

## Musings about the effects of environment on photosynthesis

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Understanding of how plants respond to their environment, particularly to extreme conditions to which their metabolisms are not adapted, is advancing on many fronts. An enormous matrix of plant and environmental factors exists from which mechanisms and assessments of quantitative responses must be developed if further progress in understanding how to improve plant (and particularly crop) production is to be achieved. This Special Issue contains assessments of different areas of plant sciences, ranging from genome to field, but with a focus on photosynthesis. Photosynthesis is central to all aspects of plant biology as the provider of energy and assimilates for growth and reproduction, yet how it is regulated by abiotic stresses, such as salinity and water deficits, and by biotic stresses, such as insect herbivory, is still unclear. Differences in responses of C<sub>3</sub>, C<sub>4</sub> and CAM plants are still uncertain and mechanisms unclarified. Gene distribution and transfer between chloroplasts and nucleus on an evolutionary time scale may reflect conditions in the cell and organelles relevant to the short-term effects of water deficits on photosynthetic rate and the function of ATP synthase. Regulation of conditions in tissues and cells depends not only on chloroplast functions but on mitochondrial activity, and their interaction and differences in responses have implications for understanding many aspects of cell metabolism. Adaptation of plant structure, such as stomatal frequency and composition of the photosynthetic machinery by changes to gene expression controlled by transcription factors, or arising from regulation of gene expression by redox state, is of major importance with implications for adaptation in the short- and long-term. The incisive and thought-provoking reviews in this Special Issue offer analyses of experimental information and develop concepts within the complex matrix, relating photosynthesis and associated metabolism to the environment and addressing mechanisms critically with a balanced assessment of the current state of the science.

**Key words:** Photosynthesis, environment, water deficit, drought, salinity, gene expression, C<sub>3</sub>, C<sub>4</sub>, CAM, ATP, RuBP.

### PLANTS AND ENVIRONMENT: PHOTOSYNTHETIC PROCESSES IN CONTEXT

Photosynthesis in the wider sense, and its behaviour in relation to environment, is considered in this collection of reviews addressing currently important scientific topics. Photosynthesis is central to the performance of autotrophic plants, not in isolation or uniquely but combined with the processes determining growth and development as part of the whole organism's function and reproductive performance and survival. Ultimately reproduction and survival of the species depend on the efficiency of the different components of the whole organism (Dieckmann *et al.*, 2004). All this is achieved by integration of many, complex, individually and finely regulated sub-processes, currently summarized with the now-familiar litany of genomics, proteomics and metabolomics (van Straalen and Roelofs, 2006), plus the less familiar but arguably (because of its integrative role) more important physiomics (Grossmann, 2005). Performance and efficiency of all sub-systems and of their integration in the system – the whole organism – are tested in evolutionary competition (Silvertown, 2005). This may involve development of – and changes to – fundamental molecular mechanisms such as association of existing regulatory mechanisms, e.g. of transcription factors, producing altered characteristics in plant

development and flower formation (Scutt *et al.*, 2007). Biological success requires effective regulatory mechanisms in all processes, at all levels of organization. The plant kingdom is enormously diverse and widely distributed (Rosenzweig, 1995) and plants have achieved world dominance by 'doing their thing' effectively within particular environments (Breckle, 2002). However, environments are not constant anywhere; conditions fluctuate rapidly around a long-term mean, and there are extremes, i.e. conditions that occur infrequently as defined statistically. Of course, 'extreme' is a relative term: it can only be understood in relation to the ability of the species (actually its sub-systems and their integration into the whole organism) to survive and to perform adequately within a particular environment over a long period in the face of competition (van Straalen and Roelofs, 2006). In addition, plants must cope with long-term changes in the mean and range (including extremes) of conditions. Ultimately, it is the ability of individuals of a species to adapt to extremes that determines distribution of the species. Limited adaptation may decrease the frequency of occurrence of a species and its contribution to the community and total biomass. Inability to adapt results in total elimination of the species from the area where those conditions occur, or if they occurred for a limited period, the species is absent for a span of time determined by the rate of recolonization and the frequency of the extreme conditions and competitive ability (Aarssen and Keough, 2002).

