

REVIEW PAPER

Plant hormone interactions: innovative targets for crop breeding and management

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Abstract

Here we highlight how both the root and shoot environment impact on whole plant hormone balance, particularly under stresses such as soil drying, and relate hormone ratios and relative abundances to processes influencing plant performance and yield under both mild and more severe stress. We discuss evidence (i) that abscisic acid (ABA) and ethylene act antagonistically on grain-filling rate amongst other yield-impacting processes; (ii) that ABA's effectiveness as an agent of stomatal closure can be modulated by coincident ethylene or cytokinin accumulation; and (iii) that enhanced cytokinin production can increase growth and yield by improving foliar stay-green indices under stress, and by improving processes that impact grain-filling and number, and that this can be the result of altered relative abundances of cytokinin and ABA (and other hormones). We describe evidence and novel processes whereby these phenomena are/could be amenable to manipulation through genetic and management routes, such that plant performance and yield can be improved. We explore the possibility that a range of ABA-ethylene and ABA-cytokinin relative abundances could represent targets for breeding/managing for yield resilience under a spectrum of stress levels between severe and mild, and could circumvent some of the pitfalls so far encountered in the massive research effort towards breeding for increases in the complex trait of yield.

Key words: Abscisic acid, crop breeding, cytokinin, drought, ethylene, grain-filling, hormone, stomata, water use, yield.

Introduction

The ways in which crop plants generate, transport, and regulate both local and long-distance hormone-based chemical signals are important research targets. Similarly, the mechanisms by which these signals are translated into adaptations such as changes in root and shoot biomass and architecture, changes in plant water transport and gas exchange, and more direct yield-related parameters such as alterations in grain-filling rate, are being intensively studied. Such knowledge can greatly impact on the ways in which we design, or screen for more water-productive or stress-tolerant genotypes (Araus *et al.*, 2008; Mittler and Blumwald, 2010), and can allow the development of novel ways of treating/managing crops in the field (Davies *et al.*, 2002; Belimov *et al.*, 2009; Dodd, 2009). This type of research is becoming increasingly important in the context of food and energy security, because traditional or empirical

breeding for the primary trait of 'yield' is both slow (it can only be measured at plant maturity) and complex (yield is controlled by many different genes, each making a small contribution, such that taken as a whole, yield manifests low heritability and can react unpredictably to environmental change). Screening genotypes for a molecular marker or phenotype that can act as a surrogate for yield is potentially more rapid. However, determining which genes or phenotypes could act as yield surrogates requires in-depth knowledge of processes that impact on yield. Here we discuss the influence of plant hormone status in this context, and the influence of both the roots and the shoots on this balance.

Genetic linkages to drought-tolerance features such as stomatal closure, reduced canopy expansion, 'stay-green', and deep-rooting; as well as the biochemical processes that

control them, have been investigated in relation to yield maintenance or high water productivity under drought. Examples of single genes (or traits) associated with increased plant survival rates under relatively severe droughts, are those controlling cellular desiccation tolerance (Nelson *et al.*, 2007; Yang *et al.*, 2007) and changes in hormone concentration (Iuchi *et al.*, 2001; Rivero *et al.*, 2007; Thompson *et al.*, 2007). Genetic manipulation of these features induced yield gains under stress when tested under specific experimental conditions. However, this did not always translate to yield gains (and indeed sometimes gave rise to yield penalties) under field conditions, where water was freely available at least some of the time, and where there are other important genotype by environment (G×E) interactions which affect yield. One reason for the elusive nature of a ‘magic bullet’ for minimizing drought-induced yield impediments is that breeding for the conservative use of water frequently equates to breeding for plants with reduced rates of transpiration, through more closed and/or fewer stomata. This commonly leads to a reduction in photosynthesis and carbon fixation, therefore giving rise to smaller plants, at least where soil moisture levels are not limiting. There are, however, significant exceptions to this problem: Rebetzke *et al.* (2002) identified commercially applicable wheat genotypes with yield increases of 10% in dryland systems (below 500 mm of rain per annum), by screening for leaf level transpiration efficiency. Secondly, Thompson *et al.* (2007) demonstrated the possibility that high yields and low transpiration can, after all, be achieved simultaneously through genetic approaches (in tomato plants over-expressing *LeNCED1* and exhibiting increased abscisic acid [ABA] concentrations), because transpiration is more sensitive to stomatal closure than photosynthesis under many environmental conditions (whether it is induced by managed manipulation of the environment or by genetic control). Here *partial* stomatal closure resulted in large increases in water use efficiency, with no significant difference in biomass accumulation after 10 weeks growth in either droughted or well-watered plants.

