Effect of partial rootzone drying on the concentration of zeatin-type cytokinins in tomato (*Solanum lycopersicum* L.) xylem sap and leaves

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Abstract

Decreased cytokinin (CK) export from roots in drying soil might provide a root-to-shoot signal impacting on shoot physiology. Although several studies show that soil drying decreases the CK concentration of xylem sap collected from the roots, it is not known whether this alters xylem CK concentration ([CK<sub>xyl</sub>]) in the leaves and bulk leaf CK concentration. Tomato (*Solanum lycopersicum* L.) plants were grown with roots split between two soil columns. During experiments, water was applied to both columns (well-watered; WW) or one (partial rootzone drying; PRD) column. Irrigation of WW plants aimed to replace transpirational losses every day, while PRD plants received half this amount. Xylem sap was collected by pressurizing detached leaves using a Scholander pressure chamber, and zeatin-type CKs were immunoassayed using specific antibodies raised against zeatin riboside after separating their different forms (free zeatin, its riboside, and nucleotide) by thin-layer chromatography. PRD decreased the whole plant transpiration rate by 22% and leaf water potential by 0.08 MPa, and increased xylem abscisic acid (ABA) concentration 2.5-fold. Although PRD caused no detectable change in [CK<sub>xyl</sub>], it decreased the CK concentration of fully expanded leaves by 46%. That [CK<sub>xyl</sub>] was maintained and not increased while transpiration decreased suggests that loading of CK into the xylem was also decreased as the soil dried. That leaf CK concentration did not decline proportionally with CK delivery suggests that other mechanisms such as CK metabolism influence leaf CK status of PRD plants. The causes and consequences of decreased shoot CK status are discussed.

Key words: Cytokinins, long-distance signalling, partial rootzone drying, tomato, xylem ABA concentration, zeatin.

Introduction

During soil-drying episodes, roots growing in the dry upper soil layers generate chemical signals, which can alter shoot physiology in the absence of changes in shoot water relations (reviewed in Davies and Zhang, 1991; Dodd, 2005). One way of demonstrating the potency of these root-sourced chemical signals has been to grow plants with roots split between two soil columns (Blackman and Davies, 1985; Gowing et al., 1990; Sobeih et al., 2004). After plant establishment, water is applied to both columns (well-watered control; WW) or one (partial rootzone drying; PRD) column and water is withheld from the other column in the PRD treatment. Such experiments have shown stomatal closure and leaf growth inhibition of PRD
plants even though they maintain a similar leaf water potential ($\psi_{\text{leaf}}$) to control plants. Further evidence that the roots controlled shoot responses in such split-root plants was provided by an experiment showing that excising the roots in drying soil allowed growth recovery and stomatal re-opening of apple (Malus × domestica) seedlings (Gowing et al., 1990). Root excision cannot make more water available to the plant, but it can remove the supply of root-sourced chemical signals.

Much research has focused on identifying these chemical signals, and many reports implicate increases in xylem abscisic acid (ABA) concentration ([ABA$_{\text{xyl}}$]) in the regulation of shoot processes as the soil dries (reviewed in Dodd, 2005). Although decreased cytokinin (CK) delivery to the shoot has long been considered an important root-to-shoot signal of soil drying (Davies et al., 1986), relatively few reports implicate changes in xylem cytokinin concentration ([CK$_{\text{xyl}}$]) or delivery (the product of [CK$_{\text{xyl}}$] and xylem flow rate) in regulating the physiology of plants grown in drying soil (Itai and Vaadia, 1965; Bano et al., 1993; Shashidar et al., 1996; Hansen and Dörfling, 2003). Reasons for this lack of attention include the multiplicity of CK forms (Incoll and Jewer, 1987), the greater volumes of sap needed for CK analysis than ABA analysis (Bano et al., 1993), and that [ABA$_{\text{xyl}}$] can respond more quickly and sensitively to soil drying. Mild soil drying [soil water potential ($\psi_{\text{soil}}$) decreased from −0.3 to −0.6 MPa] of sunflower (Helianthus annuus) plants increased [ABA$_{\text{xyl}}$] 3-fold but did not change the zeatin riboside (ZR) concentration of root-derived xylem sap, but the decrease in the whole plant transpiration rate (presumably mediated by the increased [ABA$_{\text{xyl}}$]) decreased xylem ZR delivery to the shoot. More severe soil drying ($\psi_{\text{soil}}$ of −1.2 MPa) decreased the xylem ZR concentration by 85% (Shashidhar et al., 1996). However, similar experiments in sunflower suggest that CKs can respond sensitively to soil drying. The xylem ZR concentration decreased exponentially as the hypocotyl water potential ($\psi_{\text{h}}$) decreased from −0.1 to −0.3 MPa, while ABA was ascertained not to increase until $\psi_{\text{h}}$ decreased below −0.4 MPa (Hansen and Dörfling, 2003). Thus there remains a need to evaluate the relative sensitivities of xylem ABA and CK concentration to soil drying in other species.

