradients or transpiration rates.

(*a*) *Description of the root model*

(i) *Tissues and compartments included*

Our model considers entire root systems consisting of cortical cells and cells of the central stele (figure 1). We do not distinguish between rhizodermal cells, cortical parenchyma cells and endodermal cells within the cortex which is assumed to be composed of homogeneous apoplasm, cytosolic and vacuolar com-

partments. Rhizodermal cells are taken into consideration by increasing the plasma membrane surface area of this cortical parenchyma cell layer. The central stele (with apoplasm, cytosol and vacuole) consisting of pericycle cells, sieve elements, phloem parenchyma cells and xylem vessels is not divided into different cell types. Endodermal cells (cortex) and pericycle cells (central stele) are connected by plasmodesmata. The apoplasm of the stele is composed of the water free space of the stele (wet cell walls, water-filled dead tracheids and sclerenchyma cells) and water in the xylem vessels. Thus, the apoplasm of roots is divided into a cortical water-free space which ends at the endodermal Casparian strip and a separate water-free space within the central stele. The latter exports xylem sap into model leaves (mathematical formulation in Slovik *et al.* (1992)) that are connected to the modelled root system. All tissue and compartment volumes and surface areas of this model root are derived from measurements on roots of greenhouse-grown *Valerianella locusta* L.

(ii) *ABA fluxes at membranes*

We assume that ABA fluxes across root cell membranes occur strictly according to the anion trap concept and the Henderson–Hasselbalch equation, with undissociated ABA (ABAH) as the predominantly permeating molecule. The role of carriers is assumed to be negligible (Fleming *et al.* 1991). Therefore, flux-creating gradients at membranes are determined by the absolute pH at both sides of the membrane, by the pK_a of abscisic acid and by the permeability of the membranes to ABA. The fact that permeability coefficients for ABAH differ between membranes (plasma membrane and tonoplast) and root tissues (cortex and central stele) is taken into account.

(iii) *Compartmentation of ABA metabolism*

There is a net synthesis of ABA in the model root which is necessary to maintain a constant ABA content in the root tissue despite of net ABA export via the xylem sap and leaching of ABA from cortical cells into the rhizosphere. Cortical cells and cells of the central stele are assumed to be equally capable of conjugating or mobilizing ABA.

(iv) *Flux balances of different root tissues*

The water conductances of the stomata and of the epidermal cuticles together with the leaf and root area indices define the transpiration rate and thereby the export rate of dissolved ABA from the stele apoplasm (xylem sap) of the whole root system. Concomitantly, a symplastic mass flow imports dissolved ABA from the shoot via the phloem sap into the cytosol of the central stele. The mass transport of ABA from the rhizosphere into the cortex apoplasm is defined by the actual transpiration rate and the ABA concentration in the soil water. At the same time, there is ABA diffusion from the cortex apoplasm to the rhizosphere (leaching). In the model root, cortical cells and the cells of the central stele are connected by a distinct number of plasmodesmata. They balance the mass

