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Long-distance ABA Signaling and Its Relation to Other Signaling Pathways in the Detection of Soil Drying and the Mediation of the Plant's Response to Drought

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ABSTRACT

In this article we review evidence for a variety of long-distance signaling pathways involving hormones and nutrient ions moving in the xylem sap. We argue that ABA has a central role to play, at least in root-to-shoot drought stress signaling and the regulation of functioning, growth, and development of plants in drying soil. We also stress the importance of changes in the pH of the leaf cell apoplast as influenced both by edaphic and climatic variation, as a regulator of shoot growth and functioning, and we show how changes in xylem and apoplastic pH can affect the way in which ABA regulates stomatal behavior and growth. The sensitivity to drought of the pH/ABA sensing and signaling mechanism is emphasized. This allows regulation of plant growth, development and functioning, and particularly shoot water status, as distinct from stress lesions in growth and other processes as a reaction to perturbations such as soil drying.

Key words: Long-distance drought signaling; Abscisic acid; Ethylene; Cytokinin; pH; Deficit irrigation; Water use efficiency (WUE)

INTRODUCTION

The growth, development, and yielding of crop plants is highly sensitive to a reduction in water availability in the soil (see, for example, Boyer 1982). There is general agreement among plant scientists that much reduction of yield due to "drought stress" occurs at the wet end of the spectrum, well before plants are themselves stressed in the conventional sense of the word (see, for example, Richards 1993). This is because plants can sense and respond to changes in their immediate environment (for example, soil drying) and then regulate growth and functioning, for example, to

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avoid shoot dehydration stress. To sustain yield as soil dries, which will be necessary as demands grow for judicious water management in agriculture, the plant biologist must initially address these regulatory processes, rather than focusing on processes that underlie stress-induced lesions in functioning and development or that contribute to desiccation resistance. For example, the limitation of leaf growth as soil dries around the roots is often highly attuned to a reduction in soil water availability, with the result that biomass production is often substantially reduced when there is still plenty of water in the soil. In this case, if we can reduce the sensitivity of the plant's environmental sensing mechanisms or its regulatory response to the stress, we can then stabilize vegetative crop yield between years and enhance yield per unit cropping area, which will be necessary if we are to sustain food production on an ever-decreasing stock of cultivatable land.

Recent research has suggested that regulation of plant growth and development as the soil dries commonly involves long-distance chemical signaling that can close stomata to sustain shoot water status. This kind of signaling can also restrict shoot growth, although the signaling pathways involved in these two responses may be distinctly different. The observation that shoot water status can be regulated by long-distance signaling argues that hydraulic signaling (reduced uptake of water with an adverse effect on shoot water status) cannot be an important component of the most sensitive growth-regulating processes, although hydraulic responses are clearly key components of the processes leading to plant water stress, as regulation fails when soil drying becomes more severe. We examine below various components of some of the plant's long-distance signaling processes. These can be key components of stress detection and the plant's systemic responses to perturbations in the above- or below-ground environments. We emphasize the central role played by ABA in several signaling cascades and the possible interactions between ABA signaling and long-distance signaling by other hormones and other chemical species in the xylem or the apoplast.

LONG-DISTANCE ABSCISIC ACID SIGNALING

It is now well known that stomatal behavior can be closely related to changes in soil water availability even when the leaf water status is not affected by soil drying. Using split-root techniques, with one drying root half and one well-watered root half (later referred to as ''partial root zone drying,'' PRD) Blackman and Davies (1985) showed that despite an unchanged leaf water potential as soil dried around half of the root, leaf conductance was restricted. Most physiological work has suggested that abscisic acid is likely to be a major chemical root-to-shoot stress signal in plants in drying soil (for review see, Davies and Zhang 1991). More recently, experiments with transgenic plants that have a low capacity for ABA biosynthesis have confirmed this view. Grafting ABA-deficient sunflower roots to ABA-sufficient shoots generates a stomatal response that is distinctly different from that of ABA-sufficient shoots grafted onto ABA-sufficient roots (Borel and others 2001). Some rather different results have been obtained with grafting experiments with tomato (Holbrook and others 2002), and these results emphasize the importance of shoot-sourced chemical signals (and hydraulic influences) in the regulation of growth and gas exchange. Some very recent work in our own laboratory (Ian Dodd, unpublished) with tomato shows that grafting of ABA-sufficient roots onto deficient shoots generates clear phenotype reversal. It is apparent that in certain situations root signaling effects can become apparent, whereas in other conditions shoot influences may dominate, precisely the kind of stress defense reaction that we might expect evolution to throw up in plants. Further work will be necessary in this area, but from the balance of evidence it seems reasonable to conclude that roots can ''measure'' the water availability in the soil and transfer this information to the shoot, where such a measure can contribute to the regulation of plant growth and development. This may occur via increased ABA biosynthesis in roots and/or through stimulation of ABA transport from the roots (that may not necessarily be root-sourced hormone) and release into the xylem vessels.

Abscisic acid can be synthesized in the cytosol of all root cell types, with comparable rates in the different cell types. High ABA concentrations in root tips are a result of the low vacuolization of the root-tip cells (shown for maize and runner bean roots by Hartung and others 1999). Besides drought stress, other factors that are characteristic of soils of arid climates, such as special nutrient relations (variation in nitrate and ammonium supply, phosphate and potassium deficiency) soil strength, and high salt concentrations of the soil solution, all stimulate ABA formation in roots, release to the xylem, and translocation to the leaves. In only some cases does this extra ABA accumulate in the leaves. It has been shown that at least in the case of phosphorus deficiency (Jeschke and others 1997) and potassium deficiency (Peuke and others 2002) ABA is metabolized to phaseic acid after action on stomata. Therefore, although ABA acts in these situations as an effective root-to-shoot signal, no accumulation in the leaves can be observed.

In addition to the ABA that is synthesized in the roots, much of the ABA found in the xylem actually originates in the leaves. It is synthesized in the leaves and can be loaded into the phloem and transported to the roots. Here, some of it may be deposited in the tissue or metabolized, another part, however, may be loaded to the xylem vessels and recirculated to the shoots. Salt stress, phosphate deficiency, and ammonium nutrition enhance the percentage of ABA arriving in the phloem that is recirculated from the roots to the shoots (Jeschke and others 1997r; Peuke and others 1994). Most interestingly, recent work by Soar and others (2004) suggests that much of the ABA found in grapevine leaves is made *in situ* and stays there.

Both free and conjugated ABA can also be taken up by the roots from sources external to the plant and then loaded into the xylem vessels. Both free ABA and ABA-glucose ester (ABA-GE) have been detected in the soil solution under a range of crop plants in concentrations up to 10 nM or 30 nM, respectively (Hartung and others 1996; Sauter and Hartung 2000). External, free ABA can be readily taken up by roots. It also participates in maintaining an ABA equilibrium between roots and the external medium, and this can be crucial in ensuring that long-distance ABA signaling operates optimally (Hartung and others 1996). The uptake from the soil of conjugated ABA is strongly dependent on the extent and properties of apoplastic barriers/bypass conduits in the hypodermis.

Until recently, little information was available regarding the transport paths and transport mechanisms of ABA into, out of, and through roots. It was assumed that radial ABA transport occurs exclusively in the symplast (Jackson and others 1996). In this case the intensity of the ABA signal in the xylem depends strongly on the lateral water flow through roots. Abscisic acid would be diluted drastically when transpirational water flow was increased. Computer simulations by Slovik and others (1995) support such assumptions. Modeling of a symplastic ABA transport pathway for the root-to-shoot-signal has indicated dramatic changes in the ABA concentration of the xylem sap, even on a diurnal basis. In the case of field-grown maize, however, Tardieu and others (1992) found relatively constant xylem ABA concentrations during the diurnal day/light cycles, indicating that mechanisms must exist that maintain some ABA homeostasis in the xylem.

Steudle and co-workers (for example, see Steudle and Peterson 1998) have suggested that some water and solutes pass into the root exclusively through the apoplast. This observation and other careful experimentation has resulted in the development of the so-called composite-transport model, which describes water and solute flux through roots and adequately accounts for the various physiological demands of roots. The model suggests that ABA could be translocated to the xylem by solvent drag with the transpirational water stream through the apoplast. Such a bypass flow could buffer the dilution that could be caused by increased water flow. The model of Slovik and others (1995) predicts that a small contribution (1%) of such an apoplastic bypass flow will compensate for any ABA dilution caused by symplastic transport combined with high rates of water transport.

Experimental evidence for the presence of an apoplastic ABA bypass flow was provided by Freundl and others (1998, 2000). These workers applied ABA in the physiological concentration range of 5 nM up to 100 nM to media bathing excised root systems of maize (*Zea mays*) and sunflower (*Helianthus annuus*). The xylem sap was then collected by application of sub-atmospheric pressure to the cut surface of the mesocotyl (Freundl and others 1998). An intensified radial water flow (J_{Vr}), induced by the pressure gradient, did not reduce the ABA concentration of the xylem sap and even increased it.

Being a weak acid ($pk_a = 4.8$), ABA is distributed within the apoplast and the symplast of root tissues according to the anion-trap concept and the Henderson-Hasselbalch equation. Acidification of the apoplast, as occurs in ammonium-supplied roots (Gerendás and Ratcliffe, 2000), should redirect ABA to the cytosol of root cortical cells. It should then be translocated predominantly in the symplast. An increased water flow J_{vr} should therefore dilute the signal. Indeed, as shown by Peuke and others (1994), the concentration of ABA in the xylem sap is significantly higher in plants supplied by NH_4^+. However, the ABA concentration is reduced whenever water flow is increased (Hose and others 2002).

In most roots, hypodermal cell layers can build up Casparian bands in their radial cell walls (Peterson 1988), in addition to those in the endodermis. Hose and others (2001) demonstrated that Casparian bands in the hypodermis caused a significant reduction in radial ABA flow through exodermal roots, especially at high water flow rates. On the other hand, roots lost significantly smaller amounts of ABA to the surrounding media when a Casparian band was present in the hypodermis. Hose and others (2002) compared the efflux of ABA from exodermal and non-exodermal maize roots using compartmental efflux analysis. The half time of isotope exchange from the apoplast of exodermal roots was 2–3 times higher than in the non-exodermal controls. This indicates that the Casparian band of the maize exodermis is an effective barrier for ABA, slowing down ABA efflux to the surrounding medium. Lower efflux results in an increased ABA concentration in the root apoplast. Such ''extra ABA'' can stimulate the hydraulic conductivity of root cortical plasma membranes (Hose and others 2000). It is also available for apoplastic translocation by solvent-drag directly into the xylem vessels.

pH as a Moderator of the ABA Signaling Process

Alkalization of xylem sap is a common response to soil drying in some plants (for example, tomato, barley, and grapevine; see Wilkinson and others 1998 for tomato data) and supplying detached leaves with neutral or alkaline buffers (pH ≥ 7) via the transpiration stream can restrict transpiration (Wilkinson and Davies 1997; Wilkinson and others 1998). Apparently these buffers can increase apoplastic pH, which will result in an increase in apoplastic ABA concentrations (ABA will distribute between compartments of the leaf according to the Henderson-Hasselbalch equation). Then, pH-induced increases in apoplastic ABA concentration will ultimately close the stomata (Wilkinson and Davies 1997). Stomatal closure in response to xylem-supplied alkaline buffers was shown to be ABA-dependent, as leaves detached from an ABA-deficient tomato mutant (*flacca*) did not show stomatal closure when fed pH 7 buffers (Wilkinson and others 1998). In wild-type (WT) plants, it is possible that increased xylem sap pH could elicit ABA-dependent stomatal closure without the need for increased xylem ABA delivery. In other words, there will always be enough ABA to close stomata, even in the well-watered plant, but the response to that ABA will be highly pH dependent. Temporal changes in xylem sap pH and ABA concentration during a soil-drying episode have been measured, and increased xylem sap pH can precede (Bahrun and others 2002; Sobeih and others 2004) (Figure 1) or follow (Liu and others 2003) increased [X-ABA].

Increased xylem sap pH can also correlate with drought-induced leaf growth inhibition in barley, where feeding leaves alkaline buffers via the xylem inhibits leaf growth (Bacon and others 1998). Feeding alkaline buffers to an ABA-deficient barley mutant (*Az34*) did not inhibit leaf growth unless an ABA concentration typical of well-watered plants was also present in the buffer. These responses are directly analogous to the effect of alkaline buffers on detached leaf transpiration. In grass leaf bases, increased sap alkalinity presumably allows ABA access to sites of action within the leaf elongation zone, inhibiting growth. In well-watered plants with a more acidic, apoplastic pH, ABA is presumably partitioned into alkaline compartments in the symplast and away from sites of action regulating leaf growth.

It interesting to note that the pH of xylem sap of all plants is not increased as soil dries. This may particularly be the case in woody plants (see for example, Wilkinson and Davies 2002) and to understand these differences, it is important that we understand the mechanistic basis of the change in xylem pH as the soil dries. Clearly, the buffer capacity of the sap is important here. There is very little information on the relationship between xylem pH and the pH of the apoplast of the leaves. The proposal made above for long-distance pH signaling requires that there should be a fairly tight relationship between the two. We have recently demonstrated that this can be the case (Jia and Davies, unpublished). The use of fluorescent pH dyes viewed under the confocal microscope shows that soil drying increases the pH of the xylem sap leaving the roots of tomato, and qualitatively similar changes are also observed in the leaf apoplast. Interestingly, the nutrient status of the soil and the vapor pressure difference (vpd) around the shoots also had significant effects on leaf apoplastic pH. Some recently published work also highlights the effect of climatic factors on the pH of the apoplast/xylem sap within the shoot. High photon flux density can alkalinize the sap of the woody plant *Hydrangea*, with a concurrent reduction of stomatal conductance presumably in response to a redistribution of ABA (Wilkinson and Davies 2002). Although this may suggest a rather exciting way for the plant to integrate the impacts of climatic and edaphic factors, these observations do complicate rather simple proposals that information on soil water availability can be communicated directly and unambiguously to shoots.

ABA Conjugates as Root-to-shoot Stress Signals

Munns and King (1984), Netting and others (1992), and Munns and Sharp (1993) have postulated that ABA conjugates (sometimes referred to as the ABA

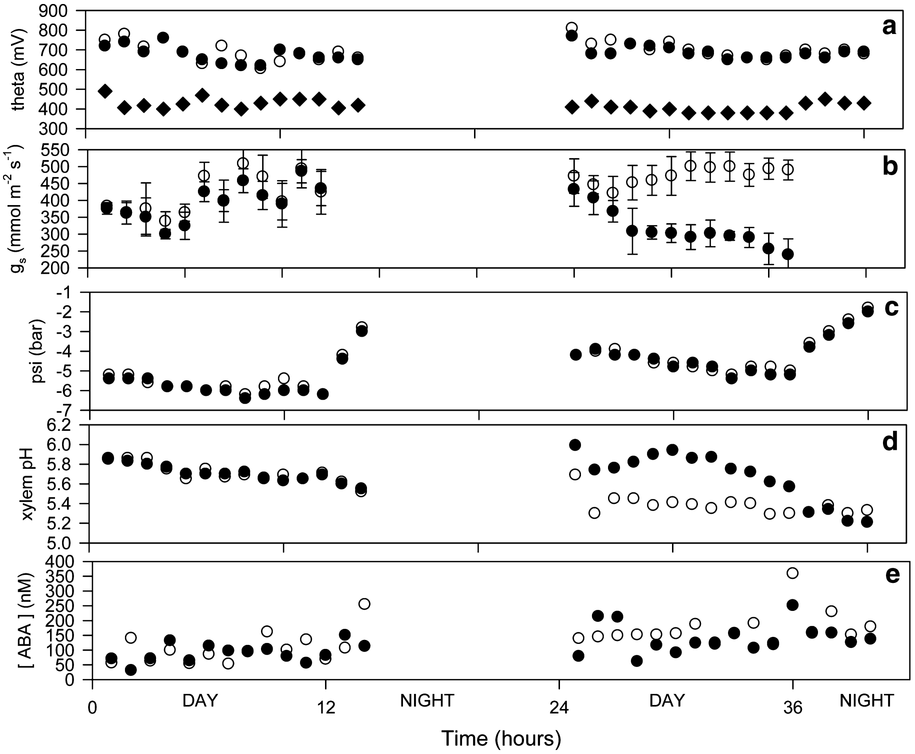

Figure 1. Effects of partial root drying on functioning of tomato leaves. Moisture content of the upper 6 cm of potting compost from pots watered daily (indicated by arrows) on both sides of the split-pot (open circle), and from the watered (filled circle) and drying (filled diamond) sides of plants watered daily on one side of the split-pot (**a**). Stomatal conductance (**b**), leaf water potential (**c**), xylem sap pH (**d**), and xylem ABA concentration (**e**) of fully expanded leaves at node 9. Points are from individual wild-type (cv. Ailsa Craig) plants watered daily on one (filled circle) or both (open circle) sides of the split-pot (**c–e**). In (**b**), points are means ± S.E. of five leaflets per leaf. From Sobeih and others (2004).

adduct) can act as an additional root-to-shoot stress signal. Until now, the chemical structure of this adduct has not been elucidated, but ABA glucose esters have been found in xylem, and these compounds may play an important signaling role.

Bano and others (1993, 1994) identified enhanced concentrations of glucose esters of ABA, phaseic acid, and dihydrophaseic acid in the xylem sap of stressed sunflower and rice plants. The concentrations of free and bound ABA decreased again after relief of the stress condition. Dietz and others (2000) found a 4–5-fold stimulation of ABA-glucose ester (ABA-GE) concentration in the xylem sap of barley under salt stress. Five alkali-hydrolyzable conjugates of ABA were detected in the xylem sap of well-watered sunflower plants (Hansen and Dörffling 1999). In plants under stress, a sixth conjugate appeared, and the concentration of the others was enhanced significantly. Drought stress increased the amount of ABA conjugates with β-glucosidic linkages, predominantly ABA-GE. More data on ABA conjugates in the xylem sap of stressed plants are cited and discussed by Sauter and others (2002).

External conjugated ABA-GE cannot be dragged with the water flow across the hydrophobic endodermis, as is the case for free ABA. In particular, aeroponically cultivated maize roots with a complete exodermis are not able to take up external ABA-GE. Both tissues, the exodermis and the endodermis are good barriers for ABA conjugates. However, hydrolytic enzymes of the root cortex apoplast are able to cleave the conjugate (Sauter and Hartung 2000). The released free ABA can then be translocated in the apoplast to the xylem vessels. Translocation of endogenous ABA-GE must occur within the root symplast. Once it has arrived at the xylem parenchyma cells, the conjugate can be released into the xylem elements because the plasma membranes of stelar cells show a fivefold higher permeability than those of the cortical cells. The mechanisms of membrane transport of ABA-GE are still unknown. Because ABA-GE can be loaded into the xylem elements and transported to an even greater extent under stress conditions, the conjugate can be considered a long-distance signal. Dietz and others (2000) have investigated the fate of the ABA-GE after it arrives in the leaf apoplast, and results show that esterases in the leaf apoplast release free ABA from its conjugates. The activity of these esterases is increased substantially under salt stress, thus making more free ABA available in leaves and linking the long-distance signals in the xylem (ABA-conjugate) with enzyme activity in the apoplast of the leaves.

Interaction of ABA Signaling with Other Hormonal Signaling Cascades: Cytokinin Signaling

Since the early days of phytohormone research (for references, see Kamboj and others 1998), we have known something of the importance of cytokinins as a hormonal signal that apparently originates mainly in the roots and is transported in the xylem to the shoot (see also Incoll and others 1989; Hare and others 1997). Until recently, virtually nothing conclusive was known about the pathways of cytokinin biosynthesis in plants or of the enzymes that control it (Prinsen and others 1997). Since 1991, when the capacity of soil microorganisms to produce cytokinins was established (Arshad and Frankerberger 1991), the possibility has been discussed that these microorganisms and not plants themselves but might be the source of cytokinins for plants (Holland 1997). However, the discovery of plant genes coding isopentenyltransferase (Kakimoto 2001) (an enzyme catalyzing cytokinin biosynthesis) put an end to doubts concerning the capacity of plants themselves to produce cytokinins. Because roots were shown to be the main site of the enzyme expression, the results provide new support for the old suggestion that cytokinins are involved in root/shoot signaling. Cytokinin content of plants was shown to be responsive to changes in the root environment (see references cited by Jackson and others 1996). Thus NO_3 re-supply to N-deprived roots rapidly stimulated cytokinin biosynthesis and transport to the shoot (Takei and others 2001) and cytokinins were suggested to serve as long-distance signals mediating molecular responses to changes in NO_3 availability. This suggestion has been supported by the recent work of Rahayu and others (2005), whereas Dodd and others (2004) provide compelling evidence from work with branching pea mutants that shoot nitrogen status is more important than xylem cytokinin delivery, at least in the regulation of leaf growth.

Cytokinins are commonly discussed as antagonists of ABA action on stomata. External application of cytokinins has been shown to maintain stomata in an open state (Blackman and Davies 1985), but there are questions about the importance of these hormones as regulators of growth and development under drought conditions. This is because the effects of exogenous cytokinins on stomatal conductance are less clear than those for ABA and the fact that very severe stresses are often required to significantly reduce cytokinin delivery to shoots (contrast the increase in delivery of ABA, which can be more than an order of magnitude in response to quite mild soil drying).

Certainly, elevated levels of leaf cytokinins can be shown to correlate with stomatal opening in some plants (Vysotskaya and others 2004). In keeping with the proposed role for cytokinins in the moderation of stomatal behavior, transgenic tobacco plants, which overproduce cytokinins, are characterized by increased transpiration (Teplova and others 2000) and even wilting (Thomas and others 1995). Stoll and others (2000) have found a 50% reduction of zeatin and zeatin riboside in both the shoots and stems of grapevines grown under partial root-zone drying (PRD) conditions, and these authors related changes in plant development to changes in the abacisic acid:cytokinin (ABA:Ck) ratio. They also concluded that cytokinin transport in the xylem should be reduced by soil drying. However, information about cytokinins in the xylem of plants under drought stress is rare in the literature. Masia and others (1994) and Sashidar and others (1996) failed to find significant decreases in xylem cytokinins of drought-stressed sunflower plants (perhaps because of the extent of the drought stress applied), whereas Bano and others (1994) investigated this problem with sunflowers, and under drought stress they found a decrease in the xylem sap of sunflower of trans-zeatin, zeatin riboside (ZR), isopentenyl adenine, and isopentenyladenosine. The discrepancy of results in this important area might be a function of the existence of at least 20 naturally occurring cytokinins in plants (Chen 1997). We do not have a clear idea of which cytokinins will be affected by drought stress or, even more specifically, which transport forms should be measured in the xylem.

