Perspective

Towards a conceptual ABA ideotype in plant breeding for water limited environments

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Abstract. A huge amount of information had been accumulated on abscisic acid (ABA). Laboratory and some field research with ABA-enhanced transgenic plants generally conclude that ABA is a drought resistance hormone, since it causes stomatal closure, reduces transpiration and results in 'water saving' under drought stress. This recurring conclusion is hard to accept in the agronomic domain considering the many direct and indirect negative effects of ABA on plant growth and reproduction. In order to formulate a conceptual phenotypic ABA ideotype for plant breeding, this paper begins by briefly reviewing the phenomics of ABA relative to plant function and productivity. Consequently, it is recognised that ABA enhancement is important in controlling the isohydric ('water saving') plant model, whereas plant hydraulics are more important in controlling the anisohydric ('water spending') plant model. Subsequently, the respective isohydric and anisohydric ideotypes appropriate to specific dryland crop drought stress scenarios are proposed. It is concluded that ABA can by no means be universally defined as a 'drought resistance hormone'. Its benefit or damage depends on the crop drought stress profile and the dynamics of the seasonal regimen of ABA in the plant. The isohydric ideotype might have an advantage in the harshest environments, whereas the anisohydric one will perform relatively better under more moderate drought conditions.

Introduction

ABA was first discovered as an abscission-promoting substance in cotton (*Gossypium hirsutum* L.) buds (Ohkuma *et al*. 1963), which would translate to the farmer as a problem. Research on ABA moved into high gear when it was realised that it was produced in plants under various stresses and most prominently under drought stress. Currently, the genomics of ABA is being studied extensively in order to resolve its molecular function and networking under drought stress.

Endogenous ABA concentration increase under drought stress is due to the expression of ABA biosynthesis genes. The increase in ABA reprograms gene expression patterns, resulting in a cascade of genes that regulate various physiological and metabolic responses. ABA is therefore defined as a 'drought stress messenger' (e.g. Raghavendra *et al*. 2010; Daszkowska-Golec and Szarejko 2013), interfacing the plant and its environment, as well as communicating between various plant organs and cells. The effect of the message as received by molecular receptors and transcribed through the network depends on ABA concentration and the sensitivity to ABA at the receiving end. Here, 'ABA enhancement' is defined as an increase in ABA concentration or an increase in plant sensitivity to ABA.

Great advances were made in understanding the metabolism and molecular networks involved in ABA production, transport and sensing. Expert reviews on the subject are abundant (e.g. Sreenivasulu *et al*. 2012; Daszkowska-Golec and Szarejko 2013; Finkelstein 2013; Kuromori *et al*. 2014). Nearly 5000 studies have been published in the past decade referencing just ABA and *Arabidopsis* (*Arabidopsis thaliana* (L.) Heynh.). Major recent breakthroughs include the identification of multiple receptor classes, transporters, metabolising enzymes and numerous regulatory pathways. Almost 200 loci regulate ABA responses and thousands of genes are regulated by ABA in various contexts (Finkelstein 2013). In the interest of space, all this information will not be repeated here.

This analysis is interested in the scaled up phenotypic impact of ABA on the projected field performance and productivity of crop plants subjected to drought stress and the lessons for plant breeding in this respect. Most research on ABA-responsive genes and the associated molecular networks in *A. thaliana* and other plants concluded almost unanimously that ABA enhancement confers drought resistance. Many of these studies were performed under limited laboratory and greenhouse conditions, and too often were subjected to unreliable drought stress phenotyping protocols (Blum 2014). Major conclusions that attempted to emphasise the importance for agriculture were based on the fact that ABA-enhanced transgenic plants closed stomata, used less water from the pot and thus did not wilt as quickly as the wild-type within a few days. Only very few such transgenic plants were subjected to real field drought stress conditions, with variable results (see

below). Moreover, ABA is recognised for its many negative effects on plant growth and fertility, which trace back to its historical discovery in abscising cotton buds. Therefore, when plant breeding considers the final integrated effect at the whole-plant and crop level, there is a gap between the huge molecular and genomic information on ABA and the limited information on its real impact on crop improvement under water-limited conditions. One important singular application of ABA knowledge in agronomy was related to the use of a partial root drying irrigation procedure designed for improving irrigation water-use efficiency (WUE) (e.g. Dodd *et al*. 2006).

Sreenivasulu *et al*. (2012) tried to resolve the 'contradictory role of ABA known to mediate two opposite face[s] of [a] coin: regulating abiotic stress tolerance and imparting growth retardation'. They tried to develop some answers by a separate analysis of the short- and the long-term effects of ABA under drought stress conditions, which is an important step in scaling up the role of ABA from the molecular and cellular level to the whole-plant level. However, although their excellent review ploughed deeply into the molecular networking of ABA, they seem to have had difficulty in harvesting conclusive answers to their question. Evidently, their difficulty in proposing some practical conclusions is the *a priori* acceptance at face value that ABA universally regulates abiotic stress resistance.

Another review that addressed ABA and plant breeding (Xiong 2007) stated that 'to date, limited success has been achieved in regulating plant ABA action for increasing plant drought tolerance'. It added that more research was needed for 'revealing the mechanisms of ABA action in stress adaptation'. That review concluded that 'without some certainty as to the contribution of ABA levels to drought tolerance, breeding ABA levels using conventional approaches for enhancing drought tolerance may not work well.' Xiong's review also accepted at face value that ABA was a universal stress resistance factor.

