The contribution of internode and mesocotyl tissues to root-to-shoot signalling of abscisic acid

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Abstract

The xylem of first internode of runner bean and of previously etiolated maize mesocotyl segments was perfused with media containing abscisic acid (ABA) or abscisic acid glucose ester (ABA-GE) in concentrations as they occur under stress conditions. ABA-GE passed through the internode and mesocotyl segments unchanged. Within 10 min the concentration of ABA-GE	extsubscript{xyl} rose to a level similar to that in the external perfusion medium. By contrast, 30–40 min passed before the concentration of free ABA in the xylem sap [ABA	extsubscript{xyl}] reached the level in the external medium. When ABA-free media were used, ABA was released from the xylem parenchyma to the xylem vessels resulting in an [ABA	extsubscript{xyl}] of 13–23 nM (runner bean internode) or 1–6 nM (maize mesocotyl). The total perimeter and, hence surface area, of the xylem elements was measured microscopically and from these measurements it was estimated that, in both bean internodes and maize hypocotyls, the flux of ABA to the xylem was 1 pmol m	extsuperscript{−2} s	extsuperscript{−1}. The ABA efflux from the stem and mesocotyl parenchyma into the xylem could be increased when the tissues were treated with tetcyclacis, an inhibitor of ABA degradation, but also by changing the pH from its normal value of about pH 5.8 to pH 7.0 and by adding 100 mM NaCl to the perfusion medium. If 100 nM ABA was added to the perfusion medium the above treatments had only small effects on the release of ABA from the tissues into the xylem.

Key words: ABA and ABA glucose ester, Phaseolus coccineus L., xylem sap, Zea mays L.

Introduction

The role of abscisic acid (ABA) as a root-to-shoot stress signal is well established. Root tips appear to measure the soil water potential as the soil is drying (Zhang and Davies, 1987, 1989; Hose et al., 2001) by increasing the biosynthesis of ABA and releasing it into the xylem so that it is transmitted to leaves and shoot meristems. The fate of the ABA signal on its way from the root to the target cells in the shoot has recently been investigated in detail, as far as the lateral transport in the roots (Freundl et al., 1998, 2000) and the transport in the leaves is concerned (Hartung et al., 1998; Daeter and Hartung, 1990).

Very little information is available about the fate of ABA during its long distance xylem transport through stems. Above nodes [ABA	extsubscript{xyl}] tends to become lower since a significant amount of ABA is diverted to the leaves (Jeschke et al., 1997a, b; Jokhan et al., 1999). The experiments reported in this paper were done to see if internode and mesocotyl parenchyma cells modulate the ABA signal during its transmission. A technique for perfusing the xylem of stem segments (Freundl et al., 1998) was used. The fate of ABA and its glucose ester during passage through segments taken from the first bean internode and from maize mesocotyls was observed. The experiments have been performed under some of the conditions that might be expected to occur under stress (i.e. increased accumulation of endogenous ABA, increased salt concentration, alkalinization of the sap perfusing the xylem).

Materials and methods

Plant material and culture

The culture of maize with etiolated mesocotyls has been described previously (Jeschke et al., 1997b). Seeds of maize (Zea mays L. cv. Helix, Kleinwanzlebener Saatzucht AG,

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Abbreviations: ABA, abscisic acid; ABA-GE, abscisic acid glucose ester; \( C_{ABA}^{xyl} \) [ABA	extsubscript{xyl}] = ABA concentration in the xylem; \( C_{ABA-GE}^{xyl} \) [ABA-GE	extsubscript{xyl}], ABA-GE concentration in the xylem; \( J_V \), volume flow through the segments; \( J_{ABA} \), ABA flow into the xylem.

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Einbeck, Germany) were germinated in sand, irrigated with nutrient solution (1.5 mM KH$_2$PO$_4$, 2.0 mM KNO$_3$, 1.0 mM CaCl$_2$, 1.0 mM MgSO$_4$, 18 μM FeNaEDTA, 8.1 μM H$_3$BO$_3$, and 1.5 μM MnCl$_2$ at a pH of 5.8) for 5 d at 25 °C in the dark. These growing conditions provoked etiolation where seedlings developed 6.0 ± 1.0 cm long mesocotyls after 5 d. Subsequently, plants were supported with a stick and cultivated in the greenhouse with an additional light source (mercury vapour lamp; 200 μmol m$^{-2}$ s$^{-1}$; day/night 16/8 h; 25/17 °C) for 7 d. During that time plants developed three to four leaves. The mesocotyl stopped elongating and turned red due to an anthocyanin deposition.

Seeds of runner bean (Phaseolus coccineus L., Weißer Riese) were germinated on filter paper soaked with 0.5 M CaSO$_4$ for 4 d at 25 °C in the dark. When seedlings had developed primary roots up to 2 cm in length they were transferred to 0.5 l pots filled with sand and watered with the above-mentioned nutrient solution. The plants were also kept in the greenhouse with an additional light source (mercury vapour lamp; 200 μmol m$^{-2}$ s$^{-1}$). At 8 cm root length up to 2 cm in length they were transferred to 0.5 l pots with a large diameter. The total inner vessel surface of bean internodes was much larger than in maize mesocotyls which, on the other hand, had a high percentage of metaxylem vessels smaller than 6 μm diameter without clearly visible and stainable cell wall thickenings were neglected. The radii were used to calculate the total inner surface and the total volume of the xylem vessels. They were assumed to have a cylindrical shape with the length equal to that of the segment. Radii, surfaces and volumes are given in Table 1. In bean internodes the number of xylem vessels was much larger than in maize mesocotyls which, on the other hand, had a high percentage of metaxylem vessels with a large diameter. The total inner vessel surface of bean internodes was five times larger than that of the maize mesocotyl.

The total volume of xylem elements of a bean segment was much larger than in maize mesocotyl. Anatomical parameters Freehand cross-sections of either 20 maize mesocotyls or first bean internodes were stained for 5 min with 0.5% (w/v) toluidine blue. The cellular dimensions were measured under a microscope (Leitz Diaplan, Leitz, Wetzlar, FRG). Tiny vessels filled with sand and watered with the above-mentioned nutrient solution. The plants were also kept in the greenhouse with an additional light source for a further 10 d. They developed first internodes 8.8 ± 1.5 cm long.

Table 1. Numbers and dimensions of xylem vessels of first runner bean internodes and etiolated maize mesocotyls determined microscopically (n = 20)

<table>
<thead>
<tr>
<th></th>
<th>Bean internode</th>
<th>Maize mesocotyl</th>
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<tbody>
<tr>
<td>Number of vessels</td>
<td>205 ± 46</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Number of small vessels</td>
<td>191 ± 40</td>
<td>51 ± 9</td>
</tr>
<tr>
<td>Radius: 6.3–21.3 × 10$^{-6}$ m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of large vessels</td>
<td>14 ± 10</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Radius: 22.5–51.3 × 10$^{-6}$ m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average vessel radius</td>
<td>12.4 ± 1 × 10$^{-6}$ m</td>
<td>14.4 ± 1.3 × 10$^{-6}$ m</td>
</tr>
<tr>
<td>Total inner vessel surface</td>
<td>145.3 ± 50.6 × 10$^{-9}$ m$^2$</td>
<td>29.1 ± 3.1 × 10$^{-3}$ m$^2$</td>
</tr>
<tr>
<td>Total vessel volume</td>
<td>10.4 ± 4.5 × 10$^{-9}$ m$^3$</td>
<td>2.8 ± 0.4 × 10$^{-9}$ m$^3$</td>
</tr>
</tbody>
</table>

Perfusion technique To study the longitudinal ABA and ABA-GE transport bean internodes and maize mesototyls were fixed to a perfusion apparatus (Fig. 1) as described and used for intact root systems (Freundl et al., 1998; Sauter and Hartung, 2000). A sub-atmospheric pressure of −0.02 MPa (bean internodes) and −0.005 MPa (maize mesocotyls) was applied to the xylem of these excised segments. The suction pressure maintained a longitudinal water flow $J_v$ through the internodes and mesocotyls, as occurs in a transpiring plant (Table 2). The upper cut surface of each segment was attached to an acrylic (perspex) block connected with a capillary and vacuum pump. The suction pressure could be regulated with a valve and a manometer. The basal cut surface was left in the nutrient medium. The applied suction pressure caused xylem sap flow into the capillary where it could be collected with a syringe. It should be mentioned that sub-atmospheric pressure was used as reference (zero pressure) throughout this paper. The amount of xylem sap was determined by weighing the harvested xylem sap fractions. The water flow was steady after 10 min. This sap was discarded. Afterwards xylem sap was collected at 10 min intervals. After 40 min the perfusion solution was exchanged for one that was supplemented with either ABA, ABA-GE, tetacyclacis, NaCl or medium with increased pH (suction medium enriched with MOPS-KOH buffer, 10 mM, pH 7) for another 80 min.