Nevertheless, much of the progress in yielding under reduced water availability has come from breeding for traits not so obviously related to water stress, such as the modification of rates of phenological development at sensitive stages. For example, reducing the length of the anthesis silking interval (ASI; Bolanos and Edmeades, 1996; Badu-Apraku *et al.*, 2011) reduces the period of stress susceptibility, and thus the likelihood of a stress-induced yield reduction. This impressive gain in crop resilience is summarized well in a recent Royal Society report, ‘Reaping the benefits’ (The Royal Society, 2009).

More recently, the chances of engineering drought-resistant genotypes where yields are not penalized under less stressful water environments (Hammer *et al.*, 2006) have recently been improved through the use of stress-inducible promoters to drive the expression of drought-tolerance genes. Here, the chosen genes are only expressed during the stress period (Nelson *et al.*, 2007; Rivero *et al.*, 2007; Araus *et al.*, 2008; Qin *et al.*, 2011), and genes

inducing timely changes in hormone biology are among those shown to be effective.

The progress of breeding for tolerance to severe drought has also been hindered by the fact that any yield advantages gained from engineering/selecting for drought-tolerance traits will probably be very small in terms of grain weight, even if this is double what had previously been possible: doubling very little still equates to very little (Condon *et al.*, 2004; Fisher and Edmeades, 2010). At the other end of the spectrum, a promising alternative may be to breed for less conservative genotypes in terms of reductions in transpiration, under relatively mild drought. Such genotypes may well fare poorly under severe drought (and indeed they will deplete soil moisture faster and a mild drought may become a more severe drought), but when matched with environments that are likely to experience mild or intermittent droughts only, this may not be problematic (Collins *et al.*, 2008). Under rain-fed agriculture, even relatively mild droughts, such as those experienced in the UK, can reduce maximal yields (found under fully irrigated conditions or in wetter years) by as much as 30–60% (Dodd *et al.*, 2012). Even where yield gaps are smaller than this in terms of percentage yield loss, they can often be large in terms of actual grain weight, reflecting a change at the upper end of the scale. Reducing relatively small yield gaps by maintaining carbon gain at the expense of transpiration efficiency (when expressed in terms of water loss per unit leaf area or whole plant water use) may be a readily accessible breeding/management target where we can predict some hormonal influence.

Another set of problems encountered when breeding for improvements in leaf-level water use efficiency (WUE), is that these may not always translate into higher crop canopy WUE or yield. If attempts to improve WUE are made through increasing the sensitivity of stomatal closure to drought, canopy temperature can increase as stomata close and drive transpiration harder (Jarvis and McNaughton, 1986; Condon *et al.*, 2004), especially in hotter climes. Thus, just as much water escapes (via an increased leaf to air diffusion gradient) from the canopy of the stressed crop despite individual leaves having more closed stomata. Leaf level WUE is thus often uncoupled from canopy or ecosystem WUE (Condon *et al.*, 2004; Niu *et al.*, 2011). This increased canopy temperature may also have a more direct negative effect on yield, as cooler canopies have been associated with improved yield (Lopes and Reynolds, 2010). This problem might also be avoided by breeding for reduced sensitivity of stomatal closure under mild drought, particularly in warm climates.

Increased plant productivity under mild drought has indeed been correlated with the ability of plants to maintain high rates of growth and transpiration (Fischer and Edmeades, 2010). Over-expression of a maize transcription factor gene, *ZmNF-YB2*, conferred greater photosynthesis and stomatal conductance, lower leaf temperatures, and increased tolerance to such mild droughts, in transgenic maize plants in the field (Nelson *et al.*, 2007). Thus more open stomata can be associated with enhanced yield.

Here we identify some promising advances in our understanding of ABA, ethylene, and cytokinin signalling-response pathways, and interactions between them, that impact on crop performance and yield. We provide novel insight into how such new knowledge can be exploited through innovative crop management and crop breeding strategies. We discuss whether changes in ratios and relative abundances of hormone concentrations, rather than changes in the concentration of a single hormone per se, may represent useful targets for improving stress tolerance or, indeed, rapidly accessible surrogates for yield. This is because modelled plant responses (including yield responses in cereal and energy crops) to the changeable environment can be made to match measured responses more closely, when combinations of signals rather than single hormones/signals are used in model parameterization, both under experimental conditions and in the field (Tardieu and Davies, 1993). This is because signals often act in concert, either antagonistically or synergistically, when responding to environmental change/stress or developmental cues (Tardieu and Davies, 1992, 1993; Ghassemian *et al.*, 2000; Sharp, 2002; Yang *et al.*, 2006; Acharya and Assmann, 2009; Wilkinson and Davies, 2010). Furthermore different abiotic stresses, and indeed biotic stresses, often induce plant responses through shared or overlapping hormone signal transduction pathways (Mittler and Blumwald, 2010; Lee and Luan, 2012) and/or gene expression profiles (Seki *et al.*, 2001). Here we argue that breeding/managing for particular ranges of ABA:ethylene and ABA:cytokinin ratios or relative abundances, as biochemical markers that integrate the products of, and/or responses to such multi-gene regulated, multi-stress-responsive protein networks, may also be an appropriate strategy.

