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REVIEW PAPER

# Photosynthesis and drought: can we make metabolic connections from available data?

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## Abstract

**Photosynthesis is one of the key processes to be affected by water deficits, via decreased $CO_2$ diffusion to the chloroplast and metabolic constraints. The relative impact of those limitations varies with the intensity of the stress, the occurrence (or not) of superimposed stresses, and the species we are dealing with. Total plant carbon uptake is further reduced due to the concomitant or even earlier inhibition of growth. Leaf carbohydrate status, altered directly by water deficits or indirectly (via decreased growth), acts as a metabolic signal although its role is not totally clear. Other relevant signals acting under water deficits comprise: abscisic acid (ABA), with an impact on stomatal aperture and the regulation at the transcription level of a large number of genes related to plant stress response; other hormones that act either concurrently (brassinosteroids, jasmonates, and salycilic acid) or antagonistically (auxin, cytokinin, or ethylene) with ABA; and redox control of the energy balance of photosynthetic cells deprived of $CO_2$ by stomatal closure. In an attempt to systematize current knowledge on the complex network of interactions and regulation of photosynthesis in plants subjected to water deficits, a meta-analysis has been performed covering >450 papers published in the last 15 years. This analysis shows the interplay of sugars, reactive oxygen species (ROS), and hormones with photosynthetic responses to drought, involving many metabolic events. However, more significantly it highlights (i) how fragmented and often non-comparable the results are and (ii) how hard it is to relate molecular events to plant physiological status, namely photosynthetic activity, and to stress intensity. Indeed, the same data set usually does not integrate these different levels of analysis. Considering these limitations, it was hard to find a general trend, particularly concerning molecular responses to drought, with the exception of the genes ABI1 and ABI3. These genes, irrespective of the stress type (acute versus chronic) and intensity, show a similar response to water shortage in the two plant systems analysed (*Arabidopsis* and barley). Both are associated with ABA-mediated metabolic responses to stress and the regulation of stomatal aperture. Under drought, ABI1 transcription is up-regulated while ABI3 is usually down-regulated. Recently ABI3 has been hypothesized to be essential for successful drought recovery.**

**Key words:** ABA, carbon metabolism, drought, photosynthesis, sugars, stress imposition rate and intensity.

## Introduction

Dictated by evolution, plant success in unfriendly environments (including drought) involves a plethora of responses, from early responses to longer term metabolic and physionomic alterations that can sustain acclimation and survival (Lawlor, 2009). This requires a tight coordination at the whole plant level.

From the work being produced in the last decade, it became apparent that plants perceive and respond rapidly to alterations (even small) in water status via a series of physiological, cellular, and molecular events developing in parallel (Chaves *et al.*, 2009). The responses at various levels are modulated by the intensity, duration, and rate of

progression of imposed drought. From the methodological point of view, this complexity poses additional challenges to the compilation and integration of the available data. This brings about the need to define and monitor the drought that plants are facing in each experiment, in terms of water availability in the substrate, photosynthetic activity, plant water status, as well as the radiation to which plants are subjected, since this combination will determine the elicited responses. Only such fine monitoring of conditions will allow the results to be reproduced and correctly interpreted, and for distinct data sets to be compared (Jones, 2007; Dehyolos, 2010).

Often the studies of molecular responses of plants to drought use very artificial systems of stress imposition, such as an instantaneous decline in water availability produced by detaching organs or removing the plants from substrates. Such experimental conditions cannot provide information on relevant acclimation processes that might occur under field conditions. Furthermore, rapid alterations (within hours) would not necessarily reflect a response to a long-term water shortage but instead a short-term adjustment to a new environmental condition. Although some of the effects of a rapidly imposed water deficit might be common to those when the deficit is imposed slowly, reproduction of slowly imposed water deficits under field conditions is required when considering a crop's response to drought. This type of study will allow the evaluation of acclimation processes in mature plants as well as plant resistance to a multistress situation that often is the cause of dramatic losses in agricultural production. Recent studies revealed that molecular and metabolic responses of plants to a combination of stresses are unique and cannot be extrapolated from the separate study of individual stresses (Mittler, 2006). Moreover, from an agricultural perspective, drought is ultimately defined in terms of its effects on yield, since this is the relevant issue when addressing the improvement of crop production under water-limited environments (Passioura, 2007). Consequently, the timing of water deficits during the season (e.g. sowing, crop establishment, flowering, or grain filling) may have a much larger impact on yield than the intensity of drought *per se*.

As the key process of primary metabolism, photosynthesis plays a central role in plant performance under drought (see reviews by Chaves *et al.*, 2003, 2009; Flexas *et al.*, 2004; Lawlor and Tezara, 2009). The decline observed in leaf net carbon uptake as a result of plant water deficits is followed by an alteration in partitioning of the photoassimilates at the whole plant level, corresponding in general to an increase in the root to shoot ratio. This is the result of the decline in shoot growth and the maintenance of root growth under decreasing water in the soil (Sharp, 2002). Such a response is mediated by hormonal control, namely by abscisic acid (ABA), ethylene, and their interactions (Wilkinson and Davies, 2010), as will be discussed further on. The changes in the root–shoot ratio as well as the temporary accumulation of reserves in the stem that occur in several species under water deficits (Blum *et al.*, 1994; Chaves *et al.*, 2002) are accompanied by alterations in carbon and nitrogen metabolism in the different organs (Pinheiro *et al.*, 2001; António *et al.*, 2008), whose fine regulation is still largely unknown. In this context, sugars are likely to be key players in the integration, at the whole plant level, of the cellular responses to internal and environmental alterations. They act as substrates and modulators of enzyme activity in carbon-related pathways and via the control of expression of different genes related to carbon, lipid, and nitrogen metabolism (Koch, 1996; Gibson, 2000; Rolland *et al.*, 2006). The interplay of sugars with other stress elicitors, such as redox and hormone signals, is at the forefront of present research efforts (Couée *et al.*, 2006; Usadel *et al.*, 2008; Bolouri-Moghaddam *et al.*, 2010). Sulpice *et al.* (2009) recently suggested that starch is a major integrator of plant metabolism and growth, in response to changes in development or the environment, reflecting a regulatory network that balances growth with carbon supply (see Discussion and Fig. 1 below).

In the present paper, the current status of the physiological limitations to photosynthesis under drought was revised and a meta-analysis (covering >450 papers published in the last 15 years) was performed with the goal of strengthening our understanding of the complex network of interactions and regulations of photosynthesis in plants subjected to water deficits. The working hypothesis is that if a general/unifying response exists it will emerge from these data and would be a useful starting point for future experiments.