One of the difficulties with demonstrating a role for xylem CKs in influencing shoot physiology is that changes in [CK] in xylem sap emanating from the roots need not necessarily influence [CK] of the leaves. For example, the ramosus (rms) pea (Pisum sativum L.) branching mutants have increased (rms2) and decreased (rms4) xylem [CK] (relative to wild-type plants), yet all lines maintained a similar leaf [CK] (Dodd et al., 2004). Similarly, root hypoxia substantially decreased xylem ZR concentration in bean (Phaseolus vulgaris) and hybrid poplar (Populus trichocarpa × deltoides), yet had no effect on leaf CK concentration (Neuman et al., 1990). The lack of correlation between the changes in [CK$_{\text{xyl}}$] and bulk leaf [CK] indicates a complex interaction between transport and metabolism along the transport pathway (Burkle et al., 2003).

Several studies measured [CK] in sap exiting the root system of plants grown in drying soil without parallel measurements of leaf [CK] (Itai and Vaadia, 1965; Bano et al., 1993; Shashidar et al., 1996; Hansen and Dörfling, 2003). Our objective was to measure [CK] in both leaf tissues and xylem sap collected from the leaves of plants exposed to drying soil, using a species (tomato) in which sufficient sap could be collected from leaves detached and pressurized in a Scholander-type pressure chamber. This sap collection method is commonly used in experiments on root-to-shoot signalling (Sobeih et al., 2004), is suitable for field use (Dodd et al., 1996), and provides a reasonable estimate of the concentration of xylem sap constituents within the transpiration stream (Borel and Simmoneau, 2002). Zeatin (Z, and its derivatives) was chosen for study since it represents the major xylem-transported CK in tomato (Davey and van Staden, 1976). A partial rootzone drying treatment was implemented to minimize leaf water deficit experienced by plants grown in drying soil.

Materials and methods

Plant material and culture

Tomato (Solanum lycopersicum L. cv. Ailsa Craig) plants were raised in a single walk-in controlled environment room (3 m × 4 m) at the Lancaster Environment Centre under a 12 h photoperiod (09.00 h to 21.00 h) and 16–26 °C temperatures. Atmospheric evaporative demand varied between 0.2 and 1.2 kPa. Metal halide lamps (HQI-T 400N, Osram, St Helens, UK) were 1.2 m above bench height and provided 220 mmol m$^{-2}$ s$^{-1}$ photosynthetic photon flux density (PPFD) at bench height. Seeds were sown in a well-watered peat-based compost (Levingtons M3, Levington Horticulture Ltd., Ipswich, UK) in seedling trays, with a single seed in each separate compartment (30 mm deep × 20 mm × 20 mm). After 14 d, when the first true leaf had emerged, seedlings were transferred to ‘net pots’ (Teku™, Poppelmans Plastiques, Poppelmans, France) of 50 mm diameter × 50 mm deep with 5 mm × 7 mm pores in the sides. After 1 week, plants in the ‘net pots’ were transplanted into custom-made split pots comprising two plastic columns (335 mm deep × 70 mm × 70 mm) that were taped together. Where the two individual columns adjoined, part of the plastic wall of the columns (50 mm deep × 50 mm wide) had been removed to allow each ‘net pot’ to be inserted into the potting compost, minimizing seedling disturbance. Both ‘net pots’ and split pots were filled with a commercial potting compost (John Innes No. 2, J Arthur Bowers, Lincoln, UK) and watered daily until each experiment commenced.