This perspective paper does not share the above views about the application of ABA knowledge in breeding for drought resistance. More research on ABA in stress adaptation may be needed but it is not the heart of the problem. The impact of ABA on the phenotype is becoming clearer and what is required is a scaled up view of the possible ABA-regulated phenotype at the field level. If such a phenotype can be resolved with respect to its environment, then plant breeding can begin to formulate a conceptual ABA ideotype for drought resistance and plant production. When a specific phenotypic ideotype is drafted, then genomics can perhaps help to engineer it towards final assembly on the breeding production line.

The phenomics of ABA-enhanced plants – a summary

The information compiled on ABA phenomics has been scanty since the publication by Davies and Jones (1991). In more than a few cases, as reviewed below, there is no apparent consensus about the exact direction of ABA enhancement on the given trait, whereas in other cases, consensus is solid. In some cases, a cellular level phenotype cannot yet be transcribed into a scaled up phenotype.

Research on the effect of ABA enhancement in plants has sometimes been performed by spraying plants with ABA solutions of varied concentration. Besides the inherent problem of ABA's *modus operandi* under such treatment

compared with endogenous ABA, there are technical issues such as ABA penetration into the leaf under the effect of light, temperature, the concentration used and the different plant response immediately after a single spray and a short time afterwards (e.g. Travaglia *et al*. 2010). Endogenous ABA accumulation is not normally subjected to bursts of concentration followed by a decline. Therefore, reported experiments involving the use of ABA sprays have been subjected to close inspection regarding their methodology and conclusions before being cited here.

Plant water relations

ABA enhancement causes stomata to close, with a resultant decrease in leaf gas exchange. This is a major point of consensus and it is also the first point of the evident clash between survival and productivity. Stomata also close in response to leaf turgor reduction and there is an ever-existing interaction between turgor and the effect of ABA on stomata, where ABA can promote turgor by stomatal closure, although low turgor might generate gene expression leading to ABA enhancement. Attempts to prioritise these interactions beyond their molecular level are not at hand. It is, however, absolutely clear that ABA enhancement serves to close stomata with the aim of maintaining turgor, at the cost of reduced assimilation.

When plants are subjected to drought stress, the bulk of the ABA is generated in three main organs: the root, the leaf and the growing seed (or fruit). ABA may also be generated in the stomata guard cells under the effect of low vapour pressure deficit (VPD) (Bauer *et al*. 2013). However, stomatal sensitivity to high VPD may involve both ABA and hydraulic signals (Assmann *et al*. 2000; Kholová *et al*. 2010).

As the soil dries, ABA is produced in the root and transported to the shoot in the xylem sap, and will cause stomatal closure before a significant reduction in leaf water status takes place. The huge amount of information on this hormonal root signal is not cited here, but it is a point of consensus since it was reported by Blackman and Davies (1985), besides a few cases where such a signal was not evident. ABA production in the root under drought stress is mainly induced by soil drying and hardness. It was suggested that ABA production is largely determined by the average soil moisture content at the root level (Puértolas *et al*. 2013). However, most importantly, this signal can also be generated by dry topsoil even when deeper soil still contains available moisture (Blum *et al*. 1991). In either case, dry topsoil can generate ABA signalling and stomatal closure while shoot water status is hardly affected due to deeper soil moisture availability. This is a very common drought stress profile in dryland fields, especially in row crops that expose the topsoil to evaporation. Under such conditions, root ABA constitutes an early warning system, which, in the eye of the farmer, might be too early. At the same time, ABA enhancement under soil drying improves root hydraulic conductance (Hose *et al*. 2000; Kudoyarova *et al*. 2011), with or without the involvement of root aquaporins (Javot and Maurel 2002; Mahdieh and Mostajeran 2009). Root growth may or may not be enhanced by ABA when the whole root system is considered and not only a single root axis (Leach *et al*. 2011 and references therein; Yamaguchi and Sharp 2010).

Thus, root-sourced ABA's message under soil drying can enhance water uptake by the root on one end while reducing water loss on the other. However, once stomata close and transpiration is reduced in the ABA-enhanced plant, the advantage to the plant of enhanced root conductance or root growth under the effect of ABA is greatly diminished, if not lost altogether. The maintenance of high water potential in the leaves (by stomatal closure) reduces the water potential gradient between leaves and roots, which is a prime condition for soil water uptake. Therefore, the cost for this high alert to the dryland crop plant can be twofold: there is available water in the soil that is not being used for plant production on one end and a reduction of assimilation by stomatal closure on the other.

ABA exported from roots to shoots might raise another crucial problem. Plants that have an inherent capacity for osmotic adjustment (OA) will accumulate solutes in direct relations to the rate of reduction in leaf water potential (Jones and Rawson 1979). Without this hydraulic signal, appreciable OA will not take place. Whether ABA has any direct effect on OA is discussed below, but OA will not take place at high leaf water potential. The advantage of OA in coping with drought stress and in sustaining plant production under drought stress is well documented (Blum 2010). OA retains turgor and facilitates the extraction of more water from the soil. This is avoided by the hormonal early warning system, leading to a quiescent state in response to drought stress. The farmer would say that the plants are sitting there looking beautiful but not doing their job.