## Revisiting drought constraints to photosynthesis

Several recent review papers have dealt with this issue in a comprehensive way (Chaves *et al.*, 2009; Lawlor and Tezara, 2009). However, there is still some controversy regarding the relative importance and timing of the main physiological targets responsible for limiting photosynthesis under drought. Decreased $CO_2$ diffusion from the atmosphere to the site of carboxylation is generally considered the main cause for decreased photosynthesis under mild to moderate water limitation (Chaves *et al.*, 2003, 2009; Flexas *et al.*, 2004; Grassi and Magnani, 2005). This limitation includes a stomatal and a mesophyll component (Flexas *et al.*, 2008). The magnitude of the latter is still under debate, with criticisms arising over methodological issues related to the estimation of the intercellular or the chloroplastic $CO_2$ concentration (Bunce, 2009; Lawlor and Tezara, 2009). Mesophyll conductance ($g_m$) comprises physical (solubility of $CO_2$, surface area of the apoplastic, and symplastic routes of $CO_2$) and metabolic components (aquaporins and carbonic anydrase). Both are dependent on the species concerned, presumably as a result of differences in the relative contribution of anatomical versus biochemical components and on the experimental conditions, namely water deficits and temperature. It has been shown that soil water deficits can substantially reduce $g_m$ (see Flexas *et al.*, 2008), although in general $g_m$ is less sensitive to water stress than $g_s$ (Bunce, 2009).

In species well adapted to dry environments the feed-forward responses of stomata to soil and atmospheric dryness are important components of plant water saving (Maroco *et al.*, 1997; Chaves *et al.*, 2003, Chaves and Oliveira, 2004; David *et al.*, 2007). Stomata act as pressure regulators that prevent xylem pressure from runaway cavitation thresholds (David *et al.*, 2007). This is visible in the midday closure of stomata on hot days or in the decreased stomatal conductance in response to mild soil dehydration, in plants whose tissue water status is high. Both responses seem to be mediated by ABA synthesized in or transported to the leaves (from dehydrating roots) and are modulated by numerous internal and external factors, as will be discussed later on (see also a recent review by Wilkinson and Davies, 2010). Under field conditions, together with the intensity and duration of midday depression of stomatal conductance, how early in the day it starts to appear is an important indicator of the degree of stress being endured by the plant.

Furthermore, when decreased stomatal conductance is combined with sustained high irradiance, leaves are subjected to excess incident energy relative to the available intercellular $CO_2$, and the rate of reducing power production can overcome the rate of its use by the Calvin cycle. Under such circumstances, down-regulation of photosynthesis, or even photoinhibition, can be a powerful defence mechanism in $C_3$ plants. Such protection may be achieved by the regulated thermal dissipation occurring in the light harvesting complexes, involving the xanthophyll cycle (Demmig-Adams and Adams, 1996; Demmig-Adams *et al.*, 2006) and the lutein cycle (Garcia-Plazaola *et al.*, 2003). These photoprotective mechanisms compete with photochemistry for the absorbed energy, leading to a down-regulation of photosynthesis evidenced by the decrease in quantum yield of photosystem II (PSII) (Genty *et al.*, 1989). If the limitation of the rate of $CO_2$ assimilation is accompanied by an increase in the activity of another sink for the absorbed energy, for example photorespiration (Harbinson *et al.*, 1990; Wingler *et al.*, 1999) or the Mehler-peroxidase reaction (Biehler and Fock, 1996), the decline in non-cyclic electron transport will be proportionally less than the decrease observed in the rate of $CO_2$ assimilation. This type of response has been well documented in $C_3$ plants native of semi-arid regions and less so in $C_4$ plants (Ghannoum, 2009). Recent evidence suggests that the equal or even stronger susceptibility to water deficits observed in $C_4$ plants as compared with $C_3$ plants, in spite of the $CO_2$-concentrating mechanism in the former, may be ascribed to the limited capacity of photorespiration or the Mehler reaction to act as alternative electron sinks for excess reducing power (Ghannoum, 2009).

The biochemical component of the limitation of photosynthesis under water deficits is generally estimated as much smaller than the diffusion limitation (Galmes *et al.*, 2007*a*). However, its importance should not be underestimated. Indeed, alterations in gene expression may develop early on in response to the decline of plant water status, preceding acclimation mechanisms, although the impact on metabolites may not occur immediately (Chaves *et al.*, 2009). Energy balance was also recognized as a key component of cell functioning under limited supply of $CO_2$ and high light (Lawlor and Tezara, 2009; Pfannschmidt *et al.*, 2009). Under such conditions, Tezara *et al.* (1999) had found an impaired ATP production and thus ribulose bisphosphate (RuBP) regeneration, and recently reactive oxygen species (ROS) generated under highly reduced conditions in the chloroplast were shown to damage ATP synthase (Lawlor and Tezara, 2009).

As for the impact of water deficits on Rubisco, although the results are very variable, it has generally been found that its activity and quantity are affected under severe stress (Maroco *et al.*, 2002; Parry *et al.*, 2002; Flexas *et al.*, 2006*b*), although there is also evidence of alterations at the transcript level under milder stress (Supplementary Table S5 at *JXB* online). The drop in Rubisco activase is presumably a key factor in slowing down Rubisco activity (Lawlor and Tezara, 2009). A recent study by Galmés *et al.* (2010) in 11 Mediterranean species suggests that low chloroplastic $CO_2$ concentration (Cc) occurring under water stress could induce de-activation of Rubisco sites, the threshold of Cc triggering de-activation of Rubisco being dependent on leaf characteristics. It is also suggested that species adapted to functioning at low Cc can maintain active Rubisco under more intense drought.

The transcriptional control of photosynthetic genes by transcription factors (TFs) in response to abiotic stresses was recently reviewed (Saibo *et al.*, 2009), pointing out the role of several TFs belonging to the MYB family in both stomatal and non-stomatal limitations of photosynthesis. They are involved in the regulation of stomatal number and size, and of metabolic components of the photosynthetic system. As highlighted by Pourkeirandish and Komatsuda (2007), breeding for increased crop yield seems to have altered the functionality of some TFs, with potential impact on their responses to the environment.

### *Adding the whole plant photosynthesis dimension*

To study the response of crop photosynthesis to drought, it is relevant to approach it at the canopy scale as well, since crop productivity is dependent on photoassimilates produced at the whole plant level. It is known that the decline in stomatal aperture is accompanied by the adjustment of leaf area at the whole plant level. It occurs either via the inhibition of new leaf growth or via the earlier senescence of older leaves, in the case of prolonged stress. This reduction in foliage dimension leads to decreased transpirational area but also to lower intercepted radiation throughout the growing season and ultimately to decreased biomass production (Pereira and Chaves, 1993). In many crops, alteration of the leaf angle with dehydration, towards smaller angles, will also diminish total intercepted radiation and therefore carbon assimilation by the plant, but will have an important protective role against excess solar energy.

Photosynthetic resilience to drought is known to vary with leaf age (Chaves, 1991). Younger leaves tend to be more resistant to drought than older leaves, and this increased tolerance may be particularly relevant in plants where a severe reduction in the size of the leaf canopy occurs as a result of shedding of older leaves, because it allows a fast recovery following rehydration (Pereira and Chaves, 1993).

In addition to a plant's ability to avoid and/or endure water stress, photosynthetic recovery following rehydration is pivotal to dictate a plant's resistance to drought and to prevent dramatic declines in crop yield (Chaves *et al.*, 2009). It was shown that recovery from a severe stress was a two-stage process: the first stage occurs during the first hours or days upon re-watering, corresponding to the improvement of leaf water status and stomatal re-opening (Pinheiro *et al.*, 2005; António *et al.*, 2008; Hayano-Kanashiro *et al.*, 2009); and the second stage lasts several days and requires *de novo* synthesis of photosynthetic proteins (Kirschbaum, 1988). Previous stress intensity and/or duration are crucial factors affecting both the velocity and the extent of recovery of photosynthesis (Miyashita *et al.*, 2005; Flexas *et al.*, 2006*a*). Long-term down-regulation of $g_s$ after re-watering may be derived from limited recovery of leaf-specific hydraulic conductivity (Galmés *et al.*, 2007*c*). From the molecular point of view, the comparison between susceptible and tolerant genotypes suggests that drought tolerance is associated with a rapid modulation of genes from different TF gene families during recovery. For example, the greatest difference between drought-tolerant and drought-sensitive maize genotypes was observed in the speed of transcriptional down-regulation during recovery from drought (Hayano-Kanashiro *et al.*, 2009).

## The respiration connection: support for photosynthesis recovery?

Net carbon gain that ultimately dictates plant growth and development reflects the balance between photosynthesis and respiration (in auto- and heterotrophic tissues). Indeed, 30–70% of the $CO_2$ fixed per day by net photosynthesis in well-watered plants is released back into the atmosphere by plant respiration, the larger part through the leaves (Aktin and Macherel, 2009).

The impact of water deficits on dark respiration is still far from clear, with reports in the literature comprising decreases, maintenance, or increases in the rates of this process (Gimeno *et al.*, 2010). Inhibition of respiration under drought has been observed in actively growing roots and mature leaves of crops and herbaceous species (e.g. Haupt-Herting *et al.*, 2001; Ribas-Carbo *et al.*, 2005; Galmes *et al.*, 2007*b*). Decreased availability of the substrate to the mitochondria under conditions of low photosynthesis as well as inhibition of leaf growth may explain reduced respiration, mostly in its growth component (Flexas *et al.*, 2006*a*; Gimeno *et al.*, 2010). However, a higher demand for respiratory ATP under severe water stress (to compensate for the lowered ATP production in the chloroplasts) may be required to support photosynthesis repair mechanisms, as suggested by Flexas *et al.* (2005, 2006*a*) and Atkin and Macherel (2009). Higher respiration rates, mainly as the maintenance component, are then observed in droughted plants, underlying acclimation mechanisms of drought (Gratani *et al.*, 2007; Slot *et al.*, 2008). Finally, a third response pattern, with no alterations in the rates of dark respiration under drought, was reported in several species, mostly in evergreen perennials (Galmes *et al.*, 2007*b*; Gimeno *et al.*, 2010).

Elaborating on such contrasting results, Atkin and Macherel (2009) proposed a model where mitochondrial respiration dictates plant survival and rapid recovery of productivity under water stress conditions, by ensuring survival under extended periods of drought. According to some authors (Gimeno *et al.*, 2010), shrubs and trees that possess long-lived leaves are likely to show slower responses to drought than short-lived species that need to optimize their carbon gain over shorter periods and therefore may respond quickly to water scarcity, lowering their respiration rates.

From the biochemical point of view it has been reported that the electron partitioning towards the alternative respiration pathway sharply increases under severe drought, even when total respiration rates are not greatly affected (Ribas-Carbo *et al.*, 2005). Unlike many other stresses, water stress does not affect the quantity of mitochondrial alternative oxidase protein, suggesting that a biochemical regulation causes this mitochondrial electron shift. This shift may have a physiological significance, since evidence is accumulating to support a role for the alternative oxidase in the prevention of the formation of ROS (Lambers *et al.*, 2005).

Overall, the changes observed in respiration in response to drought are smaller as compared with the large decreases in photosynthesis; therefore, as carbon uptake becomes more limiting under water scarcity, respiration increases proportionally, leading to increased leaf intercellular $CO_2$ and altered plant carbon balance (Lawlor and Tezara, 2009). As already mentioned, the ratio between the respiratory needs for growth and maintenance will also change in plants under water stress, the component devoted to shoot growth being drastically decreased (Flexas *et al.*, 2006*a*).

## Drought and photosynthesis: the metabolic connections

Although impressive advances have been made in the last decade with respect to the nature of events occurring in plants subjected to drought, an integrative picture of the metabolic regulation taking place is still missing (Rolland *et al.*, 2006; Shinozaki *et al.*, 2007). This is partly related to the disparate experimental conditions of the studies being done and the very artificial conditions of the applied stress, frequently acute and/or too severe. Moreover, in many studies, particularly those dealing with the molecular responses to drought, plant water status, leaf conductance,

and photosynthetic rate are usually not measured, which makes comparative analysis of these data very difficult to perform.

Details on regulatory mechanisms and interactions are available for specific situations, although systematic information on common/general effects is still scarce. It is compelling that the vast amount of information on plant transcriptomes under drought has not yet been translated into genotype selection. This is for the most part due to low correlations between transcript abundance and corresponding protein and enzyme activities, as well as plant physiological performance, the question 'what do these genes contribute to stress tolerance?' still being largely unanswered (Chaves *et al.*, 2009; Deyholos, 2010). New experimental and computational methods are starting to allow multilevel analysis that can integrate physiological, transcriptome, proteome, and metabolome data, thus providing a more detailed view of the cellular events (Eberhard *et al.*, 2008), and contributing to disclosure of the existence of common metabolic features in photosynthetic responses to drought.

### *Drought effects on photosynthesis: description of the meta-analysis*

For a comprehensive analysis of the effects of drought on photosynthesis and related molecular and metabolic events, a literature survey (from 1995 to February 2010) was performed using the tools MedScan Reader v3 and Pathway Studio v7 from Ariadne Genomics (www.ariadnegenomics.com). This meta-analysis integrates information from most studies on drought and photosynthesis published during the 15 year period mentioned above and allows a picture to be obtained of the main processes involved and how often they have been reported. A total of 469 publications for proteins/genes and 515 for metabolites associated with drought and photosynthesis were screened. Taking into consideration the number of publications that relate the involvement of a particular protein, gene, or metabolite in photosynthesis responses to drought, those associations can be considered as strong (high number of records), weak (few records), or inexistent, the latter indicating either a weak association at the biological level or few references available.

Using this tool it was possible to identify proteins, genes, or metabolites (small molecules) that are most strongly associated with drought and photosynthesis (the complete list of relationships extracted from the literature is available as Supplementary Table S1 at *JXB* online). It includes sugars, hormones, and ROS pathways. By focusing on the association of such pathways with drought and photosynthesis, it was possible to identify 389 relationships at the metabolite level and 256 relationships at the protein/gene level (Supplementary Table S2). In order to disclose strong associations, the relationships with high connectivity (which imply that a given identity is associated with several of the processes) are considered (Fig. 1).

### *Drought, photosynthesis, and strongly associated pathways*

At the metabolite level (Fig. 1A), a high level of connectivity is found between drought, photosynthesis, ROS, ABA, sucrose, and starch. Cytokinin-related processes are not well documented, presumably because they have not been studied to any great extent. Interactions between the different hormone pathways are also noticeable, namely for ABA, auxins, and ethylene. At the protein/gene level (Fig. 1B), sugars, starch, ROS, and ABA pathways are well represented in this network relating drought and photosynthesis. In contrast, auxins and ethylene appear with weaker associations (Supplementary Table S2 at *JXB* online). The literature survey reveals proteins with regulatory functions that connect photosynthesis responses and drought, as is the case for the TFs T6L1.5, HY5, AHBP-1B, and GBF3, members of the bZIP (basic leucine zipper domain) family, and one TF belonging to the ABI3 (abscisic acid-insensitive 3) family. The involvement of the bZIP and ABI3 transcription factors, both ABA dependent, in the connection between drought and photosynthesis has recently been reviewed (Saibo *et al.*, 2009). Members of the bZIP family are associated with Rubisco regulation (Saibo *et al.*, 2009); more specifically, the bZIP TFs found in the present analysis were found to be associated with: photomorphogenesis and cytokinin pathways (HY5); salicylic acid-mediated responses and defence genes (AHBP-1B); the sucrose-sensing pathway; the ABA pathway; and interactions with HY5 and AHBP-1B (GBF3) (wikigenes platform, www.wikigenes.org; Hoffmann, 2008).

The transcriptional regulator ABI3 is associated with the regulation of stomatal aperture, involved in the auxin pathways (Brady *et al.*, 2003), and interacts with ABI1 (Parcy and Giraudat, 1997). ABI1 codes for a serine/threonine phosphatase and is related to stomatal regulation and ABA-mediated responses. ETR1 (ethylene response 1, a protein histidine kinase) is responsive to ABA, auxins, ethylene, cytokininis, and gibberellins, and was shown to be involved in the regulation of stomatal movement, the glucose-sensing pathway, and $H_2O_2$ biosynthesis. This gene and the protein it encodes can thus link ROS, sugar, and hormone pathways.

All TFs identified in the meta-analysis as strong connections, linked drought and photosynthesis to the regulation of stomatal aperture. The literature survey also highlights strong associations concerning Rubisco ($CO_2$ assimilation), catalase (ROS detoxification), nitrate reductase (nitrate assimilation; NO synthesis), invertase (sucrose breakdown through an irreversible reaction), sucrose synthase (which catalyses a reversible reaction depending on the cellular homeostasis), and amylase (starch metabolism) with photosynthesis under drought.

Several questions arise from this analysis. How do the readjustments of the mentioned metabolic pathways affect and/or are affected by photosynthesis? Do they contribute to the plant's ability to cope with the stress? How are such adjustments coordinated? Are such effects strongly dependent on transcription or could they be achieved through metabolic reorganization?

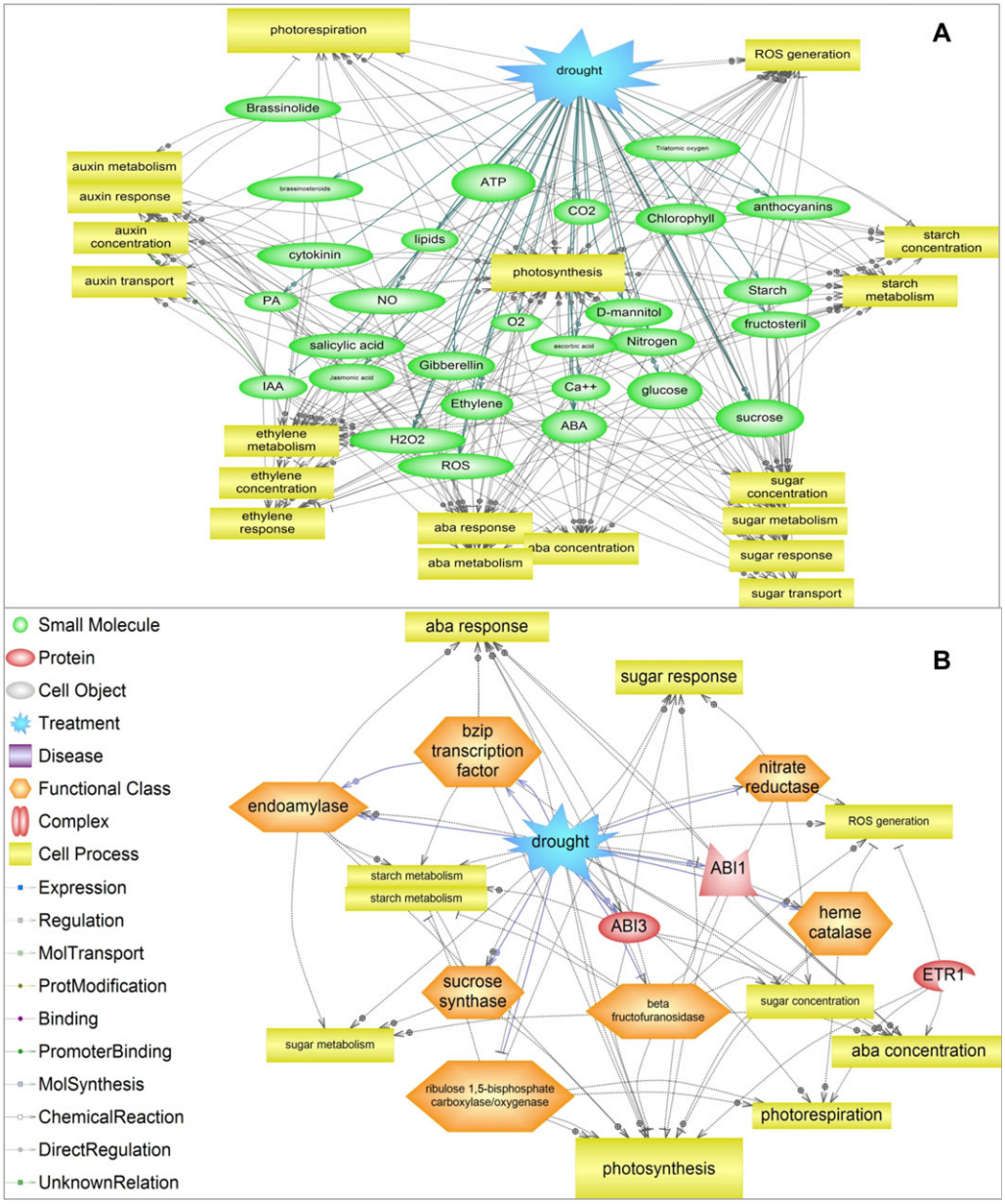


**Fig. 1.** Biological networks generated for drought and photosynthesis interactions considering the literature available (1995 to February 2010) by making use of Pathway Studio software. The complete list of interactions as well as details of the pathway is available in Supplementary Tables S1 and S2 at *JXB* online. A, small molecules (loc al connectivity ⩾6); B, proteins and/or genes (local connectivity ⩾ 6).

### *Effects at the transcriptional level: (i) methodological information*

The results can be further explored by making use of the publicly available databases on drought effects at the transcription level. This approach can allow the detection of potentially relevant trends to be considered in future studies.

One of the points of regulation in the photosynthetic response to drought is at the gene transcription level, and there is evidence indicating that although most photosynthetic genes are down-regulated in response to a multitude of stress conditions, there are certain subsets of photosynthetic genes linked with protective functions that are up-regulated. How drought affects transcription of the genes coding for the proteins represented in Fig. 1B was analysed, aiming at (i) identifying a general trend that would unify these responses; and (ii) finding out whether responses at the transcription level were different according to the stress imposition rate (fast versus slow) and the intensity (mild versus intense drought).

The publicly available data deposited in the EMBL-EBI ArrayExpress (www.ebi.ac.uk/microarray-as/ae/) were exploited, and by using the keywords drought (62 experiments), water stress (34 experiments), or dehydration (9 experiments), 56 distinct experiments for several species

were detected, 26 of them in *Arabidopsis* (Supplementary Table S4 at *JXB* online). Several experiments were eliminated due to several drawbacks: use of osmotic stress rather than drought (five data sets); no information on stress type and duration (two data sets); only recovery data reported (one data set); and only mutant information available (six data sets). In the 12 remaining *Arabidopsis* arrays, five experiments were done using soil and seven using paper/detached leaves. Relative water content is only available for three assays and none has information on leaf conductance or photosynthesis. From the six rice data sets only two are suitable for this analysis (one in paper; one in soil). For barley, three of the four experiments were considered and represent soil experiments.

In order to be able to use the array information, *Arabidopsis* genes/proteins (detailed information on their identity is available as Supplementary Table 3 at *JXB* online) were translated to the corresponding probe set IDs via the Plant Expression database (www.plexdb.org/modules/glSuite/gl_main.php; Wise *et al.*, 2007). Since in Pathway Studio there was no information for barley proteins, the *Arabidopsis* and rice probe sets were respectively translated from the *Arabidopsis* and rice microarray platform to the barley platform (http://www.plexdb.org/modules/MPT/mpt_Input.php); the identity of the resulting barley probe sets was confirmed (www.plexdb.org/modules/PD_probeset/GO_annotation.php).

### *Effects at the transcriptional level: (ii) drought, photosynthesis, and strongly associated pathways*

Supplementary Table S1 shows the effect of drought at the gene level in the model plant *Arabidopsis* and the crop plants rice and barley for the genes/proteins represented in Fig. 1B. The main findings may be summarized as follows. (i) The stress imposition method (soil versus paper) significantly affects the responses. The principal component analysis bi-plot generated with the *Arabidopsis* arrays shows that the soil data sets group together, while paper data sets are more dispersed and not mixed with the soil experiments (data not shown). (ii) In the same data set, distinct effects are observed within the same gene family, which allows the hypothesis that at the protein level the effects can be cancelled out. Furthermore, protein activity is modulated at many levels including post-translational modifications (see Eberhard *et al.*, 2008), with activity being modulated via substrate flux and availability and cellular compartmentation (Deyholos, 2010). (iii) The drought effect in a given gene is highly variable, with few exceptions (notably *ABI1* and *ABI3*). (iv) Usually the observed responses (up- or down-regulation) are not reversed with stress progression.

The present meta-analysis (Fig. 1), taken together with the transcriptomic data (Supplementary Table S5 at *JXB* online), highlights the difficulties faced when searching for metabolic events associated with stress and in gaining insight into the relevant pathways, because not all biological and methodological variables are considered in the different experiments. Although the association of a given response with stress perception, intensity, tolerance, or sensitivity is still rare, the analysis allowed recognition of some potentially relevant features. For example, ABI1 is up-regulated under water deficits in both *Arabidopsis* and barley plant systems and stress types, while for ABI3 the opposite trend is observed. ETR1 showed a similar response to ABI1, although not so marked, and seems not to respond to acute stress. These genes are related to stomatal closure regulation and provide a link between several hormone pathways. Recently, Khandelwal *et al.* (2010) highlighted a new target for ABI3 action in *Physcomitrella patens* (in an acute stress experiment). They inferred that several transcripts produced during ABA pre-treatment (necessary for *P. patens* desiccation survival) are necessary for recovery (ABI3 mutants do not survive). This may be linked with previous findings of gene expression required for stress recovery being already operative during desiccation (Bray, 1993). Accordingly, very few rehydration-specific proteins are known (Bartels and Salamini, 2001), and in the leaves of two resurrection plants (*Xerophyta humilis* and *Craterostigma wilmsii*) recovery is largely independent on *de novo* gene transcription and protein translation (Dace *et al.*, 1998; Cooper and Farrant, 2002). In lupins (Pinheiro *et al.*, 2005) and wheatgrass (Gazanchian *et al.*, 2007), it became apparent that the proteins needed for early plant recovery could already be present during the severe stress phase.

Regarding the invertase multigenic family, for three genes (one coding for cell wall invertase and two for neutral invertases) it was possible to distinguish the effects from acute (paper) stress and soil stress experiments. The genes AT3G13970 (cell wall invertases) and AT4G09510 (neutral invertase) are down-regulated under soil water stress but up-regulated with acute stress; the gene AT3G06500 (neutral invertase) is up-regulated in both systems but it seems to be affected more at the very early stages of the acute stress (1–2 h). This is an example of differences in plant response to the velocity of stress imposition—sucrose metabolism will be affected in distinct ways when plants acclimate to slowly imposed water or with a fast response to a dramatic change in tissue water status.

Moreover, the light regime under which plants are grown may also drastically influence the results. For example, when water deficits were imposed on plants adapted to low light, as in a recent study with *Arabidopsis* (180 μmol $m^{-2}$ $s^{-1}$), the expression of a set of sugar-responsive genes indicates increased, rather than decreased, carbon availability (Hummel *et al.*, 2010). Indeed, under such conditions photosynthesis was not affected under severe stress (because it was light limited) and the concomitant inhibition of shoot growth gave rise to a surplus of carbon, which was redirected to root growth. It must be emphasized that these results cannot be extrapolated to field conditions where net carbon uptake will be decreased and carbon limitation will be apparent.

### *Co-expression transcription analyses*

The tool 'Correlated Gene Search' available at PRIMe (http://prime.psc.riken.jp; Akiyama *et al.*, 2008) was also used in order to detect correlated transcription of the *Arabidopsis* genes described in Supplementary Table S3 at *JXB* online. Co-expression analysis asks the question 'what are the genes that show similar expression profiles across many experiments to my gene of interest?' Genes that are highly co-expressed may be involved in the biological process or processes of the query gene.

Considering the available experiments (AtGenExpress arrays that deal with acute stress treatments) the following correlation groups were found. (i) ABI1 transcription is correlated with sucrose synthase genes (AT5G20830, AT4G02280), neutral invertase (At3g06500), β-amylase (AT3G23920), and the TF GBF3 (AT2G46270); this group has a branch where GBF3 and SUS3 (AT4G02280) also correlate with another neutral invertase (AT4G34860), the neutral invertase and SUS3 also correlating with AMY1. (ii) This group relates several α-amylase genes to each other (AT5G04360, AT1G69830, AT2G39930, and AT4G09020). It also associates ISA3 (AT4G09020) with catalase transcription (AT1G20630, AT1G20620). (iii) In this group, another catalase gene (AT4G35090) is associated with a β-amylase (AT5G18670) in an independent node. (iv) In this group, ABI3 is associated with SUS2 (AT5G49190). This exercise shows the interplay between ABA, sucrose, starch, and ROS metabolism and points to the role of neutral invertase (not as well studied as the acid form) in stress responses.

The meta-analysis presented here documents the strong association between drought, photosynthesis, sugars, hormones, and ROS (Fig. 1), but more importantly demonstrates that it is necessary to make additional efforts in stress characterization and quantification to be able to associate the alterations firmly with stress type and intensity. Moreover, comparisons between tolerant and susceptible genotypes are still scarce and need to be strengthened because this is a faster way to produce information to design more efficient breeding programmes to produce genotypes better adapted to water-limiting conditions.

## Signals and metabolic cross-talk

In the chain of events triggered by drought, one relevant issue relates to signals and signalling cascades and their interactions. Although these terms (signals and signalling cascades) are often used, the biological rationale and supportive data are not always obvious. Some questions remain elusive. Are the observed effects a signal or a consequence of the stimulus (direct or indirect)? How do such changes lead to metabolic rearrangements in photosynthesis?

### *Hormones*

Water deficit affects biosynthesis, accumulation, and redistribution of major plant hormones, with ABA (synthesized either in leaves or in roots) playing the major role in controlling stomatal aperture and therefore photosynthetic carbon uptake under conditions of water scarcity (Dodd, 2003; Hirayama and Shinozaki, 2007). Stomatal sensitivity to ABA is modulated by a number of external drivers, such as temperature, ozone, nitrogen nutrition (often altered in drying soil), and endogenous components, including cytosolic pH or hydraulic signals that can either reinforce or moderate ABA-based signals (Wilkinson and Davies, 2002, 2010; Parent *et al.*, 2009). Recent reports show that stomatal function is also dependent on other hormones (auxin, cytokinin, ethylene, brassinosteroids, jasmonates, and salicylic acid) and on the degree of their interactions (see the reviews by Acharya and Assmann, 2009; Wilkinson and Davies, 2010). In general, auxin, cytokinin, or ethylene tend to inhibit ABA-mediated stomatal closure, whereas brassinosteroids, jasmonates, and salycilic acid display a concurrent action with ABA. Moreover, all these hormones modulate the expression of different drought-related genes (Shinozaki and Yamaguchi-Shinozaki, 2007; Huang *et al.*, 2008). Multiple cascades of cellular biochemical events have also been associated with the regulation of stomatal guard cells, such as the activation of G-proteins, the production of ROS (ABA stimulated) and NO, cytosolic $Ca^2$, protein phosphorylation/dephosphorylation, and reorganization of the cytoskeleton (Acharya and Assmann, 2009). The phosphatases ABI1 and ABI2 were shown to be crucial for ABA-mediated stomatal regulation (Merlot *et al.*, 2001) and are one of the strong connection points in photosynthesis responses to drought ABI1 in Fig. 1B. Still, many questions remain to be clarified, namely the molecular basis of cross-talk among different hormones or the underlying causes for dual roles played by some of them, such as ethylene or even ABA, as recognized by Parent *et al.* (2009).

Recent attention was given to the interactions observed between hormonal and circadian networks, since it has been demonstrated that a large proportion of transcripts involved in hormonal metabolism, catabolism, and signalling are also regulated by the circadian clock (Dodd *et al.*, 2007; Robertson *et al.*, 2009). Daily rhythms have been recognized for a long time in different plant processes, namely photosynthesis and stomatal aperture, and this may have resulted from an evolutionary pressure in order to prevent physiological responses that might be counterproductive during some parts of the day, when temperature and radiation are excessive. Circadian clocks may therefore moderate or produce antagonistic effects relative to hormones, such as those they produce with sugars, as is highlighted further on.

### *Redox signals*

Maintaining homeostasis of redox and adenylate systems is essential for cell functioning. Whenever an imbalance develops between capture of light and its utilization via $CO_2$ and $NO_3^-$ reduction, as may happen under drought, redox signals from photosynthetic electron transport and

production of ROS may occur (Lawlor, 2009) (see Fig. 1). It has now been extensively demonstrated in several biological systems that these redox signals and ROS have an important function in the plant's acclimation to stress (Buchanan and Balmer, 2005; Hayano-Kanashiro *et al.*, 2009). ROS are produced in plant tissues due to the partial reduction of oxygen as, for example, in the photosynthetic and the respiratory electron chains or the photorespiration pathway, or they accumulate as a result of the activity of peroxidases, membrane-located NADPH oxidases, etc., and this production increases dramatically under environmental stress (Mittler *et al.*, 2004). On the other hand, the intensity, duration, and localization of the different ROS signals are determined by the interplay between the ROS-producing and ROS-scavenging pathways of the cell, as highlighted in Fig. 1B (nitrate reductase and catalase). Further, antioxidants such as ascorbate, tocopherol, or glutathione (Fig. 1A; Supplementary Tables S1, S2 at *JXB* online) are able to control the lifetime of ROS signals and therefore participate in the overall redox regulation that ultimately controls the energy balance in plants (Foyer and Noctor, 2009). Although ROS can modulate many pathways (e.g. mitogen-activated protein kinase cascades) and influence the activity of TFs, redox control over photosynthesis is still largely unknown. It may occur, at least partly, through the monitoring of the cell redox status by several molecules in different cellular compartments, reporting the functional state of the chloroplast to the nucleus (Pfannschmidt *et al.*, 2009), as suggested by Jaspers and Kangasjarvi (2010), since ROS are mostly ephemeral molecules.

### *The sugar connection*

Soluble sugars play a central role in plant metabolism as sources of carbon and energy in cells, and their pools are continually adjusting as a result of the balance between supply and utilization of carbon at the whole plant level and of the cell sucrose–starch partition, which is under the control of several factors, including drought (Chaves *et al.*, 1991). Nowadays, it is also recognized that sugars are important signalling molecules (see the recent review by Hanson and Smeekens, 2009) and may play important roles in the adaptive mechanisms to stress, including, for example, sucrose induction of stress defences (Ramel *et al.*, 2009). Different neutral sugars and sugar intermediates are known to be sensed by specific sensors, although most still remain unidentified.

It was proposed by Usadel *et al.* (2008) that plants respond in an acclimatory manner to the balance in the supply–use of sugars and that signalling events may be initiated by small changes in carbon status. The intracellular concentrations of sugars exert a feedback control on the rate of photosynthesis, these feedback mechanisms leading to significant changes in enzyme activities and gene expression (Koch, 1996). In general, source activities such as photosynthesis, nutrient mobilization, and export are up-regulated under low sugar conditions, as a result of gene de-repression, whereas an accumulation of sugars has the opposite effect (Pego *et al.*, 2000). Contrastingly, sink activities such as growth and storage are up-regulated under carbon abundance. Eberhard *et al.* (2008) suggest that remodelling of photosynthesis induced by a high sugar content may play an important role in minimizing the deleterious effects of excess light under conditions in which no net photosynthesis is required (e.g. when growth is arrested, as may happen in the early stages of water deficits). Moreover, quantitative studies of biochemical and physiological traits in plants under stress revealed that pre-stress sugar concentrations were correlated with subsequent stress tolerance (Ramel *et al.*, 2009). Among the different sugar traits, the sucrose concentration at the end of the day was found to be particularly important for stress tolerance.

A recent study by Meyer *et al.* (2007) also suggests that metabolic signals and not the availability of metabolic substrates determine the growth rate, with fast-growing species using available carbohydrates in growth and thus having low tissue sugar levels. In contrast, slow-growing plants save carbon resources that might be used under unfavourable conditions.

Strong evidence is also available for the role of sugar signalling in the regulation of diurnal gene expression, with sugar content affecting 25–50% of transcripts that are subjected to circadian regulation (Blasing *et al.*, 2005). This also explains major sugar control of photosynthetic activity during the day/night cycles, with photosynthesis-related transcripts displaying the lowest abundance during the night.

Sugar metabolism in plants is highly dynamic as it varies with the stage of development and in response to biotic and abiotic stress (Rolland *et al.*, 2006). For example, phloem carbohydrates are known to control reproductive development. Indeed, starch mobilization and an increase in leaf carbohydrate export to the shoot apical meristem underlie flowering induction (Corbesier *et al.*, 2002). Figure 1 show that starch is a connection point for drought, photosynthesis, ABA, and ROS, and in *Arabidopsis* transcription of several amylase, sucrose synthase, and neutral invertase genes correlates with ABI1 and with GBF3 genes, while in another group it is possible to associate amylase and catalase gene transcription.

Under water limitation, the balance between photosynthetic carbon uptake and the use of photoassimilates by the sinks is affected, leading to alterations in the pools of carbon (sugars) in the various compartments of the plant. The concentration of soluble sugars in leaves may increase (under the initial stages of moderate stress; Pinheiro *et al.*, 2001), stay constant, or decrease (under intense stress). Inhibition of growth and export explains why under low carbon assimilation sugars may increase in leaf blades (Chaves and Oliveira, 2004). Starch synthesis is generally repressed under water deficits (Chaves, 1991), but there are indications that in the early stages of water stress a transitory increase in its concentration may occur (authors' unpublished results). Alterations in the magnitude of the sugar pools are supported by changes in the enzyme activities involved in sugar- and starch-elated pathways

such as α-amylase, sucrose synthase, and invertase, their association with drought and photosynthesis being highlighted in the literature survey (Fig. 1B). An increase in total acid invertase activity, coinciding with the rapid accumulation of glucose and fructose, was reported in leaves of maize plants (Trouverie *et al.*, 2003) and of lupins (Pinheiro *et al.*, 2001) subjected to drought. Transcription analysis (Supplementary Table S5 at *JXB* online) shows that the same stress has distinct effects within the gene family, which can be easily justified due to the fact that the invertase gene family comprises three types of invertase enzymes distinct in terms of cellular location and kinetic properties. However, Supplementary Table S5 also show that drought effects are variable for the same gene in several data sets, not allowing a general response to be extracted. Possible exceptions are one cell wall invertase and two neutral invertase genes. While acute stress lead to up-regulation of two invertases (cell wall AT3G13790 and neutral AT4G09510), slowly imposed stress leads to down-regulation. Although stress leads to the up-regulation of the neutral invertase gene At3G06500, acute stress induces higher alterations very early (1–2 h).

It is well known that ADP-glucose pyrophosphorylase (AGPase; Supplementary Table S1, S2 at *JXB* online), a key enzyme in starch synthesis, is highly regulated by sugars (Geigenberger *et al.*, 2005), with starch breakdown (namely that which occurs each night) being a major source of glucose signals (Rolland *et al.*, 2006). On the other hand, KIN10 and KIN11 protein kinases are regarded as central in the coordination of several plant responses to sugars and stress, whereas bZIP TFs (such as GBF3, identified in Fig. 1, that can interact with the sucrose-sensing pathway) were shown to mediate effects of sugar signalling on gene expression and metabolite content (Hanson and Smeekens, 2009). The interconnection between starch and sugar metabolism is highlighted when looking at the correlated transcription of GBF3, ABI1, several amylases, sucrose synthase, and neutral invertase genes under stress. It can be concluded that the imbalance between sugar production (photosynthesis) and utilization (growth) observed under moderate drought constitutes an important signal to modulate photoassimilate investment in different organs of the plant (increasing, for example, the root versus the shoot) or, by accumulating in leaves, to play a protective role against oxidative stress, as is discussed below.