Thirty-nine days after germination, two different watering regimes were imposed in four replicated experiments (experiments 1–4). Control (WW) plants received 100% of the previous day’s mean evaportranspiration (determined gravimetrically) applied equally between both soil columns, while PRD plants received 50% of the previous day’s evaportranspiration applied to one soil column. Water was withheld from the other column in the PRD treatment to expose some of the root system to drying soil. Water was replenished either daily (at the beginning of the photoperiod) or twice daily (at 09.00 h and 17.30 h) according to the transpirational needs of
the plant. Treatments were randomly arranged in the controlled environment room.

Whole plant transpiration was determined gravimetrically, and corrected for evaporation from ‘blank’ pots (without a plant). Leaf samples comprising the terminal leaflet of leaf 9 (numbering from the base of the plant) were removed, weighed, then placed in liquid nitrogen for subsequent analysis. Following measurement of \( \psi_{\text{leaf}} \) using a Scholander-type pressure chamber (Plant Moisture Systems, Santa Barbara, CA, USA), an overpressure of 0.4 MPa was applied to leaves for 60–120 s to collect xylem sap. To gain sufficient xylem sap for CK analysis, three leaves per plant (typically leaves 6–8) were sampled from 5–6 replicate plants per treatment between 10.00 h and 15.00 h. Xylem sap was pooled from each leaf number and 400 \( \mu \)l was typically used as a sample for CK analysis. For each day of sap collection, four samples (two leaves per plant x two treatments) were analysed. Following completion of sap collection, the whole shoot was removed just below the cotyledons, and the area of all leaves was recorded with a Li-3100 Area Meter (Li-Cor Inc., Lincoln, NE, USA), allowing transpiration to be calculated on a leaf area basis.

Whole pot gravimetric water content (0) at shoot harvest was estimated after measurement of combined pot and soil weight, and assuming that each pot was of uniform weight and contained a uniform weight of dry soil. Values for the combined weight of pot and dry soil were obtained from a subsample of pots for each experiment, after oven drying the soil at 60 °C until constant mass. \( \psi_{\text{soil}} \) at known gravimetric soil water contents (made by adding known volumes of water to known masses of oven-dry soil in a cup, which was sealed with parafilm and allowed to equilibrate overnight) were measured with a soil psychrometer (Model WP4, Decagon Devices Inc., Pullman, WA, USA). A calibration curve plotting 0 versus \( \psi_{\text{soil}} \) was constructed.

Single leaf gas exchange of six plants per treatment was performed between 10.00 and 12.00 h on leaves 5 and 7 (during experiments 2 and 4, respectively) on days 2 and 4 of the experiments. An infrared gas analyser (CIRAS-2, PP systems, UK) was used, and leaves were allowed to equilibrate at ambient temperature, constant CO2 concentration (37.5 Pa), and PPFD (350 mmol m\(^{-2}\) s\(^{-1}\)) for 3 min before data were recorded. After measurement, the leaf was excised for \( \psi_{\text{leaf}} \) determination and collection of xylem sap as described above. An aliquot of each sap sample was retained (prior to pooling sap for CK analysis) to quantify ABA.

**Hormone analysis**

Sap ABA concentration was measured with a radioimmunoassay (Quarrie et al., 1988), using the monoclonal antibody AFRC MAC 252 (kindly provided by Dr G Butcher, Babraham Bioscience Technologies, Cambridge, UK).