Leaf and shoot growth

ABA reduces growth directly and indirectly. The direct effect of ABA on cell expansion most probably operates through a decrease in cell wall extensibility (Van Volkenburgh and Davies 1983). The imported root-sourced ABA might have an initial positive effect on leaf growth, possibly through sustained high leaf water potential. However, as stomata close and assimilation is reduced, growth is reduced and the final result is a reduction in total plant biomass (Tardieu *et al.* 2010). This effect can also be seen in the reduction of tiller bud growth in oats (*Avena sativa* L.) (Harrison and Kaufman 1980), which could also result from an interaction with other hormones, as seen in rice (*Oryza sativa* L.) (Liu *et al.* 2011). The reduction in leaf growth and total crop leaf area under the effect of ABA will reduce crop water use, in accordance with the effect of stomatal closure. This can present an advantage for dryland productivity in certain drought scenarios, which will be discussed later.

ABA induces dormancy in seeds (e.g. Miransari and Smith 2014), which was a very early observation in plant hormone research. However, the production of ABA in living plants exposed to drought stress can also induce dormancy, as the case is for *Poa bulbosa* L., a perennial geophytic grass (Ofir and Kigel 2007) or for certain trees (Rohde and Bhaleraoemail 2007). ABA accumulation is involved in cold hardening and freezing tolerance, partly via its capacity to induce dormancy alongside other metabolic modifications (Kobayashi *et al.* 2008; Dörffling *et al.* 2009). In some respects, the expression of ABA enhancement in drought response may also be regarded as a move towards a whole-plant dormant state, awaiting rehydration.

Metabolism

ABA's involvement with metabolism is, of course, reflected in growth, fertility and other facets of plant growth and productivity under drought stress.

Photosynthesis

The impact of ABA enhancement on photosynthesis is of prime interest with respect to plant production under limited water conditions. A meta-analysis of the physiological limitations to photosynthesis under drought (Pinheiro and Chaves 2011) did not offer a conclusive opinion on the role of ABA in photosynthesis under drought stress, besides the evident effect via stomatal closure. ABA can potentially affect photosynthesis by three routes: via stomatal closure, via CO_2 diffusion to the chloroplast and via any possible injury to the photosystem. Furthermore, Paul and Foyer (2001) convincingly argued that sink reduction (in this case by ABA-inflicted sterility and fruit abortion) could in itself constitute a feedback signal towards reduced photosynthesis.

Compared with the ample information on the repercussions of ABA on stomatal gas exchange, data on the other two routes of effect is scant and inconclusive. For example, leaf mesophyll cells of common bean (*Phaseolus vulgaris* L.), *Nicotiana tabacum* L. and *Solanum lycopersicum* L. challenged by ABA over periods of up to 5 h did not change the rate of photosynthesis (Mawson *et al.* 1981). ABA treatment of sunflower (*Helianthus annuus* L.) leaves did not reduce mesophyll conductance to CO_2 (Vrábl *et al.* 2009). Exogenous application of ABA caused an inhibition of the carboxylation of ribulose-1,5-bisphosphate in both a C_3 species (*Xanthium strumarium* L.) and a C_4 species (maize, *Zea mays* L.). However, the Rubisco activity of rapidly prepared extracts of ABA-treated leaves was as high as that of control leaves (Fischer *et al.* 1986). Rubisco activity was almost unaffected in ABA treated plants, whereas carbonic anhydrase (EC 4.2.2.1) increased more than twofold (Popova *et al.* 1996). So, although ABA may inhibit chlorophyll synthesis (Jelić and Bogdanović 1989), it is not clear if endogenous ABA production under drought stress will seriously reduce photosynthesis beyond the induced stomatal closure.

Stomatal closure reduces CO_2 supply to the photosystem and, as a consequence, the photosystem becomes subjected to excessive light stress and reactive oxygen species production (Takahashi and Badger 2011). At the same time, ABA has been noted to induce reactive oxygen species production directly, which, in turn, upregulates the native antioxidant defence system (Kuromori *et al.* 2014). Therefore, antioxidants can be important in protecting the photosystem against the direct and indirect negative effect of ABA, but it is not clear to what extent ABA results in final damage to the photosystem, if at all. It is obvious that plants subjected to enhanced ABA require effective protection of the photosystem against excessive light stress and reactive oxygen species production, a protection which is perhaps already native.

Sugar metabolism

There are strong indications for an overlap and interaction between ABA and sugar signalling (Dekkers *et al.* 2008), and that ABA may regulate cold tolerance if sufficient sugar is

present (Gusta *et al.* 2005). However, there is no sufficient information to formulate a conclusion with respect to ABA and sugars in whole-plant performance under drought stress. Some conclusions can be drawn, perhaps, with respect to reproduction, as discussed below.