ABA

ABA has long been recognized as a plant hormone that is up-regulated in response to soil water deficit around roots, amongst other stresses. It plays an important role in the ability of plants to signal to shoots that they are experiencing stressful conditions around the roots, giving rise to water-saving anti-transpirant activity such as stomatal closure and reduced leaf/canopy expansion (Davies *et al.*, 2002; Wilkinson and Davies, 2002). ABA is also involved in adaptive 'deeper' root growth, and other aspects of architectural modification, under drought (Spollen *et al.*, 2000; Giuliani *et al.*, 2005) or nitrogen deficiency (Zhang *et al.*, 2007; Vysotskaya *et al.*, 2009). It also modulates aquaporin-related root and shoot hydraulic conductivity for improved soil moisture scavenging and plant water distribution (Parent *et al.*, 2009); and up-regulates processes involved in cell turgor maintenance and desiccation tolerance such as the synthesis of osmotically active solutes and antioxidant enzymes (Chaves *et al.*, 2003).

However, a given plant can respond very differently to a given ABA concentration depending on its environment and previous stress history, to the extent that ABA may

induce opposing effects on whole plant transpiration. It rapidly limits transpiration (within hours or even minutes; Sobeih *et al.*, 2004) through its well-known ability to induce stomatal closure as discussed above; however, ABA-induced increases in hydraulic conductivity via improved aquaporin functioning and/or osmotic regulation will tend to oppose stomatal closure in the long run, or indeed may fully overcome it (Tardieu *et al.*, 2010, Travaglia *et al.*, 2010), potentially leading to sustained growth under stress. In addition, other hormones such as cytokinins (CKs), auxin, and ethylene, may alter the effectiveness of ABA to modify its target cells directly, or indirectly by altering its biosynthesis (Davies *et al.*, 2005; Wilkinson and Davies, 2010; see below). Non-hormone-based signals of environmental change, such as changes in tissue water status and changes in apoplastic pH, can also modulate the extent to which ABA penetrates to, or affects target cells such as stomatal guard cells or growing leaf epidermis (Wilkinson and Davies, 2002).

Which of these potential ABA-induced effects predominates with respect to altering/adapting plant phenotype, is likely to depend on the chronological progression of changes in ABA concentration, ABA distribution, and downstream effects. For example, stomatal closure may be an initial rapid effect resulting from ABA accumulation in the apoplast in the vicinity of stomatal guard cells, whilst phenotypic reversion to sustained, high stomatal conductance may be possible days later, after enough time has passed for osmotically active solutes to be synthesized in leaf tissue, as a result of the sustained presence of ABA in the leaf symplast. Alternatively, or in addition, ABA accumulation at sites with high hydraulic resistance may contribute to the maintenance of intra-plant water flow, via increased aquaporin activity (Kudoyarova *et al.*, 2011), to override the opposing effect of ABA on stomata (Tardieu *et al.*, 2010), giving rise to an overall null effect, or, at least, moderating ABA's effectiveness as an anti-transpirant.

ABA and crop management

Advances in ABA signalling science have already allowed refined development of crop management techniques for reducing irrigation input, exemplified by the form of deficit irrigation known as partial root-zone drying (PRD), which is practised in parts of Southern Europe and China. Standard deficit irrigation can sustain yields under reduced irrigation because photosynthesis and fruit/grain growth are less sensitive to water deficit, and water deficit-induced root-sourced chemical signals like ABA, than transpiration. With a partially reduced transpiration rate, plants can both yield and still tolerate a reduced water supply. PRD allows some of the pitfalls associated with standard deficit irrigation (vulnerability to other abiotic stresses) to be avoided: one portion of the root-zone is kept irrigated (drip or furrow irrigation) such that shoot water relations remain relatively unperturbed even though total crop water application rates are still low. It has been shown that the ABA signal from the root in the dry portion of the soil can persist to alter the

shoot phenotype in some crops, despite improved shoot water relations (Davies *et al.*, 2002; Dodd, 2009). Other potential management techniques involving altered ABA signalling could include the use of relatively acidic foliar sprays to reduce stomatal ABA sensitivity (Wilkinson and Davies, 2002); or soil/seed inoculation with ABA producing or suppressing bacteria, and the search for these is fully active.

