Chemical root to shoot signaling under drought

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Chemical signals are important for plant adaptation to water stress. As soils become dry, root-sourced signals are transported via the xylem to leaves and result in reduced water loss and decreased leaf growth. The presence of chemical signals in xylem sap is accepted, but the identity of these signals is controversial. Abscisic acid (ABA), pH, cytokinins, a precursor of ethylene, malate and other unidentified factors have all been implicated in root to shoot signaling under drought. This review describes current knowledge of, and advances in, research on chemical signals that are sent from roots under drought. The contribution of these different potential signals is discussed within the context of their role in stress signaling.

Chemical and hydraulic signaling under drought stress

Numerous studies have shown that plant roots can sense changes in abiotic factors such as soil water content [1,2], soil bulk density or compaction [3], soil oxygen content [4] and changes in the nutrient composition of soil (both enrichment and depletion) [5]. Root sensing of water deficit has been widely studied, and this review focuses on the outcomes of the early stage of water-deficit sensing: the transport through the xylem of chemical signals that ultimately reduce leaf transpiration and leaf growth. Chemical signals can be differentiated from hydraulic signals [6] but both are important because they reduce stomatal conductance and leaf growth under conditions of water deficit. Chemical signals most probably dominate during early stages of stress before hydraulic signals are produced [7], and become less important under severe drought when leaf water potential declines and leaves wilt. It is likely that the hydraulic signals that may trigger the production of the hormone abscisic acid (ABA) in leaves dominate as plants become more water stressed [8]. This review focuses on chemical signals because of their importance during the early stages of plant response to water deficit, and because their manipulation has applications in increasing water use efficiency (WUE) in agriculture.

Agricultural practices involving chemical signals: deficit irrigation and partial root-zone drying

Root to shoot signaling under conditions of both mild and severe drought is an important area for research because of its implications for agricultural production and the WUE of plants. Fresh water supplies are predominantly used for agriculture, but this usage will need to be reduced in the future as supplies become limited. Where irrigation can be manipulated, researchers have experimented with, and successfully implemented, both deficit irrigation and partial root-zone drying (PRD) [9,10]. Both of these drying regimes reduce leaf transpiration and limit vegetative growth, thereby increasing WUE, and have demonstrated efficacy in both woody perennials [9] and herbaceous crop plants [11,12] (Box 1). Although these treatments can increase the WUE of certain crop species, knowledge of the role of chemical signals sent from root to shoot during such practices is still developing [13]. Some studies have shown that chemical signals such as ABA are generated by the reductions in soil water content and act on the leaves to reduce transpiration and growth [14].

Where PRD and deficit irrigation have been used in agriculture, the most success has come from applications to tree crops and to vines (in viticulture) [9,10]. With horticultural crops, the management of individual plants is more intensive and economic return is often linked to fruit quality. In one study, the use of PRD or deficit irrigation in viticulture increased WUE by about 40% while only decreasing yield by 15% [10]. PRD improved the quality of the grape berry by increasing the anthocyanin content [10] and the quality of wine produced [1]. In tomato (Lycopersicon esculentum), PRD has been shown to increase crop quality. For example, one PRD study found a 21% increase in the Brix value of fruit (a measurement of the mass ratio of dissolved sugar to water), and a 50% increase in WUE with only a 15% decrease in yield [15]. A physiological explanation for such a small reduction in yield has been proposed on the basis that the fruit receives water and minerals from the xylem early in development, but later, more than 90% of the water supplying the fruit is transported via the phloem [15]. Particularly in arid climates, the use of precision irrigation methods such as PRD and deficit irrigation puts into practice the principles developed from basic scientific studies on how root signals increase WUE while maintaining crop quality and yields.

ABA is a key regulator of leaf stomatal conductance

The production of ABA in roots and its transport to the leaves provides the plant with a mechanism for transmitting a chemical signal to report on the water status of the soil. This system could have evolved specifically for this purpose or the chemical signal could merely be a consequence of the increased production of ABA required to maintain root growth under water deficit [16]. A dominant

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role for ABA in root to shoot signaling under drought and in the control of stomatal conductance was demonstrated in early reports [17]. Several groups have also used a bioassay approach to show that ABA that is fed to leaves decreases transpiration and leaf growth while increasing WUE with only small decreases in yield.