### *The interplay between sugars, ROS, and hormones*

Figure 1A shows that ROS generation, sugar and starch metabolism, and ABA are clearly associated. Considering ETR1, a cross-talk between sugars and ROS is becoming apparent, with either converging or antagonistic effects (see the review by Couée *et al.*, 2006). Using the tool 'Correlated Gene Search', it was possible to observe that stress induces correlated effects on several α-amylase and catalase genes, as well as catalase and β-amylases genes. Sugars are known to feed the oxidative pentose phosphate pathway that can contribute to ROS scavenging, as shown in mammalian cells, but can also increase ROS production through, for example, glucose auto-oxidation. Sucrose protection against oxidative stress seems to be partly due to activation of specific ROS scavenging systems (e.g. superoxide dismutase, Supplementary Tables S1, S2 at *JXB* online), with consequent reduction of oxidative damage, as confirmed by transcriptome analysis (Koch, 1996; Ramel *et al.*, 2009). Fluctuations in sugar content that accompany alterations in the environment will therefore influence ROS production, placing sugars as key players in the redox balance in plants. Interestingly, the relationship between ROS production under excessive light and sugar accumulation may have been the basis for the selection of the parallel induction of gene expression by light and sugar in plant cells, as suggested by Couée *et al.* (2006). However, pathways of sugar-induced responses to stress remain to be characterized, and further investigation of the interactions between metabolic (sugar) and other stress signals needs to be pursued (Gibson 2000, 2005).

The interplay between sugar and plant hormone pathways is also well established (Hanson and Smeekens, 2009). Sugars activate specific, or hormone cross-talk, transduction pathways in response to stress (Ramel *et al.*, 2009). In particular, sugar is closely related to the ABA signalling cascade and to a lesser extent with auxins and ethylene signalling, which is exemplified by ABI1, ABI3, and ETR1 (Fig. 1; Supplementary Table S2 at *JXB* online). Furthermore, under stress, co-expression was found for one sucrose synthase gene and ABI3, while ABI1 gene was co-expressed with the genes of one neutral invertase, two sucrose synthases, one β-amylase, and the GBF3 TF. Such an association can lead to amplification of the signals as, for example, sugars travelling in the xylem of droughted plants are likely to exert an influence on stomatal sensitivity to ABA and, on the other hand, ABA can regulate the activity and expression of invertases, enzymes that hydrolyse sucrose to its hexoses.

## Conclusions and the way foreward

Great progress has occurred in recent years in elucidating the nature of the various factors affecting photosynthesis in plants subjected to water deficits. The alterations that do occur in response to stress comprise the restriction of $CO_2$ diffusion to the chloroplast, as well as metabolic changes, including the modulation of the expression of photosynthesis-related genes. However, when trying to make use of publicly available data to establish which events are regulated by and/or regulate photosynthesis, the lack of stress characterization is immediately revealed, impairing the possibility to compare and integrate data. From the meta-analysis of the literature, it was not possible to establish a firm answer regarding how common are the sets of metabolic responses (genes and proteins) that are active in regulating photosynthetic activity under water stress. Exceptions are ABI3 (down-regulated under stress) that responds to both auxin and ABA, and the serine/threonine

phosphatase ABI1 (up-regulated under stress), which acts as a negative regulator of ABA promotion of stomatal closure. This highlights the role of post-translational regulation of protein activity in drought responses.

The meta-analysis also reveals how interconnected sugar and starch metabolism (directly affected by the photosynthetic performance) are with hormone and ROS pathways. The interconnections between the different pathways that act on photosynthesis in response to dehydration are being unravelled and indicate a multitude of responses acting in parallel, which may explain the flexibility and resilience of photosynthesis under drought as well as the diversity of responses across species. On the other hand, as rightly pointed out by Hanson and Smeekens (2009), sugar signalling research is likely to present new opportunities for crop improvement, by acting on pathways determining source–sink relationships or spending–saving strategies in plants under stress.

Ultimately, many questions remain unresolved regarding carbon assimilation (and plant) response to drought, partially associated with discrepancies observed with different species and experimental conditions. The physiological significance (stress response and/or tolerance) of alterations in expression observed in many drought-responsive genes, including those related to photosynthesis, are still not fully understood. Moreover, post-transcriptional and post-translational studies have been largely neglected. Proteomic and metabolomic approaches will have to be reinforced in order to obtain information on the relevance of the different metabolic responses for photosynthetic acclimation to drought. Furthermore, such approaches need to consider and integrate the physiological data in order to allow the full use of the computational methods being developed. Through the integration of multilevel data that can be compared, it will be possible to provide insights and directions on future research.

## Supplementary data

Supplementary data are available at *JXB* online.

**Table S1.** Extracted relationships at the metabolite level (A) and at the protein/gene level (B) for drought and photosynthesis with local connectivity ⩾2 using the available literature (Pathway Studio software, February 2010).

**Table S2.** Extracted relationships (A,C) and connectivity and local connectivity (B,D) for drought and photosynthesis and the several cell processes selected (available from the literature, Pathway Studio, February 2010). A, B at the metabolite level; C, D at the gene/protein level.

**Table S3.** Details of the proteins/genes found to be related to drought and photosynthesis (Fig. 1B) for *Arabidopsis*, rice, and barley (*Arabidopsis* and rice Entrez gene ID provided by Pathway Studio software).

**Table S4.** Drought-related arrays, publicly available at EMBL-EBI ArrayExpress (www.ebi.ac.uk/microarray-as/ae/). The arrays considered for detailed analysis of drought effects on transcription are in bold and blue. In red are those arrays that were excluded (see text). Keywords used: drought (62 experiments), water stress (34 experiments), and dehydration (9 experiments).

**Table S5.** Expression log ratio levels (drought/control) for amylase, sucrose synthase, invertase, Rubisco, nitrate reductase, catalase, the protein activity regulators ABI1 and ETR1, and the transcriptions factors ABI3 and bZIP (proteins TL61.5, HY5, AHBP-1B, and GBF3) taken from microarray experiments available at www.ebi.ac.uk/microarray-as/ae/). Gene ID details are presented in Supplementary Table S3 and array details in Supplementary Table S4. Arrays from *Arabidopsis thaliana*, *Hordeum vulgare*, and *Oryza sativa* were considered, being different between acute and soil experiments.

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