Analysis of free ABA and ABA-GE Xylem sap samples were taken up in TBS-buffer (TRIS-HCl: 50 mM TRIS, 150 mM NaCl, 1 mM MgCl$_2$, pH 7.8) without further purification and analysed for free ABA by ELISA as described earlier (Weiler, 1986). When the content of ABA-GE was of interest xylem sap samples were divided into two equal volumes. One part was directly subjected to ELISA; the other part was first hydrolysed with NaOH (1 M) for 1 h in the dark. The samples were then acidified to pH 3.0 with HCl and partitionated three times against an equal volume of ethyl acetate. The combined organic fractions were reduced to dryness and taken up in TBS-buffer. ELISA then determined ABA released from ABA conjugates. Tissue samples were extracted in 80% methanol for 48 h. The methanolic extracts were passed through C$_{18}$ Sep-Pak cartridges. After removing methanol under reduced pressure the aqueous residue was acidified to pH 3.0 (HCl) and partitionated three times against ethyl acetate. The organic fractions were collected and reduced to dryness, taken up in TBS-buffer and also subjected to ELISA.

Results During the passage of ABA-free medium through maize mesocotyls or runner bean first internodes, water flow through both segments ranged between 0.09 and 0.14 10$^{-9}$ m$^3$ s$^{-1}$. ABA was released from the surrounding parenchyma to the xylem elements, resulting in an
average [\(\text{ABA}_{\text{xyl}}\)] of 3.9 ± 1.8 nM in mesocotyls and 15.3 ± 3.4 nM in internodes. Exactly the same amount of free ABA was released to the xylem when the perfusion medium was enriched with 100 nM ABA-glucose ester (ABA-GE) (Fig. 2A, B). The ABA flows into the xylem calculated from \(J_{\text{ABA}}\) and the internal surface of xylem elements were on average around 1 pmol m\(^{-2}\) s\(^{-1}\) in both tissues.

When 100 nM ABA, a concentration that has been measured repeatedly in the xylem sap of stressed plants (Fort \emph{et al}., 1998; Hose \emph{et al}., 2001), was added to the perfusion medium, 30–40 min passed before [\(\text{ABA}_{\text{xyl}}\)] reached the external concentration (Fig. 3A, C). Over the same period, tissue ABA content in bean internodes (47.8 ± 8.6 pmol g\(^{-1}\) FW) increased 3-fold during perfusion compared to the control (16.0 ± 4.5 pmol g\(^{-1}\) FW). When ABA-GE was sucked through the segments harvested xylem sap contained within less than 10 min the same concentration as applied externally (Fig. 3B, D).

To avoid degradation of ABA that may be redistributed to the surrounding tissues, mesocotyl and internodal segments were treated with tetcyclacis, an inhibitor of P450 cytochrome mono-oxygenases and thus of the oxidative ABA breakdown (Zeevaart \emph{et al}., 1988; Daeter and Hartung, 1990). In this case, tissue ABA content (69.8 ± 17.4 pmol g\(^{-1}\) FW) was enhanced 4.4-fold compared to the controls. Additionally, the effects of salt stress, increased pH (7.0) and enhanced water flow were also tested. All these treatments increased ABA flow from the xylem parenchyma cells to the xylem vessels of internodal segments (tetcyclacis: +58%, 100 mM NaCl: +106%, pH 7.0: +146%, \(J_{\text{V}}\) (−0.04 MPa: 0.41 ± 0.01 \(10^{-9}\) m\(^3\) s\(^{-1}\): +175%) (Table 3). A prerequisite for calculating \(J_{\text{ABA}}\) and \(J_{\text{V}}\) was the condition of stable flows of water and ABA. Therefore, \(J_{\text{ABA}}\) and \(J_{\text{V}}\) of mesocotyl segments could not be calculated within 40 min after the start of the suction experiment since water flows were not steady over this time period. In the presence of 100 nM ABA, xylem sap was further enriched with ABA when segments were treated with tetcyclacis (+100 ± 38%). The effect of the other treatments was far less distinct (100 mM NaCl: +32 ± 20%; pH 7.0: +16 ± 20) (Fig. 4).