Desiccation tolerance as a metabolic phenomenon

Desiccation tolerance as a different trait from drought resistance is defined here as a plant's capacity to survive extreme desiccation that would cause nontolerant angiosperms to die. Typical extremely desiccation tolerant plants can reduce their water content to that of dry seed and then revive upon rehydration. Desiccation tolerance is not rare in bryophytes (liverworts, hornworts and mosses). Experiments with some nontolerant bryophytes have demonstrated that treatment with ABA-generated desiccation tolerance is similar to or even better than in some of the native tolerant species (Oliver *et al.* 2005). Resurrection plants such as *Craterostigma plantagineum* Hochst., *Xerophyta viscosa* Baker and *Sporobolus stapfianus* Gand. have long attracted the attention of botanists, and, more recently, have been subjected to a detailed dissection of the physiological, metabolic and molecular basis of their tolerance (e.g. Bartels *et al.* 2007; Moore *et al.* 2009). In several resurrection plants, ABA was found to be involved with desiccation tolerance by inducing the accumulation of stress-protective proteins (e.g. late embryogenesis abundant (LEA) proteins), the production of antioxidants and the accumulation of various sugars. However, the desiccation tolerance of these plants is not conditioned by ABA signalling only; other pathways are involved, depending on the species. There has been and probably still is some ongoing research on the possibility of using these plants as a genetic resource for improved stress resistance in crop plants (e.g. Toldi *et al.* 2009). However, besides various technical issues, the main far-reaching problem is retaining a capacity for plant production with the desiccation-tolerant phenotype. The solution, perhaps, is not in replicating the desiccation-tolerant phenotype *per se* in a crop plant but rather in deploying some of the specific ABA-regulated tolerance factors such as the LEA proteins (e.g. Chandra Babu *et al.* 2004).

Plant reproduction

Whatever the impact of the enhanced ABA ideotype might be, the main agronomic consideration in most cases is its effect on grain or fruit production.

Fertilisation and seed set

Morgan (1980) and Morgan and King (1984) were probably the first to prove that ABA accumulation in spikes of wheat (*Triticum aestivum* L.) subjected to drought stress during pollen meiosis was responsible for poor seed set. Drought stress decreased flag leaf turgor but not spike turgor. High ABA content in the spike was sourced at the flag leaf (or also perhaps at the root, as known today). The effect of ABA in this respect was pinpointed at the anthers (Lee *et al.* 1988). Westgate *et al.* (1996) found that ABA can cause wheat spike sterility by affecting both anthers and ovaries. However, Dembinska *et al.* (1992) showed that wheat seed set reduction under drought stress could be ascribed also to reduced spike

water potential. Having more sophisticated research tools at hand, Ji *et al.* (2011) confirmed very nicely that drought stress induces wheat pollen sterility via ABA enhancement. Finally, ABA's effect on pollen sterility was also found in other crops besides wheat (e.g. Shukla and Sawhney 1994) and under the effect of abiotic stresses other than drought (Oliver *et al.* 2007).

Reproductive organ abortion

Flower or fruit abortion (abscission) is a natural phenomenon known to cotton growers, where plants may produce an ample number of flowers and then adjust fruit bud numbers to the available assimilates by abortion. The difference in the rate of abortion between two cotton cultivars was associated with a respective difference in the ABA content of the aborted fruit (Davis and Addicott 1972). A drought-induced decrease in water potential and an increase in the ABA content in flowers, pods and leaves at critical developmental stage were found to contribute to pod abortion in soybean (*Glycine max* (L.) Merr.) (Liu *et al.* 2003, 2004*a*, 2004*b*). The presence of lower placed older fruit of common bean plants regulated an increase in ABA content of younger fruit at higher placement, which induced their abortion. The experimental removal of older fruits reduced ABA content in younger fruit and reduced their abortion (Tamas *et al.* 1979).

The recovery upon irrigation of kernel set in maize subjected to drought stress after pollination was ascribed to interaction between sugar availability and a reduction in ABA content in the developing kernels (Setter and Parra 2010). On the other hand, a field study with maize in Denmark (Asch *et al.* 2001) could not confirm an association between kernel abortion and the ABA content of the ovary under conditions of prolonged stress. This result could support the unique situation of maize, where kernel survival after drought stress can depend also on sugar availability (Zinselmeier *et al.* 1995) and perhaps sugar storage in the cob (Reed and Singletary 1989). More recent work at the molecular level has suggested that maize kernel abortion under drought stress is regulated by sugar availability, sugar signalling and high ABA concentration (Kakumanu *et al.* 2012).

Grain filling and fruit growth

It was initially shown that ABA effect on grain filling was not unique and that a high ratio of ABA to ethylene could promote grain filling (Yang *et al.* 2004). The ABA content of wheat grain was remarkably enhanced by water deficit and was positively correlated with sucrose to starch conversion in the endosperm (Yang *et al.* 2004*a*). Wheat grain filling under mild or severe soil drying depended on the balance between ABA and ethylene, and the direction of ABA's effect on grain filling depended on the stress intensity. Thus ABA's effect on grain filling seems to depend on hormones via interactions with stress severity (Yang *et al.* 2006). In barley (*Hordeum vulgare* L.), it has been clearly indicated that ABA levels correlate with starch content in the developing grain (Seiler *et al.* 2011).

Stem reserve mobilisation is an important source for grain filling in the absence of transient photosynthesis under stress. Irrespective of storage availability, its use depends on the signal and the capacity for mobilisation in times of need. ABA is an

important signal for reserve mobilisation towards wheat grain filling under drought stress (Yang *et al.* 2003). Fructan exohydrolase (EC 3.2.1.80) and sucrose phosphate synthase (EC 2.4.1.14) are enzymes associated with wheat stem fructan storage breakdown to more soluble C, which can be remobilised from stem to grain. The enhanced fructan exohydrolase and sucrose phosphate synthase activities in wheat stems under drought stress is regulated by enhanced ABA signalling (Yang *et al.* 2004*b*). The effect of ABA in this respect is linked to its regulation of severe leaf senescence and it might therefore be in opposition to the maintenance of the stay-green phenotype as regulated by cytokinin (Peleg and Blumwald 2011).

In conclusion, ABA enhancement increases sterility and abortion, but might help to sustain growth in the remaining grain and fruit.

Yield

Despite the huge amount of work and publications on ABA in plants under stress, information on ABA's effect on crop yield under drought stress is surprisingly meagre, leading one to suspect that many negative experimental results were not published. In this discussion, experiments evaluating yield in very small pots are not considered as finite evidence, since stomatal closure and its effect in potted plants cannot be translated to the reality of field performance (Blum 2014). The results considered here are derived from three types of experiments: (1) transgenic plants tested in the field, (2) field results from divergent selection for constitutive ABA accumulation in plants and (3) comparative studies of naturally differing genotypes in ABA content under stress.

Downregulation of the *A. thaliana* gene encoding the β-subunit of farnesyltransferase (*ERA1*) enhances the plant's sensitivity to ABA and induces stomatal closure only when drought stress occurs. Conditional and specific downregulation of farnesyltransferase in canola (*Brassica napus* L.) using theAtHPR1 promoter resulted in yield increases under the Canadian dryland conditions over three test years (Wang *et al.* 2005). The effect of stomatal closure under ABA was to reduce seed abortion. The report provided information on seasonal rainfall, which totalled 359 mm, 140 mm and 275 mm in the three test years, respectively. It might be assumed that under the very low seasonal water supply (especially in the last 2 years), stomatal closure under stress during the first part of the crop cycle moderated growth and water use so that more moisture remained for the reproductive growth stage when rainfall was lacking, resulting in reduced seed abortion compared with the wild-type. In another experiment by the same group with ABA-sensitive transgenic canola lines in two dryland field experiments in Canada (Wang *et al.* 2009), similar results were obtained. Seasonal rainfall in the two test years was very low: 145 mm and 172 mm. The explanation of the benefit of enhanced ABA sensitivity could be the same here. Since these two crops must have been grown after a fallow (145 mm and 172 mm of precipitation cannot support a canola crop), the enhanced ABA sensitivity might have helped economise use of the available stored soil moisture during the early growth stages. It would have been of great interest to know the seasonal progress of ABA concentration and crop water use in these crops.

High ABA wheat selections performed better than low ones when stress was applied at reproduction (Innes *et al.* 1984). Surprisingly, there was no effect of lines on total water use. There was no effect of lines on yield when stress was applied at the vegetative stage. There was not enough information in the report to help understand these results.

On the other hand, maize lines were divergently selected for constitutively expressed high and low leaf ABA content (Landi *et al.* 2001), a trait found later to be associated with one major quantitative trait locus (Landi *et al.* 2005). In repeated field tests performed in Italy and China, the low ABA lines were more drought-resistant and productive compared with the high ABA lines. This was also expressed in a relatively cooler canopy of the low ABA lines under stress. The high ABA lines were found to be more lodging resistant where lodging conditions occurred. (Landi *et al.* 2007).

Leaf ABA concentration under severe stress declined linearly with cycles of maize selection for yield under reproductive stage drought stress, even though this trait had not been part of the selection index (Mugo *et al.* 1999). Leaf ABA concentrations before and after flowering were negatively correlated with grain yield across selections under severe stress and under well-watered conditions, thus lending support to the above results by Landi and associates for the relative yield advantage of low ABA maize lines under drought stress.

Overexpression of the stress-responsive gene *Stress-responsive NAC1* (*SNAC1*) promoted ABA sensitivity. Single transgenic rice plants overexpressing *SNAC1* yielded more (due to reduced sterility) than wild-type plants when stress was applied at the reproductive stage (Hu *et al.* 2006). This result is interesting but difficult to understand, considering the authors' stated observation that 'the transgenic rice are more sensitive to abscisic acid and lose water more slowly by closing more stomatal pores, yet display no significant difference in the rate of photosynthesis'. However, *SNAC1* transgenic rice also transpired less before stress was applied. Therefore, depending on the seasonal water regime (not specified), it could be possible that ABA sensitivity had a role in managing seasonal water use towards water conservation for the terminal growth stage when drought stress was imposed. In following experiments by the same group, transgenic rice overexpressing the *LOS5* gene for enhanced ABA content was field-tested in short nursery rows. The transgenic line had a higher yield than the wild-type when drought stress was applied before flowering (Xiao *et al.* 2009).

Taking all of this information together, it seems that the final effect of enhanced ABA on crop yield in the field depends on the water regime profile and the seasonal progress of ABA content or sensitivity in the plant, with or without the effect of promoters. More information is needed in such experiments on plant water-relations, the environment and ABA regimen in the plant.

ABA and other hormones

ABA is known to interact with other plant hormones such as ethylene, cytokinins, brassinosteroids and auxins (Peleg and Blumwald 2011; Sreenivasulu *et al.* 2012; Wilkinson *et al.* 2012). However, this discussion does not explore the phenomics of these complex interactions and networks. The interest here is in isolating the scaled up end result of ABA

enhancement. Certainly, the signalling of other hormones under specific environmental stresses can impact the expression of ABA and its responsive genes, and thus affect the enhanced ABA phenotype. This is a complex and only partially resolved matter (Wilkinson *et al*. 2012). Predicting such interactions at the whole-plant level are beyond the present initial attempt at ideotyping an ABA-enhanced phenotype. One can expect that at a later stage, some of these interactions might be considered.