Outstanding questions

Is the source of ABA that results in stomatal closure under drought coming from the roots or the leaves?

How much variation in root to shoot signaling and xylem sap composition is there within a species?

How does the nutrient status of a plant interact with chemical signaling during water stress?

In which region of the root and in which particular root cells is ABA synthesized?

What are the different forms of cytokinins that are transported in xylem sap, and what roles do they play in root to shoot signaling?

What causes changes in pH in sap under drought and why do pH changes appear inconsistent between certain species?

Involvement of pH in signaling

Changes in the pH of xylem sap commonly observed under drought stress can be an important component of root to shoot signaling and may act synergistically with ABA. In many plant species (e.g. sunflower [Helianthus annuus] [34], Phaseolus coccineus [35] and C. communis [36]), xylem sap pH becomes more alkaline when plants are water stressed and this leads to enhanced stomatal closure and even reduced growth. The potential effects of pH have been outlined previously [37] and include: i) changes in ABA metabolism resulting in increased leaf ABA concentration; ii) direct effects on leaf water status that could alter guard cell turgor or sensitivity to leaf ABA concentrations; iii) direct effects on ion fluxes through the guard cell plasma membrane; and iv) altered distribution of ABA in the leaf, specifically an increase in the concentration of ABA in the apoplast outside of the guard cells (Figure 1). It is this change in apoplast pH that probably has the greatest role in signaling.

As ABA is a weak acid it may become protonated or deprotonated in the pH range found in the apoplasm of leaves. This was clearly shown in the early 1980s in experiments on mesophyll protoplasts that documented high rates of ABA uptake at acidic pH and almost no uptake at the more alkaline pH of 7.5 [38]. In certain plant species, the pH of the leaf apoplast increases as the soil dries because of the delivery of xylem sap that is of a more alkaline pH. The ABA that arrives from roots via the xylem will remain deprotonated under the more alkaline conditions and will not be taken up passively by mesophyll substances in the sap that act in concert with ABA to control drought-induced stomatal closure. For example, recent findings have suggested an interaction between jasmonic acid and ABA in plants under drought stress [31]. In addition, the dominant role of ABA as a root to shoot signal has been challenged by experiments showing that the ABA concentrations of xylem sap from drought-stressed plants were much lower than the concentrations of exogenous ABA required to close stomata in detached leaves [32]. It may be that the use of exogenous hormones in bioassays may exclude important components of xylem sap that act synergistically with ABA in planta, thus accounting for the higher levels of exogenous ABA that are required to reduce transpiration. Grafting experiments have also been used to determine the source of ABA in drought-induced stomatal closure. Some of these experiments suggest that leaf-sourced ABA is important for stomatal closure [22], but the influence of ABA precursors that might be transported from roots to leaves was not measured, and therefore the role of roots in chemical signaling was not completely resolved [25]. Other studies show that production of ABA in the rootstock has a strong influence on and is negatively correlated with stomatal conductance [33]. Overall, ABA is a dominate signal in controlling growth and transpiration, but other factors could also be important. The importance and role of root-sourced ABA is still controversial, but some of the conflicting findings may be due to differences in the intensity of stress imposed and the time-course of the development of water deficit.

Box 1. Partial root-zone drying and deficit irrigation in plants

Partial root-zone drying (PRD) and deficit irrigation (DI) are examples of the manipulation of root signals to enhance the water use efficiency (WUE) of agriculture. PRD is an irrigation method where half the root zone is allowed to dry while the other half is kept wet. After a period of time, the irrigation is alternated so that the previously dry root zone receives water and the previously watered side is allowed to dry. DI is an irrigation method in which the amount of water supplied is less than that required to achieve maximum crop evapotranspiration. Both methods can reduce transpiration and leaf growth while increasing WUE with only small decreases in yield.

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cells. The result is that less ABA is transported into the mesophyll cells and a build-up of ABA in the apoplast leads to enhanced stomatal closure. Under well-watered conditions, when the apoplast is more acidic, ABA would passively enter the symplast, and apoplastic concentrations would not increase as rapidly as they do under water stress. Therefore, the effect of pH is indirect in that it leads to the accumulation of ABA in the apoplast and enhances the effect of ABA on guard cells [35].

This mechanistic explanation is both attractive and intuitive. Nevertheless, the explanation sets up another hypothesis that ABA receptors are on the plasma membrane rather than inside the guard cells. Recently, three ABA receptors have been found [39–41]. Two of these ABA receptors reside inside the cell but a third was found on the cell surface [39]. Therefore, plant cells can sense both extracellular and intracellular ABA concentrations. Under conditions of drought, the increased stomatal closure that occurs because of the increased pH of sap suggests that extracellular ABA is sensed by guard cells via receptors on the plasma membrane.

Several studies have shown that pH does not act alone on guard cells or in controlling leaf growth. For example, the use of tomato [42] and barley (Hordeum vulgare) [43] mutants that are deficient in ABA biosynthesis showed that ABA was necessary for both stomatal closure and the inhibition of leaf growth. In the tomato mutant flacca, transpiration increased as the pH of artificial sap increased, suggesting that ABA is also necessary to prevent stomatal opening and water loss. In the barley mutant Az34, ABA was necessary for pH to act as a signal under conditions of drought stress [43]. Many studies that show a role for pH in stomatal closure have used tomato as a model system. It is important to point out, however, that there may be differences in response to ABA and pH between species and perhaps even between genotypes within the same species. One study compared C. communis and Arabidopsis thaliana and found that the increased pH of external solutions led to a 27% increase in stomatal opening in C. communis but had no effect on A. thaliana [44].

Both species, however, responded similarly in the presence of ABA and only changes in the kinetics of response were observed. In soybean (Glycine max) [45], xylem sap pH did not change in response to mild drought and was not correlated with decreased stomatal conductance during the initial stages of drought. The xylem sap of soybean did, however, become more alkaline during extended drought, but this alkalization occurred well after a measured decrease in stomatal conductance.

The response of xylem sap pH to drought stress does not appear to be consistent in all species or even in different experiments using the same species. One study showed that the sap pH of sunflower and C. communis did not change significantly as soil dried, whereas the xylem sap pH of tomato did increase as soil dried [46]. In maize (Zea mays) grown using ammonium as the nitrogen source, there was no change in sap pH during early [7] or even during later stages of drought (L. Ernst and D.P. Schachtman, unpublished). Other experiments using maize have been conducted in the field and have found that the sap pH of drought-stressed plants growing on a loam soil was more alkaline as the period of drought lengthened [47]. Such inconsistencies may be explained by the fact that solute concentrations within the xylem sap vary with flow rate in intact plants [48,49]. The sap solute concentrations and delivery rates that best represent those of xylem sap flow in intact plants are most accurately determined when sap is collected at rates equivalent to whole-plant transpiration. Therefore the rate of whole plant transpiration must be determined prior to detopping plants to harvest sap. External pressure must be applied to roots so that sap exudes at the flow rate of whole plant transpiration, a fact often overlooked by experiments in which only xylem sap is collected [49].

The mechanism of pH change might involve nitrate availability. As soils dry, nutrient availability is reduced because of physiochemical changes. Under conditions of reduced nitrate availability, nitrate reductase activity shifts to roots, causing changes in the pH of sap [50]. Changes in the activity of nitrate reductase that are caused by drought conditions lead to changes in organic acid production, which alter sap pH. In particular, malate concentration increases in xylem sap under drought, resulting in sap that is less acidic than that in well-watered conditions when more nitrate is loaded into the xylem. When the effects of a nitrate reductase inhibitor (sodium tungstate) were tested [51], the inhibitor was found to prevent the alkalization of the sap in tomato under drought conditions. Similarly, the addition of nitrate to tomato under water-stress conditions enhanced the alkalization of the xylem sap [46]. A synergistic effect of xylem nitrate and ABA on stomatal conductance in detached leaves of C. communis has also been shown [46]. Moreover, the results of work on maize suggest that, under some circumstances where neither compound is as effective alone, a combination of ABA and nitrate is required to elicit an effect on shoot physiology [52].

The effects of changes in the internal pH of the guard cells have been very well characterized [20]. Less is known about the effects of apoplastic changes in pH on guard cells, but one study [53] showed that stomatal opening is inhibited by acidification and that the plasma membrane potential is depolarized (which would facilitate the opening of outward-rectifying potassium channels) when the apoplast is acidified. More studies are needed to clarify the effects of changes in external pH on guard cells when the internal pH is held constant. If extracellular alkalization also leads to an increased intracellular pH, it could be an important part of the signaling process for stomatal closure.

Conjugates of ABA as potential signals

Although ABA seems to play a dominant role in root to shoot signaling under drought, it also seems likely, on the basis of several studies, that other substances are also involved. ABA is present in xylem sap in conjugated forms, such as abscisic acid-glucose ester (ABA-GE). It has been suggested that a conjugated form of ABA could serve as a transported form of the hormone and, moreover, as a stress signal [32,54]. As many as six ABA conjugates have been found in xylem sap from well-watered and water-stressed sunflower [55], and a correlation was found in one study.
Figure 1. Changes in xylem sap composition under drought. Drought causes the alkalinization of xylem sap pH in certain plant species. (a) Well-watered plant with apoplastic pH 6.0. (b) Plant under drought conditions with apoplastic pH 7.0. In plants in which xylem sap pH increases when the soil becomes dry, ABA-induced stomatal closure is enhanced. This is thought to be due to increased apoplastic concentrations of ABA. Further changes in xylem sap composition under drought are also responsible...
between increased ABA-GE concentrations and drought [56]. The importance of conjugated forms of ABA in drought responses and the mechanisms of hydrolysis [57] of such forms of ABA in the apoplast are, however, still poorly understood. A next step in establishing the importance of conjugated forms of ABA might be to genetically vary the hydrolysis of ABA conjugates. To do this, it will be essential to identify the genes encoding the apoplastic β-glucosidases that are responsible for hydrolysis before subsequent manipulation of these enzymes can proceed.

**Cytokinins as signals**

Cytokinins could also be an important signal traveling from roots to the shoots. Root-produced cytokinins are clearly involved in responses to nutrient deprivation [5] and, as they are produced mainly in roots, could be important in drought responses [58]. Despite this, there have been few reports that provide information on the cytokinin content of xylem sap and how that content changes under drought conditions. In grapevines, a 50% reduction in zeatin (Z) and zeatin riboside (ZR) was found in plants that had been subjected to PRD [59]. In a more recent study in which Z, ZR and zeatin nucleotide (ZN) were measured, PRD of tomato reduced the ZN content of the xylem sap, but the magnitude of that change and the contribution of ZN to the total cytokinin content were not shown [60]. In at least two studies on sunflower, xylem sap, combined Z and ZR and combined isopentenyladenine and isopentenyladenosine concentrations in xylem sap decreased under drought-stressed conditions [61]. It is possible that the ABA:cytokinin ratios in xylem sap are important for stress signaling [55]. The effects of cytokinins are also supported by a study showing that transpiration was higher in transgenic plants that overexpressed the isopentenyltransferase, causing the plants to produce more cytokinins after a heat shock treatment [62]. Although recent data show decreased cytokinin concentrations in the xylem under drought stress, it is still not clear that all plant species respond in the same way to cytokinin at the concentrations found in the leaf and guard cells [63].

Strong conclusions about cytokinin content are premature because the complexity of the cytokinin profiles has not been fully explored [64]. In a recent study on maize [65], we found a decrease in Z and ZR concentrations in xylem sap coming from roots of drought stressed plants as compared to well watered controls. Surprisingly, we also found high concentrations of the aromatic cytokinin 6-benzylaminopurine (BAP) in maize xylem sap, the concentration of which increased significantly as a result of water stress [65]. With the availability of increasingly sensitive mass spectrometry methods, it should be possible to explore comprehensively the changes in concentrations of many of the cytokinins, and to identify those that play a role in root to shoot signaling.

### Other chemical signals

Chemical and protein-based factors other than ABA, cytokinins or pH could also be involved in root to shoot signaling. Although it has now been established clearly that xylem sap from many plant species contains proteins [66,67], the presence of peptides in xylem sap has only recently been demonstrated [68]. The addition of xylem sap from tomato induced rapid alkalinization in a tomato cell-suspension bioassay within minutes. This approach has been used previously to identify peptides. In an experiment using maize suspension cells, alkalinization was induced by maize xylem sap, but the activity could not be purified or abolished with proteases [65]. MicroRNAs have also been implicated as potential signal molecules that move systemically [69], and recent work on Arabidopsis found that drought treatment induced the synthesis of a specific microRNA: miR-169 g [70]. The functional significance of peptides, microRNAs and proteins in the xylem sap remains largely unknown, but peptides have been shown to play important signaling roles in plants and have also been shown to move systemically via the phloem [71,72].

Many compounds, including hormones, inorganic ions, amino acids, sugars and organic acids have been identified in xylem sap [7,34], but among the organic acids found in abundance in the xylem sap, only malate has been implicated in the control of stomatal closure [73]. Stomatal opening in ash (Fraxinus excelsior) leaves could be prevented when these leaves were supplied with 0.5–3 mM malate. Similarly, elevated extracellular concentrations of malate have also been shown to close stomates in Vicia faba [74]. Further work is needed to establish the role of malate in root to shoot signaling.

Ethylene could be another important factor under drought conditions. It is known that a precursor of ethylene, 1-aminoacyclopropane-1-carboxylic acid (ACC), moves in the xylem from root to shoots [75]. Under drought stress, ACC transported in the xylem sap might result in the measured increase of ethylene evolution in leaves [76]. A role for ethylene under drought was demonstrated by the use of an ACC oxidase (ACO) antisense in tomato. In these antisense plants, ethylene evolution was much lower than normal under both well-watered and drought conditions. Under soil-drying conditions, the stomatal response in the ACO antisense plants was the same as wild type, but a decrease in leaf growth was measured in wild type but not the ACO antisense plants in response to soil drying [76]. In maize, ethylene evolution in leaves could not be correlated with reductions in leaf elongation under drought [77]. This study also showed that ABA does not play a role in reducing leaf elongation in maize [77]. This suggests that...
ethylenes may play a role in decreased leaf growth in one plant species and that ACC may be one component of long-distance root-sourced signals under drought.

Conclusions

The production of root-sourced chemical signals under conditions of water deficit has been associated with reduced transpiration and/or leaf growth. However, the identity and relative contribution to signaling of these root-sourced chemicals remains controversial. This controversy may be due to differing responses between species, the different intensities of stress treatments applied, the time at which samples were collected during the imposition of drought, and/or the different methods used for xylem sap extraction [43]. Until recently [65], few studies have provided comprehensive information on the composition of xylem sap. Such comprehensive analyses will clarify the contribution of different chemicals to root to shoot signaling, and the complexity of constituents and their interactions. As we increase our knowledge of the precise identity and biosynthesis of the primary chemical signals that are produced by roots, it will become feasible to alter plant sensitivity to soil water deficit. This will provide new molecular breeding strategies for tailoring crops to maintain or even increase yields under specific water stress conditions, such as intermittent and terminal drought.

Acknowledgements

DPS was supported in part by NSF-Plant Genome #0211842. JQDG is the recipient of an Australian Research Council Post-doctoral Fellowship (Inducrty linkage project #LP0775362).

References

1 Davies, W.J. et al. (2002) Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol. 153, 449–460
7 Goodger, J.D.Q. et al. (2005) Relationships between xylem sap constituents and leaf conductance of well-watered and water-stressed maize across three xylem sap sampling techniques. J. Exp. Bot. 56, 2389–2400
14 Dry, P.R. and Loveys, B.R. (1999) Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. Vitis 38, 151–156
15 Davies, W.J. et al. (2000) Regulation of leaf and fruit growth in plants growing in drying soil: exploitation of the plants' chemical signaling system and hydraulic architecture to increase the efficiency of water use in agriculture. J. Exp. Bot. 51, 1617–1626
31 Mahouachi, J. et al. (2007) Hormonal changes in papaya seedlings subjected to progressive water stress and re-watering. Plant Growth Regul. 53, 43–51
36 Wilkinson, S. and Davies, W.J. (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 Physiol. 113, 559–573


Kehr, J. et al. (2005) Analysis of xylem sap proteins from *Brassica napus* BMC Plant Biol. 5, 11


Hedrich, R. et al. (1994) Malate-sensitive anion channels enable guard-cells to sense changes in the ambient CO2 concentration. *Plant J.* 6, 741–748