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Environmental conditions that decrease growth, reproduction, etc. are often called ‘stresses’ but it is not the environment that is ‘stressful’. Environment is neutral: there is no such thing as a stressful environment *per se*. An environmental factor is only stressful if the organism is unable to function effectively. After all, one man’s meat is another man’s poison. Plants are not immutable: individuals may change in composition and function depending on the environment, allowing adaptations that help to maintain (or partially so) function, reproductive success, and thus ensure survival (Schulze *et al.*, 2005). Of course, survival is not the only outcome of the process of adaptation. New qualitative features or permanent quantitative changes alter behaviour of the species, and are the stuff of natural selection, part of the long-term adaptation to environment that drive changes in organisms (van Straalen and Roelofs, 2006). This is summed-up by: ‘there are horses for courses’. Environmental conditions to which species are adapted through evolution of mechanisms from gene to organism are those where they function best in the long-term, with survival the outcome (Schulze, *et al.*, 2005). Different environmental factors – abiotic such as temperature, water and light, and biotic such as insect pests or pathogens, together with the competition from other organisms – vary unpredictably, perhaps even chaotically. Species have evolved under such conditions and extant species are those best adapted. But plants (and the sub-systems that comprise them) are not able to match all conditions. Competition ensures specialization (Silvertown, 2005) and adaptation to a particular range of conditions (horses and courses again) – which gives vegetation growing and surviving successfully at high temperatures and with small rainfall (tropical deserts) or in standing water at low temperatures (boreal swamps) (Breckle, 2002). It is when environmental conditions exceed the ability of individuals in species to achieve optimal performance that the environment becomes a ‘stressor’. Under such conditions, by definition, plants photosynthesize, grow, produce and survive sub-optimally, and of course they will not out-compete better-adapted species.

Why is all this important? Because it points out the complexity of adapting to a range of conditions is one answer. Currently there is great emphasis on using direct intervention (genetic modification) in the genome of plants to improve performance for specific human requirements: increased yield of desired products to feed, clothe and protect the burgeoning human population of our increasingly over-exploited ecosystem (Arntzen *et al.*, 2003; Vaughan *et al.*, 2007). In addition, less direct intervention (plant breeding) has long been pursued to achieve these ends (Waines and Ehdaie, 2007). Both approaches have proved difficult to apply to improve production under ‘stress conditions’ such as salinity and water deficit, a major environmental factor that is particularly considered in this Special Issue. The aim is to obtain species that are ‘stress tolerant’ or ‘stress resistant’ and can produce the largest possible production for human consumption. Both ‘stress tolerant’ and ‘stress resistant’ are frequently used terms meaning ‘not being affected by’ the conditions. Given how dependent production of natural vegetation is on water supply for example, it is highly unlikely (I might even say impossible) that true resistance, as defined above, can be developed in a crop that yields well under adequate conditions.

Rather, partial tolerance or resistance might be a more realistic aim, depending on the environment. Perhaps, in considering the potential for adaptation of crops, more exact definition of terms and quantification of conditions are required. Of course, species are adapted to water supply combined with other environmental conditions: the range of natural adaptations shows it can be done. Many species are adapted to particular geographical areas where water supply is not conducive to growth by exploiting periods (often very limited and infrequent) when it is (Breckle, 2002; Schulze *et al.*, 2005). Adaptations to limited water may include small leaf area or decreased branching, which preclude large total assimilate production and so limit yield of seed, for example. Species are often very plastic, so with abundant water large and sustainable leaf area, branching, seed production and yield are possible, but they are much decreased with limited water (Neumann, 2008). Small differences in efficiency determine competitive ability and survival (Silvertown, 2005) and may be expected with water limitation. In crop species there is considerable genotype  $\times$  environment interaction, as breeders summarize the situation. The aim is to select the most productive genotype for a particular environment. There are potential pitfalls, as shown by wheat cultivars developed in the green revolution that produce smaller root systems than local land races (Waines and Ehdaie, 2007): in natural dry environments plants with such characteristics would be rapidly lost from communities. In irrigated agriculture they may be less efficient in using water. For crops, improvement in performance under adverse water conditions is possible, but it may be difficult to achieve quickly and may always be limited – after all, vegetation of deserts produces much less biomass per unit area of land over a given period than vegetation of swamps, other conditions being equal. Developing a crop with the same production with ample and with limited resources is unlikely.

Obtaining the holy grail of large agricultural production in dry and/or saline environments to which desired species are currently poorly adapted (Porter and Hay, 2006) will certainly require improvements in knowledge, both in genomics and breeding (Vaughan *et al.*, 2007; Neumann, 2008) and in details of processes deep in metabolism (Libourel and Shachar-Hill, 2008). These must be linked to detailed understanding of the environment. Assessing the potential for crop improvement realistically also depends on such understanding. How do plants adapt to their environment and what parts of the plant – genome to physionome – are susceptible? What is the weakest link? Given the number of species (and, in crops, cultivars or varieties) and the number of sub-processes (qualitative) and their ranges (quantitative), and the types (qualitative) and ranges (quantitative) of both abiotic and biotic environment factors, there is an enormous multi-dimensional matrix of possibilities. Even if for scientific study there is strong selection of species, conditions, etc. in order to try and identify mechanisms, it still leaves considerable room for uncertainty. Identifying mechanisms has progressed dramatically with the millions of person-hours and other resources invested over a considerable span of human endeavour: information has been increasing exponentially over recent decades. This explosion of information complicates the analysis of plants. Attempting to turn conceptual (qualitative) models into quantitative

models is slow and fraught with uncertainty. Knowledge is the key, but are we in a position to use current knowledge sensibly? Can we avoid a science-fiction world of hopes and dreams ill-founded in reality, or pessimism that all is too complex and progress is not possible? One way of trying to do so is to review the scientific knowledge and progress and to chip away at the edifice (or fill in the matrix) with the aim of understanding plants in relation to their environments. Hence the group of reviews presented here.

### UNRAVELLING THE GORDIAN KNOT

Currently there is a strong emphasis on unravelling the Gordian knot of cellular and sub-cellular mechanisms involved in the response of the whole plant to environment, particularly to water deficits (Neumann, 2008) but also flooding (Shimamura *et al.*, 2007). It is accepted that the same basic mechanisms are affected by particular conditions, including extremes of very different environmental factors, sometimes similarly but at other times very differently. Because of the importance of photosynthesis, the selection of papers in this Special Issue focuses on the way this process is affected by environment, particularly water, addressing basic mechanisms. The range of topics is broad, from the changes in gene expression related to conditions in photosynthetic cells, to evolutionary aspects of gene transfer from chloroplast to nucleus. There is emphasis on the effects of water deficits on photosynthetic metabolism in relation to plant performance at different levels of organization, including mitochondria and leaf cells, and consideration of CAM and C<sub>4</sub>, as well as C<sub>3</sub> photosynthetic mechanisms. Biotic conditions, such as insect feeding, affect plants via different mechanisms to abiotic but there are considerable links. From the contributions it will be apparent that plant responses are elicited by common mechanisms at different levels of organization. However, although the mechanisms may be common they are sufficiently different to allow considerable interaction, thus enlarging the matrix, and care has to be taken in assuming that 'one-size fits all'.

The review by Chaves *et al.* (2009) provides a skilful and detailed analysis of the effects of soil and atmospheric water deficits and salinity, showing how plants respond, emphasizing photosynthesis together with cell growth. It also provides a back-drop for the other reviews. The relative roles of diffusion limitations through the stomata and metabolism are addressed, including oxidative stress arising from multiple stresses. Effects of water deficits and salinity on metabolism depend on the rate of change and on the adaptability of the plant's mechanisms. In addition, rate of recovery of photosynthesis is important for production. There is a novel analysis of transcript-profiling studies in plants subjected to drought and salinity; plants perceive and respond rapidly to physiological and biochemical changes by altering gene expression in parallel to small changes in environment. Chaves *et al.* (2009) show that gene expression responds rapidly to mild water deficits and gene expression for photosynthetic components is inhibited. It seems likely that the effects are mediated through the type of mechanisms considered by Pfannschmidt *et al.* (2009), where the changes in redox state and products of an over-reduced electron transport system, such as reduced oxygen species and hydrogen peroxide, alter gene expression and re-balance

metabolism according to the energy balance. Although there are similar effects of water deficit and salinity on some genes, others differ, suggesting that water deficits and salinity are not equivalent (see Nagy and Galiba, 1995) and that differences in types and concentrations of ions are important. Similarly, Hewezi *et al.* (2008) have shown that a number of genes respond similarly to light and temperature applied separately and together, but other genes are affected by one condition and not the other, showing how complex regulation is.

The theme of energy balance emerges from the analyses presented in several reviews: the importance of maintenance of homeostasis of redox and adenylate systems in cells under different environmental conditions is emphasized, for metabolic imbalance prevents normal organ and cell functions. Redox and adenylate homeostasis in photosynthetic cells is, of course, intimately dependent on assimilate supply and demand, and thus on CO<sub>2</sub> assimilation. Very complex regulatory networks are required to keep cells functioning when stomata close (or partially so) or parts of metabolism are affected (as is likely with salinity and drought), so changing the balance between CO<sub>2</sub> assimilation and capture of light energy and its use or dissipation. As Pfannschmidt *et al.* (2009) and Lawlor and Tezara (2009) summarize, photosynthetic electron transport starts with the excitation of chlorophyll by photons, which drives electrons from photosystem II to photosystem I and then to NADP<sup>+</sup>, reducing it to NADPH (reductant). This electron transport occurs along a chain of redox components in the thylakoid membranes and is coupled to transport of protons (H<sup>+</sup>) from the chloroplast stroma into the thylakoid lumen, generating an electrochemical proton gradient. Protons move back to the stroma through the transmembrane components of the complex enzyme ATP synthase, which spans the membrane, providing the energy for ATP synthesis by a rotary mechanism (Weber and Senior, 2003). NADPH and ATP are consumed in the dark reactions of photosynthesis by the Calvin cycle, which generates RuBP. Combination of CO<sub>2</sub> with RuBP gives 3-phosphoglyceric acid, which is metabolized to sucrose and starch. Using reductant, NO<sub>3</sub><sup>-</sup> is also reduced to NH<sub>4</sub><sup>+</sup>: this is used in synthesis of amino acids and proteins. Thus, photosynthesis provides the basis of growth, reproduction and survival. Efficiency of the process depends on the balance between electron transport and NADPH and ATP synthesis and the dark reactions of CO<sub>2</sub> assimilation. Adverse environmental conditions such as excess illumination, limited CO<sub>2</sub> assimilation due to drought or salinity, or low nutrient or water availability, cause imbalance between electron transport and dark reactions and so disturb homeostasis in photosynthesis. One aspect of considerable importance is the generation of reactive oxygen species (ROS) when excitation energy and electrons are not used in CO<sub>2</sub> and NO<sub>3</sub><sup>-</sup> reduction. Because photosynthesis is such an important process, imbalance affects all cell functions. Photosynthetic organisms, therefore, have developed many, varied mechanisms to avoid or minimize imbalance and to maintain homeostasis. All mechanisms are based ultimately on gene expression: adaptation to adverse conditions requires changes in expression to alter the amounts and activities of system components to maintain or re-adjust photosynthetic efficiency under adverse conditions and to counteract abiotic and biotic factors.

Redox changes and signals from photosynthetic electron transport, and ROS or ROS-scavenging molecules produced as a consequence of imbalanced redox state, are part of normal regulation of enzymes and metabolic pathways (Buchanan and Balmer, 2005). They also play a central role in the regulation of acclimation and stress responses. Pfannschmidt *et al.* (2009) conclude that although the underlying signalling network of photosynthetic redox control is largely unknown, gene regulation by redox signals is of major importance. Such signals relate the state of the chloroplast to the rest of the cell, including the nucleus, so that changes in the environment and plant are expressed via molecular signals and gene expression to regulate and balance cellular functioning. The studies of Pfannschmidt *et al.* (2009) were on a well-defined, rapidly changed system. However, trying to unravel the responses of cells to water deficit is complicated by technical difficulties, plus multiple, simultaneous changes in different parts of metabolism. As Lawlor and Tezara (2009), and Chaves *et al.* (2009) discuss, a primary effect of mild, relatively rapid water deficit is decreased stomatal conductance, decreasing CO<sub>2</sub> concentration in leaves. Tezara *et al.* (1999) conclude that with decreased CO<sub>2</sub> assimilation but continued photosystem function and electron transport, a highly reduced state occurs. Despite increased energy dissipation, it is likely that generation of ROS occurs under such conditions. New evidence shows that ROS damages ATP synthase, offering explanation of the observations (Lawlor and Tezara, 2009) of decreased ATP synthase and therefore ATP contents with water deficit. Consequently, ribulose bis-phosphate (RuBP) synthesis by the Calvin cycle decreases and with it CO<sub>2</sub> assimilation: this is shown by decreased CO<sub>2</sub> assimilation as a function of CO<sub>2</sub> concentration in the leaf (Lawlor, 2002). If ATP synthase is particularly sensitive to conditions and ROS within the chloroplast, then the conditions under which ROS develops will be very important. Lawlor and Tezara (2009) identify differences in radiation between experiments as the reason for the much-discussed variation in responses of photosynthesis under water deficits to external CO<sub>2</sub> supply.

The importance of ATP synthase in relation to photosynthetic regulation is recognized (Wu *et al.*, 2007) and requires further examination under water deficits and other stresses. Strong evidence that ATP synthase from both mammalian (Wallace, 1999; Cadenas and Davies, 2000; Green *et al.*, 2004) and plant mitochondria (Day *et al.*, 2004) and from chloroplasts (see Lawlor and Tezara, 2009) is susceptible to ROS damage indicates that ATP synthase may be a 'weak spot' – an Achilles' heel (N. