Food production: reducing water consumption by manipulating long-distance chemical signalling in plants

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Introduction

One of the great challenges for the future is the production of sufficient food for a dramatically growing population during a period of climate change where water supplies are dwindling, and phytotoxic air pollutants such as ozone are increasing. By 2050 global grain demand is expected to double (Postel, 1998; Tilman et al., 2002; Schiermeier, 2008). Greater food production, however, will require larger areas of irrigated land unless the water use efficiency (WUE) of crop plants can be increased. To increase and sustain food production the following ecophysiological, genetic, and agronomic actions have been suggested (Postel, 1998; Tilman et al., 2002; Schiermeier, 2008).

(i) Improving irrigation practices and equipment (sprinkler, drip irrigation).
(ii) Implementation of small-scale water-harvesting, terracing, improved rainwater storage, and increased soil moisture retention by improving soil structure (for example by adding manure, mulching).
(iii) Improving crop water use efficiency by using crop varieties that are more drought- and salt resistant, and by using strategic agricultural practices.
(iv) Ensuring a more equitable distribution of food to satisfy the basic nutritional needs of all people as water shortage increases.

Plant science, especially plant eco- and stress physiology, can inform future agricultural practices under points (i) and (iii). This opinion paper suggests some new simple procedures that may amplify or suppress plant stress signalling to improve water saving in agriculture. Much research in this area is aimed at impacting on the ability of plants to produce and respond to the stress hormone abscisic acid (ABA). This is because this hormone controls many of the adaptive responses that plants have evolved to conserve water when they perceive a reduced supply of this commodity. Stomatal closure, reduced canopy area, and increased root biomass are three of the major adaptive processes that ABA regulates that can potentially be manipulated to improve crop WUE (Wilkinson, 2004).

The ABA signalling pathway

In order to manipulate the ABA signalling system successfully, it is first necessary to understand it, and unfortunately this remains a contentious issue. When plants perceive drying soil it is accepted that they synthesize ABA. This new ABA or any pre-existing ABA can be moved around the plant to induce the required adaptive response, the closure of stomata being the most studied of these. What remains under debate, however, is the source of this ABA: root or shoot. Until recently it was accepted that the roots can be a major site for ABA biosynthesis when soil dries, and that this ABA is transmitted upwards to the shoot via the xylem. Whilst it has been shown that root-sourced chemical signals can be responsible for soil-drying-induced stomatal closure or reductions in leaf growth in the shoot (Passioura, 1988; Gowing et al., 1990; Holbrook et al., 2002), that roots can and do synthesize/export ABA in response to soil drying (Davies and Zhang, 1991; Thompson et al., 2007; Dodd et al., 2008), and that root-sourced ABA can affect stomata (reviewed in Davies and Zhang, 1991; Wilkinson and Davies, 2002), current opinion is shifting to the idea that soil-drying-induced ABA signals arising in the shoot (upon receipt of other signals from the root) are more important for the regulation of stomatal aperture. This is because several authors have recently determined that leaf or shoot xylem ABA content need not be affected by root genotype in reciprocal grafting experiments (where, for example, a wild-type rootstock is grafted on to a shoot from an ABA-deficient mutant), and that shoot rather than root genotype can be more important in determining stomatal conductance (gₛ; Holbrook et al., 2002; Thompson et al., 2007). On the other hand, older studies show that rootstocks do influence shoot conductance (Jones et al., 1987; Borel et al., 2001; and see Holbrook et al., 2002; Thompson et al., 2007, for further examples demonstrating both positive and negative...
influences of rootstocks on $g_s$). A synthesis of these ideas, and the most probable scenario, is that signals emanating from roots in contact with drying soil are likely to integrate both chemical and hydraulic signals, and both root- and shoot-originating sources of ABA, depending on (i) the extent and speed of the reduction in soil water content, (ii) the prior nutritional, water, and hormonal status of the plant, (iii) the aerial microclimate, (iv) developmental stage, and (v) plant structure and form. This has been described in detail by Wilkinson and Davies (2002, 2008). Root–shoot signalling of soil water deficit will not always rely absolutely on a supply of ABA that has been newly synthesized in the root (strong hydraulic signals can increase shoot ABA biosynthesis in the absence of root biosynthesis, ABA can be transported down to the root from the shoot and recycled back up to other parts of the shoot, pH signals emanating from either roots or shoots will amplify an ABA supply from the root or the shoot even in the absence of de novo ABA biosynthesis, ABA conjugates may be transported from the root to be cleaved in the shoot), but under many circumstances, a supply of ABA from the root will be involved in stomatal closure, and an influential portion of this ABA will have been synthesized there upon the sensation of soil water deficit (as determined by early reciprocal grafting experiments).

Manipulating the ABA signalling pathway using deficit irrigation

One way of inducing plants to deploy water-saving chemical and/or hydraulic signalling systems is by using deficit irrigation techniques such as regulated deficit irrigation (RDI) and partial rootzone drying (PRD). These techniques are already being used in some sectors of the agricultural community and the relative merits of each have been discussed in depth elsewhere (Loveys et al., 2004; Dodd et al., 2006; Fereres and Soriano, 2007; Sadras, 2009). RDI works on the premise that photosynthesis and fruit/grain growth are less sensitive to water deficit, and water deficit-induced root-sourced chemical signals like ABA, than transpiration. By reducing transpiration (closing stomata or reducing leaf surface area by reducing leaf growth) plants can cope with a reduced water supply. Biomass production can be reduced by water deficit without a negative impact on harvest index up to a point (usually at around 60% of maximum biomass), because of resource reallocation to fruits/grains (Fereres and Soriano, 2007). The principle behind PRD is that if only part of the root system is dried and the remaining roots are kept well-watered, chemical signals (changes in ABA concentrations and pH of the xylem sap) produced in the drying roots reduce stomatal aperture and leaf growth, thus preventing water loss (Stoll et al., 2000; Dry et al., 2001; Dodd et al., 2006). Meanwhile, the wet portion of the root system maintains a favourable water status in the shoot such that yield is not compromised and may even be increased or qualitatively improved (Dry et al., 2001). PRD techniques have been adapted to the use of drip (fruit crops: Costa et al., 2007) and furrow (field crops: Kang et al., 2000) irrigation.

How else can we manipulate ABA concentration? Some open questions

Because PRD irrigation techniques require relatively highly developed equipment and infra structure, and because most arid agricultural land is in developing countries, easier and simpler low-grade technologies may also be required to improve WUE, such as soil-friendly ploughing methods to reduce the runoff of water (see above, point ii). The possibility is suggested here of employing several other relatively simple non-irrigation-based techniques under point (iii) above, that also impinge on the ability of the plant to generate and respond to the plant hormone ABA, whether this is synthesized in the root or the shoot. The same caveats will apply in these instances that apply to the use of deficit irrigation, however. Firstly, the sensitivity of yield to water deficit/ABA signalling depends on developmental stage (Loveys et al., 2004). Secondly, techniques designed to increase ABA signalling will, like deficit irrigation, be more appropriate for crops that do not rely on an increase in leaf biomass for an increased yield, as ABA reduces canopy area. Thus they may be more difficult to implement with spinach, alfalfa, or lettuce, for example, whilst reducing the canopy area of fruit and grain crops redirects resources to the fruit/grain as described above, and/or increases fruit radiation interception. Leafy ‘biomass’ crops may still be cultivated using less water by increasing the rate at which the crop develops (Turner, 2004). Ironically, in such cases it will be pertinent to implement mechanisms which reduce ABA signalling, such that leaf growth is more rapid.

The following suggestions are derived from a review article of Jiang and Hartung (2008) who refer to several aspects of long-distance signalling that may have an impact on the ABA concentration in the xylem, and that could be manipulated in the field.

Soil micro-organisms

Abscisic acid in the xylem originates from internal (biosynthesis) and external sources. External ABA-synthesizing organisms predominantly comprise soil fungi which can produce ABA in high amounts. This ABA is released easily to the external medium because the plasma membrane of soil fungi exhibits a high permeability to the ABA anion. In fungal systems, the chemiosmotic concept whereby ABA is trapped in alkaline compartments (see below) does not function (Hartung and Eggeling, 1984). Can we increase concentrations of fungi in the rhizosphere to intensify ABA signalling?

Recently, several free-living soil bacteria that affect ABA relations in plant roots have been isolated and investigated. Variovorax paradoxus, a rhizospheric bacterium with an increased activity of 1-aminocyclopropane-1-carboxylic acid-deaminase (ACCd) reduces not only ethylene (ACC is the ethylene precursor) but also ABA in plants (Jiang and
Growth in neutral or alkaline soils. Thus matching species/variety to soil type is an important consideration in maximizing WUE.

**ABA-glucose ester (ABA-GE)**

The extremely hydrophilic glucose conjugate of ABA, ABA-GE, is a perfect long-distance signal because it is transported over long distances without any loss to the surrounding tissues (Sauter and Hartung, 2002). Because of its hydrophilic properties, a transporter is postulated that loads ABA-GE from the cytosol of the xylem parenchyma cells across their plasma membrane to the xylem vessels. In leaves, cytosolic and apoplastic β-glucosidases have been detected that cleave the conjugate and release free, physiologically active ABA to the target cells (Dietz et al., 2000; Lee et al., 2006). Crop plants with highly active ABA-GE-specific β-glucosidases should have strong ABA-signals, deriving from ABA-GE in the xylem. It will be important for plant scientists to characterize these ABA-GE transporters.

**ABA-metabolism**

Jiang and Hartung (2008) have published an ABA-flow model of maize treated with tetcyclacis, an inhibitor of cytochrome P450 mono-oxygenases that significantly reduces the conversion of ABA to phaseic acid (PA). A treatment of maize root systems with tetcyclacis resulted in an increased net biosynthesis of ABA in roots and a 4-fold increase in the intensity of the ABA signal. Since tetcyclacis is not very mobile within plants (normally it is sprayed directly onto leaves) ABA metabolism in leaves remained unaffected. Engineering plants with reduced degradation of ABA may provide effective solutions to the problem of increasing WUE.

**ABA biosynthesis**

Much work is currently being carried out to engineer crops with an increased ABA biosynthetic capacity that exhibit improved WUE without affecting seed dormancy or drastically reducing growth. This has been discussed in depth elsewhere (Tung et al., 2008).

**How can we increase stomatal sensitivity to ABA?**

Recently, Heilmeier et al. (2007) analysed more than 60 publications to elucidate the relationship between xylem ABA (ABA$_{xyl}$) and the maximal leaf conductance ($G_{max}$) of plants of different habitats, life forms, and ecotypes. In all cases, the same relationship could be found: a narrow range of ABA$_{xyl}$ within which $G_{max}$ reacts very sensitively and a wide range where even drastic concentration changes of ABA$_{xyl}$ have no or an extremely small impact on leaf conductance. In their global analysis they pointed out that, particularly in arid regions, plants exhibit a very low sensitivity to ABA. Under such conditions an increase in the intensity of the ABA signal and/or an increase in stomatal or growing cell sensitivity to ABA could be very helpful.
Manipulating xylem/apoplastic pH

The pH of the xylem and of the apoplast has also been shown to amplify the ABA signal as it moves upwards from the roots and into the leaves, and it can also amplify the ABA signal once it has reached the leaves (Wilkinson and Davies, 2002, 2008). Under well-watered conditions and favourable aerial temperatures, xylem/apoplastic sap is relatively acidic. Accordingly, the cells adjacent to the xylem vessels and the cells of the leaf are able to extract and remove a portion of the ABA from the transpiration stream as it moves upwards through the plant. If all the ABA sent upwards from the root even of a well-watered plant were to arrive at the stomata without this modification, its concentration would be so high that these would be permanently closed (Trejo et al., 1993). When soil dries or when leaves experience a high aerial temperature, the pH of the xylem sap emanating from roots and/or of the apoplastic sap within leaves can increase in some species (Hartung and Radin, 1989; and see Davies et al., 2002; Wilkinson and Davies, 2002). This traps ABA in the xylem and/or leaf apoplast and prevents it from being extracted by the cells adjacent to the transpiration stream. More of the ABA that is sent up to the shoot, or that is already there, actually penetrates to the stomata (or the growing cells) so that a pH signal can close stomata in the absence of any extra ABA being synthesized by the plant. The transpiration stream can also become enriched with ABA released from storage sites in adjacent cells when it becomes more alkaline.

The foliar apoplastic pH of intact plants can be directly manipulated to increase the intensity of the ABA signal that finally reaches the target cells (stomata and growing cells) by applying foliar sprays or root drenches that penetrate into the xylem and/or the apoplast of the plant. Wilkinson and Davies (2008) have demonstrated that spraying phosphate buffers adjusted to a pH of 6.4 or above onto the foliage of intact Forsythia and tomato plants mimics soil drying- or high aerial temperature-induced stomatal closure and reductions in leaf growth (Fig. 1) via an effect on ABA signalling. Results from Lancaster University have shown (i) that water savings of up to 20% can be made by spraying plants with alkaline buffers, and (ii) that species which do not necessarily respond to stress by generating endogenous changes in pH are still competent to respond to an alkaline foliar spray (RG Sharp, WJ Davies, unpublished results). Spraying plants with alkaline buffers will also have the advantage that water can be saved without subjecting plants to a water deficit as in RDI. Could this method be adapted for a crop in the field?

**Fig. 1.** The effect of pH on mean stomatal conductance ($g_s$, $n=6\pm SE$; A, C) and leaf elongation rate (LER; $n=5\pm SE$; B) when intact pot-grown Forsythia (A, B) or tomato (C) plants were sprayed once daily over the foliated region with water (controls) or phosphate buffers (10 mol m$^{-3}$ KH$_2$PO$_4/K_2$HPO$_4$) iso-osmotically adjusted to a range of pH values. Figure taken from Wilkinson and Davies (2008) *Journal of Experimental Botany* 59, 619–631 and reproduced by kind permission of Oxford University Press.
Soil fertilization

Early papers of Radin (1984), Radin and Ackerson (1981), and Radin et al. (1982) demonstrated that cotton plants grown under extremely stressful conditions in Arizona increased the sensitivity of their stomata to ABA when N or P-fertilization was slightly reduced. This increase in sensitivity may result from an influence of nitrate fertilization on xylem sap pH (Wilkinson, 2004; Wilkinson et al., 2007). As nitrate concentration increases, xylem pH exhibits a biphasic response: it acidifies from deficient to optimal nitrate concentrations (see Wilkinson, 2004), and then increases again at supra-optimal nitrate concentrations (inferred from Wilkinson et al., 2007). Stomatal conductance tracks these changes in pH (Fig. 2). Plants growing in either deficient or supra-optimal nitrate should therefore react to smaller changes in water supply more quickly and more sensitively.

Manipulating ethylene production/perception

The plant hormone ethylene can influence shoot growth and, more recently, it has been shown to influence stomatal aperture (see Acharya and Assmann, 2008). When UK upland vegetation was exposed in the laboratory to elevated ozone concentrations (equivalent to those experienced across much of the Northern hemisphere on hot sunny days) stomata of some species had very weak responses to ABA (applied as a foliar spray or synthesized endogenously in response to soil/air drying) and remained more open (Mills et al., 2009; Wilkinson and Davies, 2009). Leontodon hispidus plants also produced more ethylene—a common response to ozone pollution. However, when they were pre-treated with 1-methylcyclopropene (1-MCP), to prevent ethylene binding to its receptors, the stomata were able to close in response to ABA, soil drying as normal.

Tropospheric ozone pollution resulting from continued global industrialization and increased vehicle use has already been shown to be responsible for billions of dollars worth of crop losses, and its concentration is predicted to increase alongside the incidence of drought over the coming decades (Wilkinson and Davies, 2009). In polluted areas, crops in drying soil and/or hot air will produce ABA as expected, but their stomata may become desensitized to this ABA as a result of an ozone-induced up-regulation in ethylene production. These plants will be more susceptible to dehydration and reduced yield. This may be a contributory factor to the crop losses described above, although ozone has many other detrimental effects on plants.

Use of compounds that reduce ethylene production and/or perception should improve WUE under drought and ozone stress by re-sensitizing stomata to ABA, although it must be noted that some studies (in unpolluted plants) describe an opposing influence of ethylene on stomata; it closes them (see Acharya and Assmann, 2008; Wilkinson and Davies, 2009). 1-MCP is currently being successfully used to combat drought-induced senescence and to improve yield in the field (Rohm and Haas Company, 2009. INVINSA™ crop stress protection. A new tool to manage heat and drought stress. www.rohmhaas.com/wcm/information/industries/food_food_related/agriculture/invinsa.page). Could it also be used to improve WUE, particularly in polluted areas? Ethylene diurea (EDU), which reduces ethylene production, has also been successfully used in the field to improve crop yields in ozone-polluted regions of SE Asia (Tiwari et al., 2005; Elagoz and Manning, 2005). Improvements in yield were not suggested to have been a result of improved ABA sensitivity, although it will be important to determine whether or not this was the case. A more benign method of manipulating ethylene concentrations in stressed plants may be to use soil bacteria that produce ACCd and reduce xylem ACC concentrations (Belimov et al., 2009) as described above. Results from Lancaster University (S Wilkinson, WJ Davies, unpublished data) have demonstrated that soil inoculation of potted plants with Alcaligenes xylosoxidans strain Cm4, which produces ACCd, restored the stomatal response to ABA in ozone-treated L. hispidus plants 1–3 weeks later.

Conclusions and future research

As a result of the above considerations, the use of plant growth-altering soil micro-organisms in combination with strategic fertilization, seems to be promising, especially because such knowledge can easily be applied in the field without the...
need for complicated technology. Soil bacteria that impact on ABA signalling indirectly (Variovorax paradoxus, Bacillus subtilis), that destroy ABA on the root surface, that may produce ABA, or that reduce plant ethylene production should be investigated intensively. Crops with roots that exhibit Caspian bands in the hypodermis seem to be particularly useful under arid conditions. Alternatively, Fabaceae could be particularly valuable because they never form an exodermis. Despite this being a problem, under some circumstances they should respond particularly well to soil growth-promoting bacteria. These suggestions may also inform crop breeding. Other simple techniques that deserve attention may involve the use of foliar sprays or root drenches that alter xylem and/or apoplastic sap pH, or soil and foliar treatments that alter the production (EDU) or perception (1-MCP) of ethylene.

Further ahead it is suggested that, as well as engineering plants with enhanced ABA biosynthetic capacities, plant research using molecular techniques should focus on other areas. These should include the expression and formation of apoplastic and cytosolic β-glucosidases with high specificity to ABA-GE, which cleave ABA from ABA-GE, and transporters that release ABA-GE from the xylem parenchyma cells to xylem vessels. Genetic manipulation of ABA degradation in roots should also result in crop plants with increased long-distance ABA signalling. It would also be appropriate to reduce the expression of ACC and ethylene biosynthetic genes to improve stomatal sensitivity to ABA. Maize ACC synthase mutants have already been characterized (Young et al., 2004). However, as already pointed out by Marris (2008), gains from molecular biology cannot be expected within a short or medium time period. Improved agricultural management must be developed, based on eco- and stress physiological research, as described above. This includes the revival of traditional drought-tolerant crops (millet, chickpea, and others).

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