ABA and crop breeding

NCED encodes an enzyme that catalyses a rate-limiting reaction in the synthesis of ABA. Engineering the constitutive over-expression of *LeNCEDI* in tomato resulted in increased ABA accumulation and drought tolerance (Thompson *et al.*, 2007). However, breeding for changes in ABA biology (increased concentration or perception) that increase field performance and yield in crop species remains a great challenge, because of the necessity for avoiding inadvertent/concomitant reductions in carbon gain upon stomatal closure, ABA-induced reductions in seed germination, and ABA-induced pollen sterility and abortion (Ji *et al.*, 2011). To overcome these problems it has recently been found that the up-regulation of ABA concentration/sensitivity can be confined to the stress period, through links to drought-inducible promoters. For example, drought-inducible (and indeed constitutive) over-expression of the gene *ABA3/LOS5* (integral to ABA biosynthesis) in field-grown rice resulted in significantly increased yields (Xiao *et al.*, 2009). Using drought-inducible promoters (from *Arabidopsis*) to drive the *eral* anti-sense construct in canola (*ERAI* encodes the β -subunit of farnesyltransferase for increased plant sensitivity to ABA), also resulted in enhanced yield (Wang *et al.*, 2005).

In addition to the complexities arising from opposing direct and indirect effects of ABA itself on stomatal aperture and shoot growth rates (see above), other problems with breeding for manipulation of ABA biology can potentially arise through interactions between ABA and other hormones or signalling molecules, such as ethylene and CKs (see below).

Cytokinins

Cytokinins (CK) regulate the proliferation and differentiation of plant cells (Ashikari *et al.*, 2005). They also prevent senescence (Davies *et al.*, 2005): rapid premature leaf senescence and death often occurs when water is limiting during the grain-filling period, and genotypes possessing stay-green (photosynthetically more active) traits that lead to stress tolerance have been shown to contain elevated levels of CK in xylem sap (Borrell *et al.*, 2000). Transgenic cassava plants over-expressing CK show significant drought tolerance as a result of their stay-green capacity under stress (Zhang *et al.*, 2010). The *ipt*-gene, which drives CK biosynthesis, has now been over-expressed in several plant species under different promoters, and the transgenic plants have demonstrated improved stress tolerance (reviewed by

Peleg and Blumwald, 2011), including field analyses (Qin *et al.*, 2011).

However, the relationship between delayed leaf senescence and improved plant productivity does not always hold (Yang and Zhang, 2010). Constitutive over-expression of *ipt* results in detrimental pleiotropic effects such as decreased root growth, altered flowering time, and poor tissue water relations. Targeted reductions in CK content in transgenic tobacco and *Arabidopsis* plants over-producing cytokinin oxidase in roots, driven by root-specific promoters, was shown to increase root growth and plant survival under drought (Werner *et al.*, 2010). The results are in conflict with others showing that silencing of cytokinin oxidase genes to increase CK levels in non-stressed barley led to higher plant productivity, partially through improved root biomass (Zalewski *et al.*, 2010). Thus it is clear that effects of CK on plant productivity are diverse and possibly depend on where and when their content is changed and how the changes are related to other hormones and external conditions. Indeed tomato plants grafted on to rootstocks constitutively expressing *ipt* also exhibited a decrease in root biomass under control conditions (Ghanem *et al.*, 2011). However, under salinity stress the transgenic plants yielded 30% more than the wild-type plants.

It is now understood that *ipt* expression needs to be appropriately timed. Use of maturation-induced and stress-induced promoters (*SARK*, *senescence associated receptor kinase*) prevented negative effects of constitutively high shoot CK content on flowering time; and transgenic tobacco, rice, and peanut plants displayed enhanced drought tolerance and yield as a result of delayed stress-induced senescence (Peleg and Blumwald, 2011, Qin *et al.*, 2011). Transgenic peanut plants maintained higher photosynthetic rates, higher stomatal conductance, higher transpiration rates, and greater yields than wild-type control plants under reduced irrigation in the field (Qin *et al.*, 2011).

An alternative method for increasing CK concentration has been applied in rice and barley: transgenic plants with reduced expression of cytokinin oxidase (and thus increased CK levels in shoots) showed grain yield improvements in non-stressed plants linked to an increase in inflorescence number (Ashikari *et al.*, 2005; Zalewski *et al.*, 2010).

ABA–CK interactions

Increased xylem CK concentrations have been shown to decrease stomatal sensitivity to xylem ABA (Radin *et al.*, 1982), and this effect increases as leaves age (Blackman and Davies, 1985). CK content usually declines in stressed plants (Davies *et al.*, 2005), co-incidentally with ABA accumulation. A causal relationship between these phenomena was confirmed in experiments showing an ABA-induced increase in the expression of the gene coding for cytokinin oxidase (Brugiére *et al.*, 2003) as well as in the activity of enzymes catalysing irreversible CK degradation (Vysotskaya *et al.*, 2009). Treatment of plants with an inhibitor of ABA synthesis diminished both the activation of cytokinin oxidase in stressed plants, and the decline in CKs (Vysotskaya *et al.*,

2009). Since the decline in CK content in stressed plants is likely to sensitize ABA-induced stomatal closure, it may be beneficial to prevent this CK decline (perhaps via reduced expression of cytokinin oxidase) in plants experiencing mild or erratic soil water deficit, and prevent reductions in carbon gain associated with unnecessarily sensitive stomatal closure.

In addition to maintaining carbon gain under mild drought by de-sensitizing stomata to ABA, increasing CK concentrations in droughted plants may also be directly beneficial for grain-filling under stress. This is because, in rice and wheat at least, one yield detriment comes from the strong metabolic dominance of the apical spikelets in grain-filling, and the inhibition of filling to the inferior basal spikelets. CK up-regulation may improve inferior grain-filling (Mohapatra *et al.*, 2011) by enhancing the activities of cell-cycle genes to increase cell number, and thus the sink capacity of the developing seeds. Alternatively, the authors suggest that CK could improve phloem unloading. Slow filling rates in inferior grains have been linked with low contents of the cytokinins zeatin and zeatin riboside, and auxin (Yang *et al.*, 2000; Zhao *et al.*, 2007; Zhang *et al.*, 2009); and with low ABA content and high rates of ethylene evolution (Zhao *et al.*, 2007; Zhang *et al.*, 2009). The net development of a plant organ is regulated by a balance between hormones that promote and those that inhibit development. In the context of spikelet development in rice, it is possible that application of, or breeding for enhanced gibberellic acid, CK, or auxin may increase the relative proportions of hormones that promote and those that inhibit the grain-filling of inferior spikelets. But it would seem that increased CK content should not occur at the expense of ABA, as both are linked to high grain-filling rate (Zhao *et al.*, 2007; Zhang *et al.*, 2009). Again, breeding for high ABA plus high CK seems preferable under mild drought (where stress-induced senescence is not necessarily an issue), as both improved inferior spikelet grain-filling and reduced stomatal sensitivity to ABA can be coincidental, without the potential detrimental effects that low ABA content might have on, for example, root extension rate, aquaporin activity, and the synthesis of solutes involved in turgor maintenance.

Some of the work described above, using developmental- or tissue- specific promoters to control increases in *ipt* to reduce stress-associated senescence, have coincidentally reduced ABA concentration, alongside the increases in stomatal conductance, photosynthesis, and growth of plants exposed to drought or salt stress (Ghanem *et al.*, 2011). Could some of the positive effects of increased CK on yield under stress have been the result of stronger antagonism to ABA-induced stomatal closure at the guard cells? Peleg *et al.* (2011) suggest, however, that robust root development and increased water-absorbing capacity of the transgenic peanut plants allowed a higher stomatal conductance (rather than direct effects at the level of guard cell signalling), even under drought conditions, ensuring a higher CO₂ supply to leaf tissues and therefore a higher photosynthetic rate.

Manipulation of ABA–CK interactions through management

Increasing CK concentrations via exogenous applications to crop plants has generally proved ineffective due to *in planta* regulation of CK homeostasis via cytokinin oxidase. Another way of managing CK increases in plants may be through their inoculation with preparations of CK-producing bacteria, gradually releasing CK in concentrations within the physiological range (Arkhipova *et al.*, 2005). Some strains of growth-promoting rhizobacteria have been shown to produce high concentrations of CK (Omer *et al.*, 2004). Treatment of plants with one of these strains resulted in an increase in CK content in lettuce and wheat plants accompanied by their faster growth both in well-watered conditions and under soil moisture deficit (Arkhipova *et al.*, 2006). Here we show that grain yield in field-grown wheat plants under conditions of mild drought was increased by about 30–60% using a pre-sowing seed treatment of such a bacterial preparation (Table 1).