CKs were extracted from leaves by homogenization in 80% ethanol and incubation overnight at 4 °C. After filtration and vacuum evaporation of extracts to remove all traces of ethanol, CKs from the aqueous residue were concentrated on a precoated C18 column (Bond-Elut, RP-C18) and, after washing, the column was loaded with 20 ml of distilled water and then eluted with 5 ml of 80% ethanol. After solvent evaporation, the dry residue of leaf extract and xylem sap was dissolved in 20 ml of 80% ethanol and applied to precoated Merck 50 × 200 × 0.25 mm silica gel 60 F-254 thin-layer chromatography plates which were developed in 2-butanol:14 M NH\(_4\)OH:H\(_2\)O (6:1:2 by vol., upper phase). This protocol successfully separated zeatin nucleotide (ZN; \( R_f \) 0–0.1), ZR (\( R_f \) 0.4–0.5), and Z (\( R_f \) 0.6–0.7). More than 90% recovery was obtained for Z, ZR, and ZN standards using the described elution procedure. CK-containing zones (based on the position of standards) were eluted with 0.1 M phosphate buffer, pH 7.4 for 12 h and added directly to microplate wells in serial dilutions. These were assayed using antibodies raised against ZR having high immunoreactivity towards Z, ZR, and ZN (Vysockskaya et al., 2004). The antibodies showed low cross-reactivity to dihydrozeatin (DHZ) and isopentenyladenine (iP) and their derivatives (8% and 1% cross-reactivity, respectively). Immunoassay reliability was confirmed by dilution tests, chromatographic examination of the distribution of immunoreactivity, and comparison of the results of immunoassay against physicochemical assays [liquid chromatography–mass spectrometry (LC–MS); Veselov et al., 1999].

**Statistics**

Student’s unpaired t-tests were used to determine significant effects between watering regimes on a given measurement occasion. Analysis of variance (ANOVA) was used to detect significant effects of experiment, measurement occasion, and watering regime, and any interactions. Relationships between variables were determined using correlation analysis.

**Results**

Physiological data obtained from replicate experiments were reasonably consistent. For example, analysis of all leaf water potential data from all four experiments showed no significant (\( P > 0.05 \)) effect of experiment (data not shown). For this reason, experiment 1 is shown as an example (Fig. 1). Evapotranspiration of PRD plants grown with daily application of 50% of evapotranspirational losses was reasonably stable, while that of WW plants (grown with 100% of evapotranspirational losses) almost doubled during the experiment (Fig. 1a). Expressed on a leaf area basis, and averaged over all measurement occasions, the transpiration rate of PRD plants declined by 29% in this experiment (Fig. 1b) and by 22% in experiment 3 (data not shown). Similar averaging of the transpiration rate over three measurement occasions (days 2, 4, and 5) for four replicate experiments showed that PRD decreased the transpiration rate by 22%.

The average minimum whole pot soil moisture content experienced by WW plants was 0.37 g g\(^{-1}\) (corresponding to a \( \psi_{\text{soil}} \) of −0.05 MPa), while that of PRD plants was 0.25 g g\(^{-1}\) (corresponding to a \( \psi_{\text{soil}} \) of −0.40 MPa) (Fig. 1b). This decrease in soil water content increased the xylem ABA concentration of PRD plants 2.8-fold (Fig. 1c), while \( \psi_{\text{leaf}} \) declined by 0.1 MPa (Fig. 1d). When data were averaged over the four replicate experiments, PRD increased the xylem ABA concentration by 2.5-fold and decreased \( \psi_{\text{leaf}} \) by 0.08 MPa. After 5 d of PRD, leaf area had declined by 16% (Fig. 3).

Although PRD decreased the whole plant transpiration rate in both experiment 1 (Fig. 1b) and all subsequent experiments (data not shown), no significant changes in single leaf stomatal conductance (\( g_s \)) were detected using infrared gas analysis (Table 1), probably since \( g_s \) was measured during a small proportion of the photoperiod, immediately after irrigation. Correlation analysis determined possible influences on \( g_s \). On day 2, there was no significant correlation between [ABA\(_{\text{xyl}}\)] and \( g_s \) (Fig. 2b), yet higher \( g_s \) was significantly (\( P=0.002 \)) correlated with
lower \( \psi_{\text{leaf}} \) (Fig. 2a). The latter relationship was not detected on day 4 (Fig. 2d), yet higher \( g_s \) was significantly \((P=0.002)\) correlated with lower \([\text{ABA}_{\text{xyl}}]\) (Fig. 2e). On both days, \([\text{ABA}_{\text{xyl}}]\) significantly \((P<0.05)\) increased as \( w_{\text{leaf}} \) declined (Fig. 2c, f).