**Discussion**

During its passage through stems ABA may be diverted at the nodes to the leaves, resulting in a decrease of [\(\text{ABA}_{\text{xyl}}\)], as described previously (Jokhan \emph{et al}., 1999) and demonstrated for castor bean (Jeschke \emph{et al}., 1997a; Jeschke and Hartung, 2000). ABA redistribution also takes place within the internodes. If the xylem sap contains no free ABA, a release of tissue-ABA from the parenchyma could be observed, independent of the

![Diagram](image)

**Fig. 1.** Experimental apparatus to induce a longitudinal water flow through maize mesocotyls or bean internodes. The apical cut surfaces were fixed tightly by a silicon seal to a perspex block connected with a capillary. With the vacuum pump sub-atmospheric pressure could be created, resulting in a water flow through xylem elements similar to that occurring under transpirational conditions.

**Table 2.** Abscisic acid flows (\(J_{\text{ABA}}\)) from the xylem parenchyma to the xylem and volume flow (\(J_{\text{V}}\)) through segments of first runner bean internode and etiolated maize mesocotyls

<table>
<thead>
<tr>
<th></th>
<th>(J_{\text{ABA}}) (10^{-12}) mol m(^{-2}) s(^{-1})</th>
<th>(J_{\text{V}}) (10^{-9}) m(^3) s(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bean</strong></td>
<td>1.04 ± 0.39</td>
<td>0.12 ± 0.03</td>
</tr>
<tr>
<td><strong>Maize</strong></td>
<td>0.85 ± 0.27</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td><strong>ABA-GE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bean</strong></td>
<td>1.28 ± 0.56</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td><strong>Maize</strong></td>
<td>1.06 ± 0.39</td>
<td>0.14 ± 0.05</td>
</tr>
</tbody>
</table>
Fig. 2. [ABA$_{\text{vol}}$] (A, B) and $J_{\text{ABA}}$ (C, D) in 6.0–8.8 cm long segments of runner bean first internodes (A, C) and etiolated maize mesocotyl (B, D). pH of the perfusion medium: 5.8. Data are means ± SD, $n = 6$.

Fig. 3. Time-course of ABA and ABA-GE in the xylem sap after passage through the first internodal segments of runner bean (A, B) and etiolated maize mesocotyl (B, D). The data are expressed as a % of the externally added ABA or ABA-GE and are means ± SD, $n = 5$. 
presence of ABA-GE in the xylem sap. On the other hand, substantial amounts seemed to be loaded into the internode and mesocotyl parenchyma when 100 nM ABA (pH 5.8) was perfused through the segments. A delay of 30–40 min was observed before ABAxyl reached the external ABA concentration; a time-course during which the total volume of the xylem vessels (Table 1) would have been exchanged 35 times in runner bean internodes and 106 times in maize mesocotyls. Perfusion of ABA through the xylem resulted also in a 3-fold enhanced tissue ABA content. Such a loss to the parenchyma did not occur when ABA-GE was transported in the xylem. Because of its hydrophilic character and the extremely low permeability coefficient of membranes for ABA-GE (Baier et al., 1988), the conjugate passed the stem and mesocotyl rapidly and unchanged without being redistributed. Free ABA may then even be fed to the xylem in addition.

The ABA released from the internode and mesocotyl tissues to the xylem can be increased by accumulation of tissue-ABA. In these experiments this accumulation was achieved by a tetcyclacis treatment that inhibits oxidative ABA breakdown. A similar, but smaller, effect could be observed when salt was added to the perfusion medium or the pH of the medium was increased. An increase of the apoplastic pH by more than one unit (pH 5.8 to pH 7.0) has been observed earlier under stress conditions in different plants (Wilkinson and Davies, 1997; Bacon et al., 1998). In agreement with the general principle that ABA-anions are ‘trapped’ in alkaline compartments, ABA efflux to the xylem was stimulated significantly as xylem sap alkalinized.