Ethylene

When up-regulated under stressful conditions such as heat and drought, the gaseous plant hormone, ethylene, can inhibit root growth and development, and, like ABA, it can also reduce shoot/leaf expansion (Sharp, 2002; Pierik *et al.*, 2006). It is probably best known for its involvement in stress-induced leaf senescence and abscission (Abeles *et al.*, 1992). There is also evidence that stress ethylene may directly reduce photosynthesis (Rajal and Peltonen-Sainio, 2001). Stress ethylene production, for example under heat, flooding, air pollution, soil compaction, and drought, can also induce more direct yield detriments, notably reduced grain-filling rates and/or increased embryo and grain abortion (Hays *et al.*, 2007; see Wilkinson and Davies,

Table 1. Effect of pre-sowing treatments of wheat seeds with *Bacillus subtilis* IB-22 bacterial suspensions (colony forming cells per ml) on wheat yield, where droughts were recorded over a portion of the growing season (approximately 100 mm rainfall in April–June) in a field experiment conducted in the Southern wooded steppes of Bashkortostan, Russia in 2009. Means \pm SE are shown. Yield components measured: grain weight, number of spike bearing tillers ($n = 50$) and numbers of surviving plants per bed (1.4 m² per bed, $n = 10$).

Bacteria concentration	Yield (ton ha ⁻¹) ^a	Grain weight (g plant ⁻¹) ^a	Number of spike-bearing tillers ^a	Survived plants per bed ^a
0	3.9 \pm 0.5	2.9 \pm 0.1	2.4 \pm 0.1	185 \pm 8
10 ⁵	4.5 \pm 0.4*	3.5 \pm 0.1**	3.1 \pm 0.1**	239 \pm 11*
10 ⁶	5.6 \pm 0.7*	4.1 \pm 0.2**	3.4 \pm 0.2**	256 \pm 9*
10 ⁷	4.8 \pm 0.5*	4.9 \pm 0.3**	3.0 \pm 0.2**	231 \pm 10*

^a An asterisk (*) indicates significant difference between plants grown from seeds pre-treated and untreated with bacteria (* $P < 0.05$, ** $P < 0.01$, Student's *t* test).

2010). As so many of the effects of stress on plants mirror those known to be ethylene-induced, there is now general acceptance that ethylene responses may underlie a portion of the limitation of seed crop yield under abiotic stress, and that the prevention of ethylene production or perception under stress is a desirable genetic or management target.

However, plant ethylene production also fluctuates developmentally. It stimulates seed germination, and high ethylene accumulation is traditionally associated with reproductive processes such as ripening (Abeles *et al.*, 1992). Despite the resource-conservative effects of stress ethylene described above, it can also be linked to the rapid expansion of newly initiated leaves (Hunter *et al.*, 1999). There is also growing evidence that environmentally-induced increases in plant ethylene generation can be growth-promotive under some circumstances or in some genotypes, such as during shade avoidance and plant competition, and when overcoming submergence stress (Sharp, 2002; Pierik *et al.*, 2006). It is necessary to understand how ethylene can be associated with these opposing sets of effects, before its regulation can become a successful genetic or management target. Some progress has been made recently in reconciling these opposing effects: the concentration of other hormones such as ABA can modulate the influence of ethylene on processes such as shoot and root growth and stomatal conductance (see below).

A role for ethylene in stomatal control is also gaining support. It has been shown experimentally to close stomata (Desikan *et al.*, 2006), and instances of stomatal closure via environmentally-induced ethylene accumulation have now been recorded (Vysotskaya *et al.*, 2011). Conversely, ethylene can antagonize drought- and ABA-induced stomatal closure (Tanaka *et al.*, 2005; Wilkinson and Davies, 2009). The contrasting effects of ethylene on stomata that can be demonstrated (above), alongside the clear detrimental effects of stress ethylene on leaf senescence, grain-filling, and grain abortion have, perhaps, prevented large-scale research efforts aimed at identifying a role for ethylene in drought tolerance and yield linked to stomatal control. However, as discussed above, under mild drought where effects on leaf senescence and grain abortion are less relevant to yield, the ethylene-induced antagonism of drought/ABA-induced stomatal closure (see below) may be beneficial for carbon gain and leaf cooling, both of which are clearly linked to improved yield, at least in hotter environments.

The detrimental effects of ethylene on crop performance and yield are not necessarily confined to a stressful environment, as they may also be linked to genotypic variability in productivity through plant architecture. As discussed above, slow filling rates in a percentage of the grains lower down on a rice spikelet or wheat ear, called inferior grains, have been linked with low contents of CK and ABA and high rates of ethylene evolution (Yang *et al.*, 2000; Zhao *et al.*, 2007; Zhang *et al.*, 2009). Developing grains in the lower part of the panicle/ear that are confined in the flag leaf sheath for a longer period, may be subject to the inhibitory action of ethylene more than those in the upper

part of the ear/panicle, which may be the basis of the fact that a spike or ear always contains at least some inferior, smaller, less plump grains, particularly in some genotypes. Mohapatra *et al.* (2000) used ethylene inhibitors to improve the growth and development of the inferior spikelets, while the application of ethylene promoters inhibited further growth and development. Ethylene has now also been linked to reduced grain-filling in both superior and inferior spikelets under stress (Mohapatra *et al.*, 2011), and the authors envisage that yield gains in grain crops could be made through screening genotypes for lower retention time in the leaf sheath and/or for shorter leaf sheaths.