**Discussion**

Although PRD of tomato caused minimal (no more than 0.1 MPa) changes in \( \psi_{\text{leaf}} \) (Fig. 1d) and induced no change in xylem CK concentration (Fig. 3), the bulk leaf CK concentration of PRD plants decreased by 46% (Fig. 3). Severe soil drying \((\psi_{\text{leaf}} \text{ decreased by } -1.0 \text{ MPa})\) decreased alfalfa \((\text{Medicago sativa})\) leaf CK concentration by \( \sim 40\% \) (Goicoechea et al., 1995). Shoot tip and subtending axillary bud CK concentration of PRD-grown grapevine \((\text{Vitis vinifera})\) decreased by 49% and 26%, respectively, when compared with WW plants, although both treatments showed a similar \( \psi_{\text{leaf}} \) (Stoll et al., 2000). Sunflower plants exposed to PRD also showed no change in \( \psi_{\text{leaf}} \), and xylem sap ZR and dihydrozeatin riboside (DHZR) concentrations from the roots were similar in control and PRD plants (Masia et al., 1994). Taken together, these observations suggest that relatively mild soil drying \((\psi_{\text{leaf}} <0.1 \text{ MPa})\) can exert a major effect on shoot CK status although xylem CK concentration does not change.

Recent genetic research has confirmed classical assumptions from physiological research implicating CKs in a wide variety of processes including not only cell division and expansion, but light responses, chloroplast differentiation and functioning, nutrient metabolism and mobilization, leaf senescence, and sink–source relationships (Mok and Mok, 2001; Higuchi et al., 2004; Kiba et al., 2005). Should a 46% decrease in CK concentration occur throughout the plant, profound changes in plant growth and development are likely. Understanding the physiological

### Table 1. Changes in leaf water potential \((\psi_{\text{leaf}})\), stomatal conductance \((g_s)\), and xylem ABA concentration for well-watered (WW) plants and those subjected to partial rootzone drying (PRD)

<table>
<thead>
<tr>
<th>Day</th>
<th>PRD plants</th>
<th>WW plants</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xylem [ABA] (nM)</td>
<td>( \psi_{\text{leaf}} ) (MPa)</td>
<td>( g_s ) (mmol m(^{-2}) s(^{-1}))</td>
</tr>
<tr>
<td>Day 2</td>
<td>109±20</td>
<td>-0.59±0.03</td>
<td>219±22</td>
</tr>
<tr>
<td></td>
<td>175±11</td>
<td>-0.55±0.03</td>
<td>212±34</td>
</tr>
<tr>
<td>P-values</td>
<td>0.17</td>
<td>0.37</td>
<td>0.86</td>
</tr>
<tr>
<td>Day 4</td>
<td>222±20</td>
<td>-0.58±0.02</td>
<td>118±27</td>
</tr>
<tr>
<td></td>
<td>101±21</td>
<td>-0.54±0.03</td>
<td>157±25</td>
</tr>
<tr>
<td>P-values</td>
<td>0.005</td>
<td>0.081</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Soil drying did not induce significant differences in the proportions of different Z-type CKs in both bulk leaves and xylem sap. In sap collected from detached leaves, Z, ZR, and ZN comprised 27, 32, and 41% of the total Z-type CKs respectively, while in bulk leaves these percentages were 33, 21, and 46%, respectively. The percentage of Z-type CKs as ZR was significantly \((P<0.05)\) greater in xylem sap than in the bulk leaf.