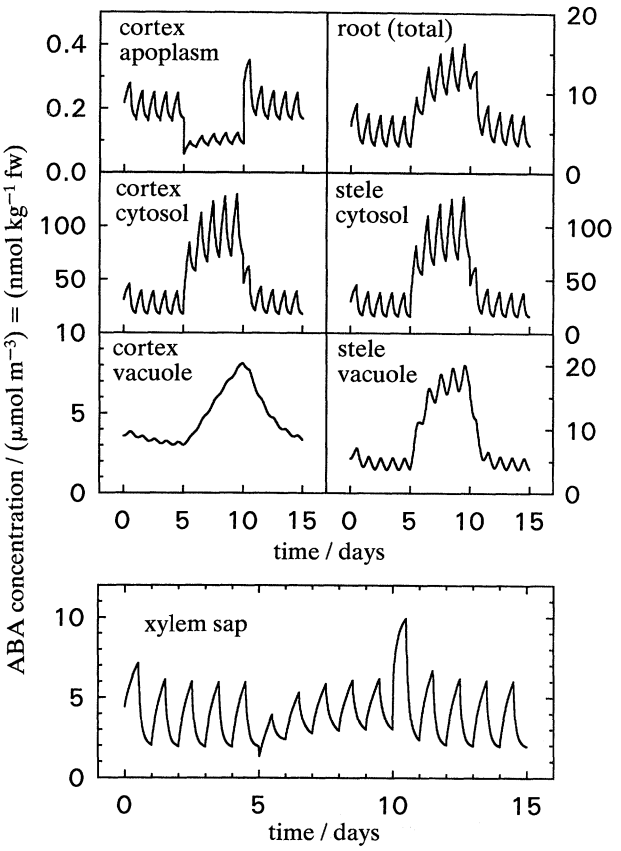

Figure 2. ABA redistribution in the root system caused by ammonium-induced compartmental pH shifts as predicted by the mathematical root model (connected to a model leaf). We assumed a 12 h day – 12 h night cycle and set the ABA concentration in the soil water to 0.1 nM. Day–night conditions were: leaf conductance 0.5–0.0 cm s^{-1}; air temperature 20–10°C; leaf temperature 23–10°C; and the relative air humidity 50–100%. During the first four days the compartmental pH values were set as follows: cortex apoplasm (ca) = 5.50, cortex cytoplasm (cc) = 7.25, cortex vacuole (cv) = 6.30, stele apoplasm (sa) = 6.20, stele cytoplasm (sc) = 7.00, stele vacuole (sv) = 6.25. At day 5 the ammonium treatment was started and caused the compartmental proton concentration to change: $pH_{ca} = 3.50$, $pH_{cc} = 7.75$, $pH_{cv} = 6.90$, $pH_{sa} = 5.75$, $pH_{sc} = 7.30$, $pH_{sv} = 6.37$. At day 10 ammonium was withhold from the root system and the pH values of the start conditions were re-established.

transport and the diffusion of ABA. All other cell types, either within the cortex or the central stele, are supposed to be connected tightly enough by plasmodesmata to form a homogeneous cortical or stelar symplasm, respectively.

It should be noted that we employ a discrete model using a standard description of reequilibration across membranes between two well stirred solutions of finite volume. The mathematical translation of the described physiological network of ABA in roots is derived from the leaf model (Slovik *et al.* 1992) and will be published separately. For an introductory description of the basic equations used in our model we refer to the above work. The aim of this communication is to present the first results of our root model, which has been formulated on the basis of measured experimental data and anatomical observations.

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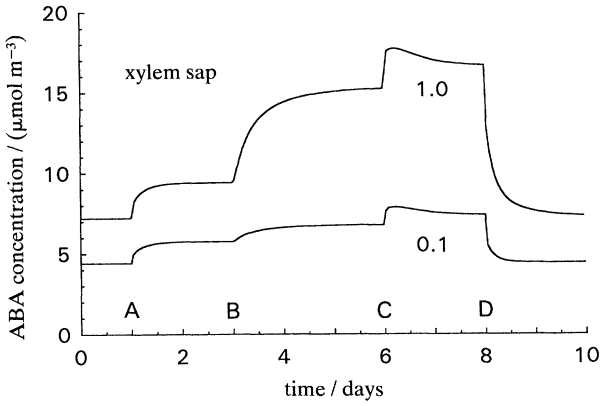

Figure 3. Drought stress-dependent changes of xylem ABA concentration predicted by the mathematical root model. For the calculations we assumed a continuous light period and set the leaf conductance to 0.5 cm s^{-1}, the air temperature to 20°C, the leaf temperature to 23°C and the ABA concentration in the soil to 0.1 or 1.0 nM, respectively. The simulation was started with a relative air humidity of 100% and compartmental pH values which we suppose to occur in unstressed roots: cortex apoplasm (ca) = 5.50, cortex cytoplasm (cc) = 7.25, cortex vacuole (cv) = 6.30, stele apoplasm (sa) = 6.20, stele cytoplasm (sc) = 7.00, stele vacuole (sv) = 6.25. At the indicated points of time the compartmental pH values were changed as follows: A, $pH_{cc} = 7.45$, $pH_{cv} = 6.50$, $pH_{sa} = 6.60$, $pH_{sc} = 7.20$, $pH_{sv} = 6.45$. B, additionally, pH_{ca} was decreased to 4.80. C, only the values of the stele cytoplasm and vacuole were switched back to the unstressed state. D, pH relations of a completely unstressed root system were re-established.