Ethylene:ABA ratios

Evidence for various interactions between ethylene and other hormones that control adaptive responses (such as grain-filling, but also other processes such as stomatal conductance and growth) to resource scarcity, have been reported. For instance, ethylene is known to prevent ABA accumulation and vice versa (Spollen *et al.*, 2000), or to modulate cellular sensitivity to ABA (Ghassemian *et al.*, 2000; Wilkinson and Davies, 2010). Ethylene accumulation can antagonize the control of gas exchange and leaf growth by drought and ABA (Tanaka *et al.*, 2005; Wilkinson and Davies, 2010). This antagonism produces an opposing effect of ethylene to the conservative effects described in the previous section (stomatal closure, growth reduction), under conditions in which ABA accumulation is also increased. As discussed above, slow filling rates in inferior grains have been linked with low ABA content and high rates of ethylene evolution (Zhao *et al.*, 2007; Zhang *et al.*, 2009). Yang *et al.* (2006, 2007) express the grain-filling rate in wheat and rice in terms of a grain ABA:ethylene (or ABA:ACC ratio – ACC, or 1-aminocyclopropane, – is the ethylene precursor), which changes according to position on ear/panicle (superior or inferior) and/or in response to soil moisture content. Filling rate increases with the ABA:ethylene ratio. Furthermore, Sharp *et al.* (2004) show that root growth in droughted maize plants is partially maintained due to an ABA-induced decrease in ethylene synthesis.

Thus, evidence is accumulating that the sensitivity of various stress responses is dependent on a ratio between ethylene and/or its precursor ACC and ABA concentration, rather than on a given concentration of either hormone alone (Acharya and Assmann, 2009; Wilkinson and Davies, 2010). We argue that organ growth, grain-filling, and stomatal behaviour (amongst other effects) respond to such ratios per se, whether they are generated by changes in air pollution, soil drying or indeed in response to any other stress that affects tissue concentrations of either hormone. Here we provide evidence for ethylene–drought interactions relevant to gas exchange in wheat. Studies of the effect of 1-methylcyclopropane (1-MCP, an inhibitor of ethylene perception) on stomatal conductance and ABA content in leaves of droughted wheat plants demonstrated a sharper decline in stomatal conductance in response to drought in 1-MCP treated plants (Table 2), which was associated with

Table 2. Effect of 1-MCP treatment on stomatal conductance ($n = 10$) and ABA content ($n = 5$) in 10-d-old wheat plants 2 d after the imposition of a soil water deficit (soil water content maintained at 30% full capacity)

Stomatal conductance is expressed as % of control values (60% soil water capacity). Plants were grown under controlled conditions: PPFD $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ under a 14 h photoperiod at 22°C .

Treatment	Stomatal conductance, ($\text{mmol m}^{-2} \text{s}^{-1}$) ^a	ABA content (ng g^{-1} fresh weight) ^a
1-MCP-untreated	78 ± 6	197 ± 23
1-MCP-treated	$36 \pm 4^{**}$	$299 \pm 31^*$

^a An asterisk(*) indicates significant difference between untreated and treated with 1-MCP plants (* $P < 0.05$, ** $P < 0.01$, Student's t test).

higher shoot water potential (not shown) and increased leaf tissue ABA concentration. A greater accumulation of ABA in the plants occurred when these could no longer perceive ethylene. Thus, at least some wheat genotypes do not respond to soil drying as sensitively as is potentially possible, due to stress-ethylene-induced reductions in ABA accumulation. The extent of stress-ethylene production may be genotype-dependent (Balota *et al.*, 2004). This provides scope for genetic or management modulation of water use and carbon gain in field crops.

Ethylene and crop management

Ethylene physiology is accessible to manipulation via crop management. Syngenta-Dow have developed a new crop management technique to reduce crop ethylene perception and reduce stress-induced grain yield losses, based on novel applications of 1-MCP. Liquid foliar applications of 'Invinsa' (AgroFresh Inc., USA), which then produces gaseous 1-MCP, can act on a range of ethylene-associated process (above). Crop management using plant-growth-regulating rhizobacteria (PGPRs), as either seed treatments or soil additions, that reduce plant ethylene accumulation under stress by metabolizing the ethylene precursor, ACC, at the root-soil interface, also increase yields of legumes in the field (Belimov *et al.*, 2009). Tests are underway to develop its applicability to improve plant performance and yield in field grain crops (AA Belimov, personal communication).