**Fig. 1.** (a) Irrigation (lines only) and evapotranspiration (symbols), (b) transpiration rate (circles) and minimum whole pot soil water content (squares), (c) xylem ABA concentration, and (d) leaf water potential of plants grown with 50% evapotranspiration (filled symbols, dotted lines) and 100% evapotranspiration (open symbols, solid lines) in experiment 1. Data are means ±SE of 5–6 replicate plants (a–c) and means ±SE of 5–6 replicate plants from which \( w_{\text{leaf}} \) was measured in three leaves per plant (d).
The effects of decreased CK status have been aided by the creation of transgenic plants overexpressing cytokinin oxidase (CKX), which show CK concentrations only 30–45% of those of non-transformed plants (Werner et al., 2001, 2003). Such transgenics show retarded shoot development and enhanced root development. Since such developmental changes are also seen in droughted plants, including tomatoes subjected to PRD (Mingo et al., 2004; Sobeih et al., 2004), decreased CK status may be partially responsible for the physiological changes induced by soil drying.

Notwithstanding the physiological implications of decreased bulk leaf CK concentration under PRD, it is important to understand the mechanisms causing this change. Instantaneous leaf CK status will depend on the balance between hormone input (from xylem sap delivery and putative leaf CK synthesis) and output (metabolism and export in the phloem). Assuming that CK delivery to the shoot of PRD-grown plants decreased in proportion to the decrease in transpiration rate (Jackson, 1993), the 22% restriction of the transpiration rate by PRD should have resulted in no more than a 22% decrease in bulk leaf CK concentration. Instead, bulk leaf CK concentration declined by 46%. That leaf CK concentration did not decline proportionally with CK delivery suggests that other mechanisms contribute to regulating leaf CK status of PRD plants, alongside decreased delivery of CKs from roots.

Although the root system has long been considered as the principal source of shoot CKs (Aloni et al., 2005), the

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**Table 2. Statistical analysis of changes in xylem sap and bulk leaf CK concentration for the data reported in Fig. 4**

Two-way analysis of variance was performed to determine significant effects of measurement occasion and watering regime, and their interaction (O×T), with P-values reported.

<table>
<thead>
<tr>
<th>CK concentration (ng ml⁻¹)</th>
<th>CK concentration (ng g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Xylem sap from detached leaves</strong></td>
<td><strong>Bulk leaf samples</strong></td>
</tr>
<tr>
<td>Occasion</td>
<td>Treatment</td>
</tr>
<tr>
<td>[Z]</td>
<td>0.58</td>
</tr>
<tr>
<td>[ZR]</td>
<td>0.14</td>
</tr>
<tr>
<td>[ZN]</td>
<td>0.014</td>
</tr>
<tr>
<td>[Total Z]</td>
<td>0.046</td>
</tr>
</tbody>
</table>

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**Fig. 2. Correlations between single leaf stomatal conductance (gₛ) and ψ_leaf (a, d); gₛ and xylem ABA concentration (b, e); and xylem ABA concentration and ψ_leaf (c, h) on days 2 (a, b, c) and 4 (d, e, f) of irrigation at 50% evapotranspiration (filled symbols) and 100% evapotranspiration (open symbols). Points are paired measurements from leaves in experiments 2 (squares) and 4 (circles). Linear regressions were fitted (where P < 0.05) in SigmaPlot for Windows 2.01.**
expression of ipt genes (for de novo CK synthesis) in the leaves (Takei et al., 2004) suggests that leaves also synthesize CKs. Modelling the xylem and phloem flows of CKs and net metabolic changes of CKs in barley plants also suggested the involvement of leaves in CK biosynthesis (Jiang et al., 2005). However, leaf CK synthesis was probably negligible in leaves in which [CK] was measured since they had ceased to expand (data not shown), while the rate of leaf CK synthesis is high in young developing leaves (Nordstrom et al., 2004). Alternatively, for several reasons, it is suggested that alterations in CK metabolism probably enhanced the decline in leaf CK status. Although CKX activity has not yet been directly assayed in water-stressed plants, transcript abundance of a maize CKX increased in response to both water deficit and ABA treatment (Brugiere et al., 2003). Furthermore, several CKX enzymes have been predicted (based on amino acid sequence analysis, and the construction of CKX green fluorescent fusion proteins) to show extracellular localization (Werner et al., 2003). Since CKX activity has an alkaline pH optimum (pH 6.5–8.5) (Motyka et al., 1996), alkalization of tomato xylem sap and the leaf apoplast following soil drying (Wilkinson et al., 1998; Sobeih et al., 2004) probably enhanced CK metabolism. Possible CK metabolism in xylem parenchyma cells (since CKX transcript abundance is greater in the vasculature; Brugiere et al., 2003) may contribute to the decreased delivery of CKs from roots, and the decreased leaf CK concentration.