Differential ABA expression and function in native breeding materials

Maize

As mentioned above, maize leaf ABA concentration has been shown to decrease with selection for drought resistance at the flowering stage (Mugo *et al*. 1999). Historical series of maize hybrids released in the USA from 1930 to 2000 were assayed for ABA accumulation in seedlings under short dehydration stress (Sanguineti *et al*. 2006). It was found that newer hybrids tended to have lower ABA accumulation than older hybrids. This was postulated to be the result of selection for drought resistance practiced in the breeding of newer hybrids.

Wheat

Normal wheat breeding materials and cultivars can differ significantly in their sensitivity to ABA (Blum and Sinmena 1995). Drought stress induced ABA biosynthesis genes in anthers and ABA accumulation in spikes of drought-sensitive wheat varieties (Ji *et al*. 2011). In contrast, drought-tolerant wheat accumulated lower ABA levels, which correlated with lower ABA biosynthesis and higher ABA catabolic gene expression.

Selected wheat landraces previously tested for drought resistance (Blum *et al*. 1989) were later bioassayed for ABA sensitivity using the growth of hydroponically grown seedlings challenged by ABA in the root medium compared with controls (Blum 1994, unpubl. data). The drought response of these landraces in the field was estimated by their midday canopy temperature under drought stress. Cooler canopy temperature under stress is usually taken as a reliable phenotypic indicator of relatively high transpiration and stomatal conductance under limited water supply. Midday canopy temperature tended to be cooler in landraces that were less sensitive to ABA (Fig. 1).

When *T. aestivum* cv. Excalibur, a drought-resistant Australian cultivar, was subjected to cyclic water deficit (Izanloo *et al*. 2008), it presented the highest level of OA, the highest stomatal conductance and the lowest ABA content compared with less resistant cultivars tested. This is probably also the only experimental indication that low ABA production under drought stress might be phenotypically associated with high OA.

Pearl millet

Drought-resistant pearl millet (*Pennisetum glaucum* (L.) R. Br.) lines had higher ABA content than susceptible lines under well-watered conditions (Kholová *et al*. 2010). However, lines did not differ in this respect under drought stress. It was suggested that ABA, as a water saving factor, served to conserve soil moisture use during the vegetative growth stage under drought stress at the flowering growth
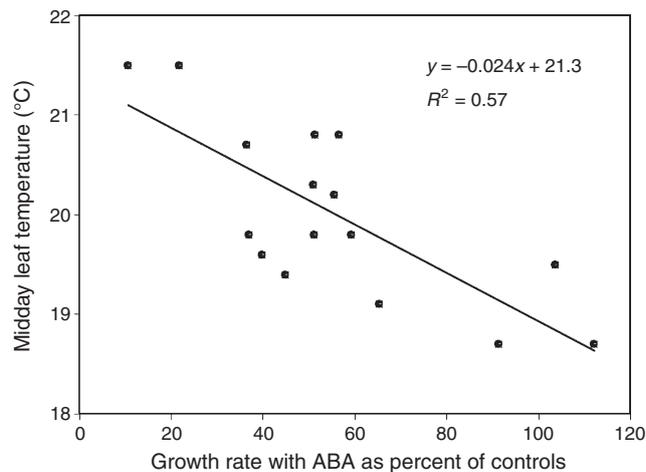

Fig. 1. The relationship between the growth rate of hydroponically grown seedlings that were challenged by ABA (at a concentration of 5×10^{-2} mol m^{-3} in the nutrient solution) expressed as percent of controls (Blum 1994, unpubl. data) and midday leaf canopy temperature under field drought stress (data from Blum *et al*.1989) in 16 wheat landraces.

stage. This explained the role of ABA in moderating seasonal soil moisture use under terminal drought stress and appears to be compatible with the transgenic canola example discussed above.

Cotton

Young fruit of *G. hirsutum* cv. Acala 4–42 contained about twice as much ABA as young fruit of cultivar Acala SJ-1, and this difference was positively correlated with a higher rate of young fruit abscission in Acala 4–42 (Davis and Addicott 1972).

Turfgrasses

Drought resistant cultivars of Kentucky bluegrass (*Poa pratensis* L.) were characterised by lower ABA accumulation, and a less severe decline in photosynthesis and stomatal conductance, as well as having better turf quality during drought stress compared with drought-sensitive cultivars (Wang and Huang 2003).

Bambara groundnuts

Two landraces of Bambara groundnuts (*Vigna subterranea* L. Verdc.) collected from different regions in Africa were found to be 'water saving' and 'water spending' types, respectively, where ABA was accumulated more in the former than the latter (Jørgensen *et al*. 2010). The 'water saving' landrace survived severe intermittent stress better, whereas the 'water spending' type was more productive.

Discussion and conclusions

ABA evolved in plants as they ascended from water to the land. Earlier plant forms controlled plant water status passively by their hydraulics (Brodribb and McAdam 2011). Later in the evolution of angiosperms ABA became functional only at complete turgor loss. In modern angiosperms, hydraulic and

hormonal controls are intertwined, and ABA is responsive to a range of plant and soil water statuses (McAdam and Brodribb 2014). Since evolution takes a conservative approach to plant survival, it is understandable that the role of ABA under drought stress is to contain the stress situation to the extent that the plant will survive and produce at least one seed. Productivity in the agronomic sense is irrelevant or even harmful. ABA's function is to conserve plant water status and reduce sink load on the plant. Reduced sink load by abortion serves to reduce C demand and even helps to sustain leaf water status (Blum *et al.* 1988).