When the volume flow was raised 3.6-fold in the bean internode by doubling the vacuum to the cut surface, more ABA was dragged from the apoplast of the surrounding tissues to the xylem and probably from the symplast as well. This may indicate that a dilution of [ABAxyl] by an increased JV, as discussed earlier (Else et al., 1994; Freundl et al., 1998), can be compensated partially by ABA originating from stem tissues. It must be noted, however, that xylem sap of unstressed plants will never be completely free of ABA. The flux of stem-ABA to the xylem will be less pronounced in vivo. It was

### Table 3. Abscisic acid flows J_{ABA} from the surrounding parenchyma of first runner bean internodes into the xylem and longitudinal volume flow J_{V} through the segments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>J_{V} [10^{-9} m^{3} s^{-1}]</th>
<th>J_{ABA} [10^{-12} mol m^{-2} s^{-1}]</th>
<th>% Stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, pH 5.8</td>
<td>0.12 ± 0.03</td>
<td>1.03 ± 0.42</td>
<td></td>
</tr>
<tr>
<td>10^{-3} M tetcyclacis, pH 5.8</td>
<td>0.16 ± 0.003</td>
<td>1.66 ± 0.54</td>
<td>58</td>
</tr>
<tr>
<td>100 mM NaCl, pH 5.8</td>
<td>0.16 ± 0.05</td>
<td>2.16 ± 0.36</td>
<td>106</td>
</tr>
<tr>
<td>pH 7.0</td>
<td>0.16 ± 0.003</td>
<td>2.58 ± 0.37</td>
<td>146</td>
</tr>
<tr>
<td>p -0.04, pH 5.8</td>
<td>0.41 ± 0.01</td>
<td>2.89 ± 0.77</td>
<td>175</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of treatments with (A) 100 μM tetcyclacis, (B) 100 mM NaCl and (C) increased pH_fy (7.0) on ABA_{xyl} of 8 cm long segments of first runner bean internodes. The segments were perfused with suction medium (pH 5.8) or with MOPS-KOH buffer-enriched medium (pH 7.0). After 40 min (in the case of tetcyclacis-treated segments after 60 min) ABA was added to the external medium. The data are expressed as a % of the externally added ABA and are means ± SD, n = 5.
clearly reduced when [ABA<sub>xyl</sub>] was increased to 100 nM, a concentration typical for stressed plants. ABA in the xylem sap may still be supplemented by release from the surrounding tissues, but only when ABA degradation was suppressed by the tetcyclacis treatment, which may maintain a steep ABA concentration gradient across the plasma membranes. Salt and increased pH had only small or negligible effects when xylem sap ABA was increased to 100 nM.

In conclusion, the data of this study demonstrate that, dependent on the conditions, parenchyma of internodes and mesocotyls play a substantial role in modulating the intensity of the stress signal in the xylem during long-distance transport. The results also show that, in contrast to the behaviour of free ABA, its glucose ester may pass for long distances in the xylem with its concentration unchanged, and may even allow supplementation of the xylem signal by influx of free ABA from surrounding tissues.

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We would like to thank Professor DT Clarkson (Long Ashton, UK) and Professor E Steudle (Universität Bayreuth) for stimulating discussions. We are grateful to Professor EW Weiler (Universität Bochum, Germany) for the generous supply of immunochemicals and to Dr R Rademacher for providing the technical assistance of B Dierich (Lehrstuh Botanik I, Universität Würzburg, Germany) gratefully acknowledged. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 251, TPA 3).

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Wilkinson S, Davies WJ. 1997. Xylem sap pH increase: a drought signal received at the apoplastic face of the guard cell that involves the suppression of saturable abscisic acid uptake by the epidermal symplast. Plant Physiology 113, 559–573.


Zhang J, Davies WJ. 1989. Abscisic acid produced in dehydrated roots plays a substantial role in modulating the intensity of the stress signal in the xylem during long-distance transport. The results also show that, in contrast to the behaviour of free ABA, its glucose ester may pass for long distances in the xylem with its concentration unchanged, and may even allow supplementation of the xylem signal by influx of free ABA from surrounding tissues.

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