The importance of measuring different forms of cytokinin in estimating the effect of drought on cytokinin supply to leaves is illustrated by the data presented in Table 1. A significant decrease in the sum of cytokinins was observed only when concentrations of O-glucosylated zeatin forms were taken into consideration. Without consideration of

Table 1. Concentration of Different Forms of Zeatin (nM) in Xylem Sap Arriving in the Leaves of Tomato Plants

	Free base	Riboside	Nucleotide	O-glucoside	Sum of Z derivatives
Control	3.1	3.2	2.3	10	18.6
Water-stressed	3.2	1.7	2.0	4.6	11.5

Mean values of cytokinin content in 6–20 samples collected 2 and 4 days after watering was stopped. Xylem sap was collected from intact, transpiring plants using the root pressure vessel.

this form the effect of drought stress was not significant (8.6 and 6.9 nM in control and stressed plants, respectively).

Concentration changes of ZR in the xylem were investigated in more detail by Hansen and Dörffling (2003). When the water potential of sunflower hypocotyl decreased, the concentration and the flux of ZR decreased substantially after a transient rise. After rewatering it rose continuously, reaching a 60-fold increase after 5 h, and it declined again afterwards. This increase was correlated with an increase of leaf conductance. No information, however, is available on the importance of cytokinins in the rooting medium, although they have been found to be present in the soil solution under a range of different plants (Frankenberger and Arshad 1995). We also lack experimental data on lateral cytokinin transport through roots and the mechanisms that regulate the intensity of the cytokinin signal in the xylem. Moreover, we can only speculate about the fate of cytokinin conjugates such as ZR. They may be cleaved by apoplastic esterases, as is the case for ABA conjugates.

There are very few reports of the impact of environmental stress on more than one endogenous hormone. This is a clear deficiency in the literature as, for example, cytokinin:ABA ratios presumably will have an important impact on plant development and functioning (see for example, Stoll and others 2000). There is much clearer information in the literature on the interaction between ABA and ethylene in the regulation of development in response to soil drying.

Interaction of ABA Signaling with Other Hormonal Signaling Cascades: Ethylene/ACC Signaling

Drought increases root and xylem concentrations of the ethylene precursor ACC (1-aminocyclopropane-carboxylic acid) (Gomez-Cadenas and others 1996). Feeding both ABA and ACC (the immediate precursor of ethylene) via the xylem to well-watered plants inhibits leaf growth additively (I. C. Dodd and W. J. Davies, unpublished results), suggesting an important role for ethylene in the regulation of leaf growth as soil dries. In plants at low water potential, these two hormones interact where ABA restricts the extent of runaway ethylene synthesis, and ABA accumulation is necessary to minimize ethylene-mediated decreases in root elongation (Sharp and others 2000). Although the delivery of ACC from the root system can account for shoot ethylene evolution (Else and Jackson 1998) and may thus limit leaf growth under drought conditions, the relationship between xylem ACC concentration and leaf growth of plants exposed to drying soil has not been defined. Stomatal behavior is apparently relatively insensitive to ethylene supply to leaves, except in very specialized circumstances.

We have evidence that the plant hormone ethylene can be involved in both the suppression of root growth during soil drying and the suppression of leaf growth via long-distance chemical signaling, again emphasizing a key role for this hormone in the regulation of plant production in dryland environments. Partial drying of the soil around the roots of tomato plants can maintain leaf water potential at values equivalent to well-watered plants for up to 2 weeks This is largely a function of partial stomatal closure in response to ABA/pH long-distance signaling from roots in drying soil. Ethylene evolution of WT tomato plants increased as soil dried but could be suppressed using transgenic (ACO_{1AS}) plants containing an antisense gene for one isoenzyme of ACC oxidase. The ACO_{1AS} plants also showed no inhibition of leaf growth when exposed to PRD (Sobeih and others 2004), even though both ACO_{1AS} and WT plants showed similar changes in other putative chemical inhibitors of leaf expansion (xylem sap pH and ABA concentration) (Figure 2). It seems likely that the enhanced ethylene evolution under PRD could be responsible for leaf growth inhibition of WT plants. The ACO_{1AS} plants showed no leaf growth inhibition over a range of soil water contents that significantly restricted growth of WT plants, but importantly, this only occurs under

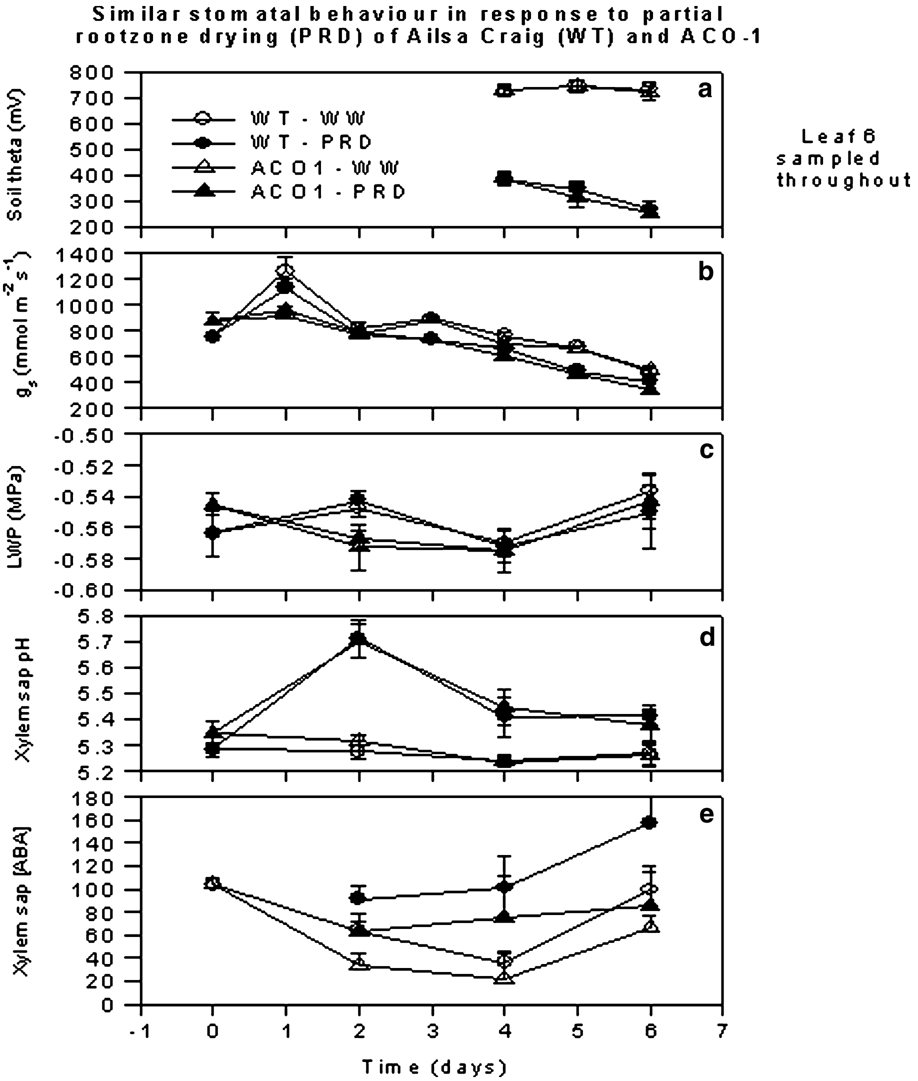

Figure 2. Effects of partial root drying on functioning of wild-type and transgenic tomato leaves. Moisture content of the upper 6 cm of potting compost from pots watered daily on both sides of the split-pot and from the drying side of the split-pot (**a**). Stomatal conductance (**b**), leaf water potential (**c**), xylem sap pH (**d**), and xylem ABA concentration (**e**) of fully expanded leaves at node 6. Points are from individual wild-type plants watered daily on one or both sides of the split-pot. (From Sobeih and others 2004).

partial root zone drying, which generates ABA signaling to regulate stomatal behavior and shoot water status.

Some studies of the effect of soil drying on tomato plants have failed to detect increased ethylene production, but there is now good evidence in the literature that enhanced ethylene production in plants in compacted soil leads to a limitation of leaf growth (Hussain and others 1999). Because an increase in soil strength is a key component of soil drying, it seems likely that this hormone is an important component of the changes in long-distance signaling capability stimulated by changes in soil water status. Nutrient stresses can also stimulate ethylene production, and another of the effects of soil drying is to reduce the uptake of some ions (Schurr and others 1992).

Again in compacted soil, ABA signaling plays a key role in moderating the plant's response to other signals. Hussain and co-workers (1999) have shown that ABA accumulation can reduce the growth-limiting effects of ethylene so that the net effect of ABA accumulation in plants at high soil strength is an increase in leaf growth relative to growth rates shown by low-ABA plants in similarly compacted soil. Sharp and co-workers (2000, 2002) have emphasized in a series of papers the interactions of ABA and ethylene in the regulation of root growth in drying soil. Again here, ABA accumulation will sustain growth by restricting run-away ethylene synthesis, which otherwise restricts root extension at low soil water potential.

The manifestation of the interaction between ABA, ethylene, and tissue water status is evident with reference to analysis by Sharp and others (2000), who point out that although a growth-inhibiting influence of ABA is apparent on primary maize roots at high water potentials, this is not the case when root water potentials are low (ABA concentrations are varied by external application of the hormone, application of fluoridone, and the use of mutants and transgenics). Presumably, these plants at high water potential have relatively low endogenous ethylene concentrations, whereas at lower water potentials, ethylene concentrations will increase. Here the effect of higher ABA concentrations is to promote growth by counteracting the ethylene effect. This contrasting effect of a single hormone as tissue water status varies highlights the importance of quantifying the interactions between hormones and other variables (in this case, plant water status) if we are to account for the impact of environmental perturbation on plant growth and development.

Conclusions

Much of the mechanistic basis of long-distance chemical signaling in plants in drying soil has been worked out with a relatively few crop species, largely in the laboratory. Attention has focused largely on a few hormones, pH, and individual ionic components of the xylem sap. Even here, our understanding of the signaling processes is very incomplete. Despite this, there are now several groups of investigators arguing for the principle of manipulation of the signaling process via irrigation practice, and these groups have now moved to the field to attempt to exploit these aspects of the plant's drought-stress biology (for example, Stoll and others 2000; Davies and others 2002). Some of this work is beginning to show impressive savings of water and associated benefits, such as increases in crop quality. More work is needed, however, particularly if we are to understand how to apply these treatments in different soils, in different climatic conditions, and to genotypes that may differ substantially in signaling capacity. It might be argued that the simple knowledge that there are root signals is enough for us to achieve improvement of plant growth and functioning under water-scarce conditions. We would suggest, however, that exploitation of the plant's physiology can be further optimized as we understand more about fundamental drought-stress physiology and biochemistry.

REFERENCES

Arshad M, Frankenberger WT. 1991. Microbial production of plant hormones. Plant Soil 133:1–8.

Bacon MA, Wilkinson S, Davies WJ. 1998. pH-regulated leaf cell expansion in droughted plants is abscisic acid dependent. Plant Physiol 118:1507–1515.

Bahrun A, Jensen CR, Asch F, Mogensen VO. 2002. Drought-induced changes in xylem pH, ionic composition, and ABA concentration act as early signals in field-grown maize (*Zea mays* L.). J Exp Bot 53:251–263.

Bano A, Dörffling K, Bettin D, Hahn H. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. Aust J Plant Physiol 20:109–115.

Bano A, Hansen H, Dörffling K, Hahn H. 1994. Changes in the content of free and conjugated abscisic acid, phaseic acid and cytokinins in the xylem sap of drought-stressed sunflower plants. Phytochemistry 37:345–347.

Blackman PG, Davies WJ. 1985. Root to shoot communication in maize plants and the effects of soil drying. J Exp Bot 36: 39–48.

Borel C, Frey A, Marion-Poll A, Tardieu F, Simmoneau T. 2001. Does engineering ABA biosynthesis in *Nicotiana* modify stomatal response to drought? Plant Cell Environ 24:477–489.

Chen C. 1997. Cytokinin biosynthesis and interconversion. Physiologia Plantarum 101:665–673.

Davies WJ, Wilkinson S, Loveys BR. 2002. Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytologist 153:449–460.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu Rev Plant Physiol Plant Mol Biol 42:55–76.

Dietz KJ, Wichert K, Sauter A, Messdaghi D, Hartung W. 2000. Extracellular β-glucosidase activity in barley involved in the hydrolysis of ABA glucose conjugate in leaves. J Exp Bot 51:937–944.

Dodd IC, Ngo C, Turnbull CGN, Beveridge CA. 2004. Effects of nitrogen supply on xylem cytokinin delivery, transpiration and leaf expansion of pea genotypes differing in xylem cytokinin concentration. Funct Plant Biol 31:903–911.

Else MA, Jackson MB. 1998. Transport of 1-aminocyclopropane-1-carboxylic acid (ACC) in the transpiration stream of tomato (*Lycopersicon esculentum*) in relation to foliar ethylene production and petiole epinasty. Aust J Plant Physiol 25:453–458.

Frankenberger WT, Arshad M. 1995. *Phytohormones in Soils.* New York, New York, USA: Marcel Dekker.

Freundl E, Steudle E, Hartung W. 1998. Water uptake by roots of maize and sunflower affects the radial transport of abscisic acid and the ABA concentration in the xylem. Planta 209:8–19.

Freundl E, Steudle E, Hartung W. 2000. Apoplastic transport of abscisic acid through roots of maize: effect of the exodermis. Planta 210:222–231.

Gerendás J, Ratcliffe RG. 2000. Intracellular pH regulation in maize root tips exposed to ammonium at high external pH. J Exp Bot 51:207–219.

Gomez-Cadenas A, Tadeo FR, Talon M, PrimoMillo E. 1996. Leaf abscission induced by ethylene in water-stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. Plant Physiol 112:401–408.

Hansen H, Dörffling K. 1999. Changes of free and conjugated abscisic acid and phaseic acid in xylem sap of drought-stressed sunflower plants. J Exp Bot 50:1599–1605.

Hansen H, Dörffling K. 2003. Root derived trans zeatine riboside and abscisic acid in drought stressed and rewatered sunflower plants: interaction in the control of leaf diffusive resistance? Funct Plant Biol 30:365–375.

Hare PD, Cress WA, Van Staden J. 1997. The involvement of cytokinins in plant responses to environmental stresses. J Plant Growth Regul 23:79–103.

Hartung W, Sauter A, Turner NC, Fillery I, Heilmeier H. 1996. Abscisic acid in soils: what is its function and which factors and mechanisms influence its concentration? Plant Soil 184:105–110.

Hartung W, Peuke AD, Davies WJ. 1999. ''Abscisic acid—a hormonal long distance stress signal in plants under drought and salt stress.'' In: Pessarakali M, editor. Handbook of Crop Stress 2nd edition New York, New York, USA: Marcel Dekker. pp 731–747.

Holbrook NM, Shashidhar VR, James RA, Munns R. 2002. Stomatal control in tomato with ABA-deficient roots: response of grafted plants to soil drying. J Exp Bot 53:1503–1514.

Holland MA. 1997. Occam's razor applied to hormonology. Are cytokinins produced by plants? Plant Physiol 115:865–868.

Hose E, Steudle E, Hartung W. 2000. Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes. Planta 211:874–882.

Hose E, Clarkson DT, Steudle E, Schreiber L, Hartung W. 2001. The exodermis–a variable apoplastic barrier. J Exp Bot 52:2245–2264.

Hose E, Sauter A, Hartung W. 2002. ''Abscisic acid in roots—biochemistry and physiology.'' In: Weasel Y, Ethel A, Kafka U, editors. Plant Roots: The Hidden Half. New York, New York, USA: Marcel Dekker. pp 435–448.

Hussain A, Black CR, Taylor IB, Roberts JR. 1999. Soil compaction. A role for ethylene in regulating leaf expansion and shoot growth in tomato? Plant Physiol 121:1227–1237.

Incoll LD, Ray JP, Jeer PC. 1989. ''Do cytokinins act as root to shoot signals?'' In: Importance of Root to Shoot Communication in the Responses to Environmental Stress. Br Soc Plant Growth Regul Monograph No. 21, pp. 185–200.

Jackson MB, Davies WJ, Else MA. 1996. Pressure–flow relationships, xylem solutes and root hydraulic conductance in flooded tomato plants. Ann Bot 77:17–24.

Justice WD, Peace AD, Pate JS, Hartung W. 1997. Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communist* L.) under phosphate deficiency and moderate salinity. J Exp Bot 48: 1737–1747.