Both Z and ZR are the substrates for CKX (Jones and Schreiber, 1997), while ZNs have been reported to be more resistant to the oxidation process catalysed by the enzyme (Laloue and Fox, 1989). In support of this differential sensitivity of different CK species to oxidation, 2 d of PRD slightly decreased the Z concentration in xylem sap of PRD plants (to 88% of control), while the ZN concentration was increased (to 215% of that of WW plants) (Fig. 4a). However, later in the experiments, no differences in the proportions of different Z-type CKs in xylem sap was observed in PRD compared with WW plants, which might be due to increased activity of enzymes that cleave ZN [e.g. 5'-nucleotidase (EC 3.1.3.5); see Chen, 1997] to make it accessible to the CKX.

Whatever the reason for the decreased leaf CK concentration of PRD-grown plants, [CKxyl] was similar in...
PRD and WW plants (Table 2; Fig. 3). It is not clear which ‘measurement’ of the CK concentration ([CKxyl] or bulk leaf CK concentration) is more important for regulating CK-dependent processes. Recent research has revealed the succession of events leading from perception of CK signals to the broad spectrum of CK responses (D’Agostino and Kieber, 1999; Kiba et al., 2005). Since CKs bind to a plasmalemma-located histidine kinase homologue (acting as a CK receptor) of which the input domain is exposed outside the cell in the apoplast (D’Agostino and Kieber, 1999), [CKxyl] may be especially important. That [CKxyl] of PRD plants was maintained at a concentration not lower than WW plants might maintain growth and other CK-dependent processes.

Since exogenous CKs can promote stomatal opening in epidermal strips (reviewed in Dodd, 2003), CKs may influence leaf gas exchange. However, changes in ψleaf and [ABAxyl] are also probably important. The decreased ψleaf of PRD plants (Fig. 1d) raises the issue of whether ψleaf itself regulates stomatal conductance (gs) via a feedback signal whereby a sensitive stomatal response to small changes in ψleaf decreases the transpiration rate thus maintaining a homeostatic ψleaf (Comstock, 2002). It seems unlikely that ψleaf controls gs since a high gs is associated with a low ψleaf (Fig. 2a), indicating that gs is controlling ψleaf and not vice versa. Later in the experiment, this relationship breaks down (Fig. 2d) and instead a high gs is associated with a low [ABAxyl] (Fig. 2e) as seen in many previous studies (Zhang and Davies, 1989; Dodd, 2005), suggesting that [ABAxyl] controls gs. In this context, it may be questioned whether xylem CKs influence gs. Assuming that guard cells detect changes in CK concentration and not delivery, the absence of changes in [CKxyl] during PRD (Table 2; Fig. 3) argues against a role for CKs.

Xylem CK concentration will be influenced by the rate of loading of CKs into the xylem by root and/or stem cells, and will decrease as xylem flow rate increases (as in Dodd et al., 2004). Assuming that these two processes are independent and that CK loading remains constant during the early stages of soil drying, the initial decrease in transpiration rate on day 2 should have increased [CKxyl]. That this may have occurred is suggested by the observation that [CKxyl] of PRD plants on day 2 was 1.4-fold higher than in WW plants (Fig. 4a). The fact that PRD and WW plants maintained a similar [CKxyl] on days 4 and 5 (Fig. 4a; Table 2), despite PRD decreasing the transpiration rate, suggests that xylem loading of CKs has also decreased, indicating that root-to-shoot signalling of CKs during soil drying is regulated by loading from the root system.

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