The information discussed above on ABA and crop performance under drought stress has been derived from genetic intervention experiments or natural variation in ABA accumulation under stress. We should be warned that reports of genotypic differences in ABA accumulation under drought stress might not always prove to be a true variable inherent capacity for ABA accumulation. Leaf ABA accumulation is a function of shoot water deficit, root exposure to soil drying or the rate of soil drying. When different genotypes are compared, ABA content might be simply a function of a difference in their rate of dehydration or soil dehydration rather than an inherent genetic ABA difference. The independence of ABA accumulation capacity from possible variation in dehydration among genotypes can be verified in some reports but not in others, and it remains important to ascertain this issue experimentally. Still, the ample amount of verified information presented above can allow one to bypass this issue and draw some conclusions.

It is unavoidable that this discussion and interpretation of ABA function at the whole plant and the field bring up the classical pre-ABA botanical concepts of isohydric and unisohydric plant models (e.g. Walter 1955).

The 'water-saving' isohydric ideotype

A common drought stress scenario typical of the Mediterranean environments and some semiarid subtropical climates is where water is available at the onset of the growing season, after which rainfall and soil moisture decline towards the end of the season and the reproductive growth stage. This terminal stress might also include high temperatures and high VPD. Terminal stress can be lethal in extreme cases. This difficult water regime, whether it is driven by limited seasonal rainfall or by limited preseasonal stored soil moisture, can be moderated by controlling crop seasonal water use. This control operates by using less water at the vegetative growth stage so that more soil moisture remains at the critical reproductive growth stage. This is where ABA enhancement seems to function well. The best evidence is the examples of the canola transgenic cultivar and pearl millet described above, appropriate to the isohydric model of 'water saving'. It was also indicated that the capacity to alkalise xylem sap as a condition for ABA responsiveness to dry soil seemed to be a characteristic of isohydric natural vegetation with enhanced ABA capacity (Sharp and Davies 2009). Although high ABA content or sensitivity would be expected to induce sterility problems in an isohydric plant, it can be assumed that the high ABA content or sensitivity at the vegetative growth stage might naturally degrade as the season progresses towards the reproductive stage. Alternatively, conditional and specific use of promoters can regulate seasonal ABA sensitivity or perhaps

even its accumulation. It can be safely predicted that the isohydric ideotype is mostly effective in dryland crop environments where rainfall is limited to the extent that only a relatively shallow soil depth exchanges moisture between plants and the received rainfall, typical of the harsher Mediterranean climates or some North American prairies.

Stem reserve utilisation is an important factor in grain filling under stress. It is therefore perhaps not a coincidence that ABA accumulation is instrumental in regulating stem reserve mobilisation to the grain as discussed above. On the other hand, the effect of ABA in this respect is linked to its regulation of severe leaf senescence and is in opposition to the maintenance of the stay-green trait as regulated by cytokinin (Peleg and Blumwald 2011). However, stem reserve utilisation might still be compatible with stay-green if the latter trait is driven by a high plant water status resulting from an improved seasonal distribution of water use (Borrell *et al.* 2014) rather than by cytokinin regulation.

The isohydric model, with its sensitive stomatal closure and reduced transpiration at times of drought stress, poses a serious hazard of severe leaf heat stress (Fig. 2). Fig. 1 demonstrates the association between the inherent high ABA sensitivity and high leaf temperature under drought stress across wheat genotypes. Transpiration is an important mechanism for leaf heat avoidance. A reduction in transpiration under drought stress can increase wheat leaf temperature at midday by $\geq 7°C$ (Blum *et al.* 1982). This effect of ABA is rarely mentioned or measured in functional ABA research, and it can be catastrophic in terms of growth and productivity. The isohydric ideotype must therefore be heat tolerant because it lacks a heat avoidance mechanism. The direct involvement of ABA in heat tolerance is not clear. There are some indications for a positive effect (e.g. Larkindale and Knight 2002; Wang *et al.* 2014) or no effect (e.g. Blum *et al.* 1997). It has been reported (Tang *et al.* 2008) that heat-induced sterility in rice was lower in a variety which had a low ABA content. ABA and heat tolerance should be explored further.

Fig. 2. Two different sorghum lines under dryland conditions photographed 1 day after a severe hot windy event. The line on the right is known to be relatively more sensitive in stomatal closure under drought stress than the line on the left (and the other materials in the background) and it therefore shows heat stress damage in the form of partially dead leaves.

The 'water-spending' anisohydric ideotype

The major basic difference between isohydric and anisohydric model is that in the former, the stomata are first regulated by ABA, whereas in the latter, they are first regulated hydraulically, with or without ABA by hydraulic control interactions (Tardieu and Simonneau 1998). It should be clarified at the onset that genetic variation between the two models within a species is possible, as the case is shown for bambara groundnuts (Jørgensen *et al.* 2010) and sorghum (*Sorghum bicolor* (L.) Moench) (Fig. 2).

Turgor is the most crucial factor in sustaining plant life and function under drought stress. Crop plants can rarely function in a dehydrated wilted state. Within the context of this discussion, there are two major ways to sustain turgor under drought stress. One is the isohydric model via stomatal closure and transpiration reduction. The other is the hydraulic anisohydric model, which is supported by the capacity for OA, by effective soil moisture extraction for maintaining plant hydration or both. OA requires a dehydration signal. Where this heritable capacity exists, it is a function of the rate and extent of dehydration (Jones and Rawson 1979). A dehydration signal is not well expressed in an isohydric plant, which maintains a high leaf water status due to stomatal closure at high leaf water potential. Dehydration exists more in the anisohydric plant and therefore it has the engine for driving OA. On the other hand, plants which inherently lack OA capacity might perhaps evolve an isohydric model in order to cope with drought stress. This difference between the two plant models is well exemplified by the comparison of anisohydric and isohydric conifer trees, where the former lost turgor at a midday water potential of 5–6 MPa, whereas the latter lost turgor at ~3 MPa (Meinzer *et al.* 2014). The benefit of OA as an underlying mechanism of the anisohydric model is in maintaining leaf turgor and stomatal conductance, and also in supporting better root soil moisture extraction (Blum 2010). Thus, the anisohydric model is fully compatible with a plant expressing an effective use of water strategy (Blum 2009). This is further supported by the suggestion that aquaporins enhance water permeability and root water uptake, and thus aquaporins were seen as a component of the anisohydric plant model (Sade *et al.* 2009; Vandeleur *et al.* 2009).

It is therefore not surprising that the isohydric model might express high WUE (Vadez *et al.* 2014) simply due to its moderated water use, whereas the productive anisohydric model will express low WUE due to its high water use. However, the high WUE of a certain isohydric plant cannot be taken as a universal advantage *per se* of high WUE for productivity, as claimed by Vadez *et al.* (2014), but as the individual case of isohydric performance against the specific prevailing harsh water regime.

A case for the two ideotypes in the form of well-known real crop plants is exemplified here by maize (relatively isohydric) and sorghum (relatively anisohydric). Sullivan and Blum (1970) already established that maize stomatal closure was more sensitive to soil drying than sorghum, and that sorghum was more capable than maize in maintaining higher leaf water status and transpiration under stress. Sanchez-Diaz and Kramer (1971) found that maize closed stomata at a higher leaf water potential than sorghum and it also wilted at a higher water saturation deficit than sorghum. On the other hand, as would be expected, maize

was more heat tolerant than sorghum in terms of leaf cell membrane stability and the chlorophyll stability index (Sullivan and Blum 1970). Beardsell and Cohen (1975) showed that upon plant dehydration, leaf ABA content increased in maize to more than double than that in sorghum at the same low leaf water potential in both. The difference between these two ideotypes is partly driven by their respective differential capacity for OA and root function. Sorghum has a higher OA capacity than maize (Fereres *et al.* 1978) and, at the same time, it also has a superior capacity for extracting soil moisture compared with maize (Farré and Faci 2006). The advantage of sorghum is this respect is mainly expressed at deep soil (Cabelguenne and Debaeke 1998). Considering the differences between the two, it is therefore an enigma why maize, as a basically isohydric plant, tends to express a generally greater yield potential than sorghum. Consider also the open pollination system of maize, which is far more susceptible to environmental hazards than the self-pollinating sorghum. Some speculate that it is a function of the greater investments made in maize breeding over time, or a difference in canopy structure and radiation use efficiency.

Another example of the contrasting features of the two plant models was observed in wheat. A collection of Mediterranean wild emmer *Triticum dicoccoides* Koern. ex Schweinf. var. *aaronsohnii* was compared with a population of cultivated wheat lines that were derived from crosses between wild emmer and wheat (Grama *et al.* 1983). When field-grown under drought stress, mean midday canopy temperature was lower in the wheat derivatives than in the wild emmer population. On the other hand, the wild emmer population tended to be more heat-tolerant than the wheat derivatives in terms of a laboratory test for membrane heat injury. Thus the wheat derivatives displayed a relative anisohydric phenotype, whereas the wild emmer displayed an isohydric phenotype, as would be expected from natural Mediterranean ecotypes. A limited number of derivatives possessed the positive traits from both the wild and the cultivated wheat parents, being an intermediate phenotype.

Finally, the seasonal dynamics of ABA enhancement are no less important than the absolute immediate rate of accumulation or sensitivity, which is measured over a few days in typical gene expression work. For example, Plaut *et al.* (2012) showed that piñon–juniper (*Pinus edulis* Engelm.–*Juniperus monosperma* (Engelm.) Sarg.), which is an isohydric tree had the capacity to sustain hydration for months under prolonged drought stress. However, it finally died due to the limited gas exchange leading to carbon starvation. It is also possible that although the accumulated ABA under drought stress induce stomatal closure in the isohydric plant, ABA content might reduce over time and the plant would revert to an anisohydric model (Brodribb and McAdam 2013). This is supported by the finding that ABA responsiveness of stomata during drought stress can be unstable and be degraded over time (Aliniaeifard and van Meeteren 2013). This raises the important point that although we recognise the two opposing ideotypes, there can be a full spectrum of intermediate variation in between the two, even within a species.

In conclusion, drought stress is not a generic phenomenon. Therefore, the resolution of the 'contradictory role of ABA known to mediate two opposite face[s] of [a] coin'

(Sreenivasulu *et al.* 2012) is tightly linked to the specific profile of the drought stress environment and the agronomic tradeoff between the advantage ABA may have in seasonal water saving under harsh stress conditions and its cost in potential productivity. Breeding of the target functional phenotypic ABA ideotype will have to apply low-scale metabolic and genomic information while navigating between the positive and negative effects on plant production.

The available evidence presented here indicates that in most dryland cropping systems (certainly not all systems), ABA as a molecular messenger of drought stress delivers bad news to the farmer. The messenger should not be killed but be directed to environments where such news might be well received.

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