Low endosperm ABA concentrations are associated with low rates of grain-filling in inferior spikelets of rice, and exogenous ABA application reverts grain-filling to rates equivalent to those in superior grains by improving assimilate partitioning to grains (Yang *et al.*, 2006). As described above, low ABA:ethylene ratios are associated with reduced grain-filling. Yang and Zhang (2010) suggest that controlled soil drying of irrigated crops in the mid- to late-grain-filling stages, to induce assimilate remobilization

of stored carbohydrates, can lead to improved grain-filling of inferior spikelets and increased harvest index (Yang *et al.*, 2007), by increasing the ABA:ethylene ratio.

Ethylene and crop breeding

Since genetic variability in stress ethylene generation exists within several species (Balota *et al.*, 2004), and this has been linked to stress sensitivity in terms of yield, leaf injury, senescence, and growth (Balota *et al.*, 2004; Hays *et al.*, 2007), it is feasible that screening and breeding techniques can be based on minimizing stress ethylene production in either roots, shoots, flag leaves and developing grains. We propose that it should be possible to determine ABA-ethylene ideotypes for the two 'ideals' of (i) conservative responses to severe drought and (ii) less conservative responses to erratic and/or mild soil drying. Conservative responses could be associated with relatively high ABA:ethylene ratios, whereas stress-insensitive stomata for improved yield under mild drought could be linked to lower ratios.

Ethylene biology has already been genetically modulated in plants with varying degrees of success in terms of stress tolerance. Ethylene-insensitive tomato mutants exhibit increased root biomass (Gallie *et al.*, 2010). Maize mutants deficient in ACC synthase (*Zmaccs6*), with lower rates of ethylene production, exhibit accelerated root elongation (Gallie *et al.*, 2009), and higher rates of CO_2 assimilation in expanding leaves (Young *et al.*, 2004). However, higher stomatal conductance and transpiration in these mutants reduced drought tolerance. We propose, however, that under milder drought, reduced stomatal closure and increased C gain in these, or similar mutants, could improve yield in the field.

By contrast, antagonism of ABA-induced stomatal closure by ethylene was suppressed in *etr1-1* and *ein3-1* ethylene-insensitive *Arabidopsis* mutants (Tanaka *et al.*, 2005). Dong *et al.* (2011) showed that an *acs7* mutant with reduced ethylene production exhibited increased tolerance to salt, heat, and drought stresses, linked with both greater ABA concentrations and the modulation of ABA stress-signalling genes under high salinity. Prasad *et al.* (2010) showed that *Arabidopsis* plants mutated in a gene that positively regulates lateral root development contained more ethylene and fewer lateral roots. The wild-type phenotype was restored by inhibition of ethylene biosynthesis and perception inhibitors, and by ABA, indicating that ethylene-induced reductions in ABA production and/or signalling were responsible for reduced lateral root formation.

Whilst such genetic manipulations are promising, it remains to be seen whether genetic modulations in ethylene biology are effective for crop improvement and higher yielding in the field.

Conclusions

Many processes that impact on crop performance and yield are controlled by plant hormone balance, which is

particular to a given organ or tissue. This balance is a function of environment and developmental stage. It can be influenced by signals/hormones emanating from and/or passing through roots in response to changes in the rooting environment, or by signals arising from altered shoot functioning in response to changes in either the root or the shoot environment. Here we highlight interactions between ABA and ethylene, which can act antagonistically to control grain-filling rate and number, stomatal conductance, and shoot and root growth. ABA's effectiveness as an agent of stomatal closure can be modulated by both ethylene and CK concentration, such that relative abundances or ratios of these hormones rather than a given concentration of one hormone alone, are most influential. Enhanced CK production can increase growth and yield by improving foliar stay-green indices under stress, and by improving grain-filling, grain number, and quality, and its effectiveness is also dependent on interactions with other hormones such as ABA. These phenomena are/could be amenable to manipulation through genetic and management routes, some of which are discussed, such that plant performance and yield can be improved. We propose that genotypes or management methods that give rise to stomata that close less sensitively in response to stresses such as soil drying when this is mild or erratic, may allow greater carbon gain, as long as the hormone balance in other tissues is not detrimental to, for example, rooting depth or grain/pod/fruit filling. The hormone balance appropriate to genetic or management-based improvements in plant performance and yield under more severe drought will be very different.

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