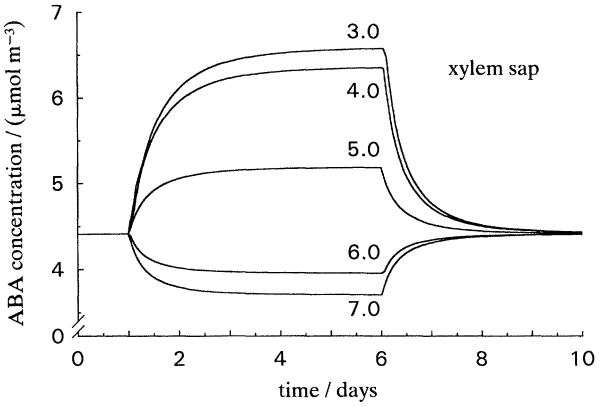

Figure 4. Changes of xylem ABA concentration depending on the rhizosphere pH. For the calculations we assumed a continuous light period and set the leaf conductance to 0.5 cm s^{-1}, the air temperature to 20°C, the leaf temperature to 23°C, the ABA concentration in the soil to 0.1 nM and the relative air humidity to 100%. The simulation was started with compartmental pH values which we suppose to occur in unstressed roots: cortex apoplasm = 5.50, cortex cytoplasm = 7.25, cortex vacuole = 6.30, stele apoplasm = 6.20, stele cytoplasm = 7.00, stele vacuole = 6.25. At day 1 the rhizosphere pH, e.g. the pH of the cortex apoplasm, was set to values between 3.00 and 7.00. At day 6 the pH in the rhizosphere was readjusted to pH 5.50.

(*b*) *Application of the mathematical root model on environmental conditions*

(i) *The effect of ammonium-induced pH changes*

In figure 2 the changes of ABA concentrations that follow ammonium-induced pH shifts are presented for six different compartments of the root hair zone. The permeability of the stele plasma membranes to ABA was assumed to be comparable to those of leaf cells for reasons described under § 4*b*.

As postulated by others (see the contribution of Tardieu in this volume) we have to expect diurnal fluctuations of ABA in the xylem sap with the night concentration approximately 3 times higher than the daytime concentration. After 5 days, when ammonium-induced changes of pH gradients occur, the ABA concentrations in the cortical and stelar compartments are significantly increased. In the xylem sap, however, only small alterations of the ABA concentration are predicted. The daytime ABA concentration in the xylem sap is increased by about 50% whereas the night concentration seems to be relatively unaffected. In preliminary experiments we have found a twofold increase in xylem sap ABA of ammonium-treated *Ricinus* root systems and a fivefold increase in ABA content of the root tissues. It must be emphasized that the aqueous solution of the rhizosphere also contains ABA as postulated earlier by Müller *et al.* (1989) and Hartung *et al.* (1990*a*). Here, we assume the rhizospheric ABA concentration to be 0.1 nM. Under this condition, ABA leaching into the soil is less severe than in the case of a totally ABA-free rhizospheric solution.

(ii) *The effect of drought stress-dependent pH changes on ABA redistribution in root tissues*

In figure 3, changes in the ABA concentration in the xylem sap are shown as predicted by the model, after drought stress-induced pH alterations. When it is assumed that the aqueous solution of the rhizosphere contains 0.1 nM ABA, only a weak stress-dependent ABA redistribution is predicted. However, if the rhizospheric solution contained 1 nM ABA, stress-dependent ABA redistribution would be much more distinct with a maximum of a 2.5-fold increase in ABA concentration in the xylem sap.

(iii) *The effect of pH changes in the rhizosphere on ABA concentration in the xylem sap*

The model predicts remarkable changes of xylem sap ABA concentration even if only the rhizospheric pH is altered (figure 4). Assuming that no other parameter of the model root is changed, the acidification of the rhizosphere from pH 6.0 to 4.0 increases the xylem sap ABA concentration by 70%.

7. CONCLUSIONS

The most important component of our model seems to be the pH gradient across the root cortex plasma membrane. Alterations of this parameter have the clearest consequences for ABA release into the xylem.

From the preliminary data obtained from the present version of our model we conclude:

1. The pH gradient-dependent ABA redistribution

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under stress accounts for a two- to threefold increase in the xylem sap ABA concentration. A further increase in xylem sap ABA must originate from biosynthesis in roots, recirculation via the phloem from the leaves or from the rhizosphere.

2. Changes of the pH gradient across the plasma membrane of cortical cells have the most distinct consequences for stress-dependent ABA redistribution.
3. ABA in the aqueous solution of the rhizosphere is an important source for that fraction of ABA, which can be redistributed when pH gradients are changed. Analysing the water from compost mixtures, ABA concentrations in the 1 nM range were found. In soils from extreme habitats like deserts, ABA concentrations seem to be significantly higher (Hartung *et al.* 1990*a*).

Additional information is badly needed on the range of ABA concentrations in the rhizosphere. Moreover, the pH values of the compartments of stelar tissue should be followed over a range of changing environmental conditions (if possible by ^{31}P-NMR measurements) and more precise data on the permeability of stelar membranes to ABA are required.

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