Kakimoto T. 2001. Plant cytokinin biosynthetic enzymes as dimethylallyl diphosphate: ATP/ADP isopentenyltransferases. Plant Cell Physiol 42:677–685.

Kamboj JS, Blake PS, Baker DA. 1998. Cytokinins in the vascular saps of *Ricinus communis*. J Plant Growth Regul 25:123–126.

Liu FL, Jensen CR, Andersen MN. 2003. Hydraulic and chemical signals in the control of leaf expansion and stomatal conductance in soybean exposed to drought stress. Funct Plant Biol 30:65–73.

Masia A, Pitacco A, Braggio L, Giulivo C. 1994. Hormonal responses to partial drying of the root system of *Helianthus annuus*. J Exp Bot 45:69–76.

Munns R, King RW. 1988. Abscisic acid is not the only stomatal inhibitor in the transpiration stream. Plant Physiol 88:703–708.

Munns R, Sharp RE. 1993. Involvement of ABA in controlling plant growth in soils at low water potential. Aust J Plant Physiol 20:425–437.

Netting AG, Willows RD, Milborrow BV. 1992. The isolation of the prosthetic group released from a bound form of abscisic acid. J Plant Growth Regul 11:327–339.

Peterson CA. 1988. Exodermal Casparian bands, their significance for ion uptake by roots. Physiol Plantarum 72:204–208.

Peuke AD, Jeschke WD, Hartung W. 1994. The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L. III. Long-distance transport of abscisic acid depending on nitrogen nutrition and salt stress. J Exp Bot 45:741–747.

Peuke AD, Jeschke WD, Hartung W. 2002. Flows of elements, ions and abscisic acid in *Ricinus communis* under potassium limitation. J Exp Bot 53:241–250.

Prinsen E, Kaminek M, Van Onchelen H. 1997. Cytokinin biosynthesis: a black box. J Plant Growth Regul 23:3–15.

Rahayu YS, Walch-Liu P, Neumann G, Romheld V, von Wiren N, and others. 2005. Root-derived cytokinins as long-distance signals for NO_3-induced stimulation of leaf growth. J Exp Bot 56:1143–1152.

Richards RA. 1993. ''Breeding crops with improved stress resistance.'' In: Close TJ, Bray EA. editors. Plant Responses to Cellular Dehydration during Environmental Stress. Rockville, Maryland, USA: American Society of Plant Physiologists. pp 211–223.

Sauter A, Hartung W. 2000. Abscisic acid conjugates–do they play a role as long-distance stress signal in the xylem? J Exp Bot 51:929–936.

Sharp RE, LeNoble ME, Else MA, Thorne ET, Gherardi F. 2000. Endogenous ABA maintains shoot growth in tomato independently of effects on plant water balance: evidence for an interaction with ethylene. J Exp Bot 51:1575–1584.

Sashidar VR, Prasad TG, Sudharshan L. 1996. Hormone signals from roots to shoots from sunflower (*Helianthus annuus* L.): moderate soil drying increases delivery of abscisic acid and decreases delivery of cytokinins in xylem sap. Ann Bot 78:151–155.

Slovik S, Daeter W, Hartung W. 1995. Compartmental redistribution and long-distance transport of abscisic acid (ABA) in plants as influenced by environmental changes in the rhizosphere—a biomathematical model. J Exp Bot 46:881–894.

Soar CJ, Spiers J, Maffei SM, Loveys BR. 2004. Gradients of stomatal conductance, xylem sap ABA and bulk leaf ABA along canes of *Vitis vinifera* cv. Shiraz: molecular and physiological studies investigating the source. Funct Plant Biol 31:659–669.

Sobeih W, Dodd IC, Bacon MA, Grierson DC, Davies WJ. 2004. Long-distance signals regulating stomatal conductance and leaf growth in tomato (*Lycopersicon esculentum*) plants subjected to partial rootzone drying. J Exp Bot 55:2353–2364.

Steudle E, Peterson CA. 1998. How does water get through roots? J Exp Bot 49:775–788.

Stoll M, Loveys BR, Dry P. 2000. Hormonal changes induced by partial root zone drying of irrigated grapevine. J Exp Bot 51:1627–1634.

Takei K, Sakakibara H, Tanigushi M, Sugiyama T. 2001. Nitrogen dependent accumulation of cytokinins in roots and the translocation to leaf: implication of cytokinin species that induce gene expression of maize response regulators. Plant Cell Physiol 42:85–93.

Tardieu F, Zhang J, Katerji N, Bethenod O, Palmer S, and others. 1992. Xylem ABA controls the stomatal conductance of field grown maize subjected to soil compaction or drying soil. Plant Cell Environ 15:193–197.

Thomas JC, Smogocki AC, Bohnert HJ. 1995. Light-induced expression of *ipt* from *Agrobacterium tumefaciens* results in cytokinin acumulaion and osmotic stress symptoms in transgenic tobacco. Plant Mol Biol 27:225–235.

Vysotskaya LB, Kudoyarova GR, Veselov SY, Jones HG. 2004. Effect of partial root excision on transpiration, root hydraulic conductance and leaf growth in wheat seedlings. Plant Cell Environ 27:69–77.

Wilkinson S, Corlett JE, Oger L, Davies WJ. 1998. Effects of xylem pH on transpiration from wild-type and *flacca* tomato leaves: a vital role for abscisic acid in preventing excessive water loss even from well-watered plants. Plant Physiol 117:703–709.

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

Zhang J, Davies WJ. 1987. Increased synthesis of ABA in partially dehydrated root tips and ABA transport from roots to leaves. J Exp Bot 38:2015–2023.

Zhang J, Davies WJ. 1989. Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. Plant Cell Environ 12:73–81.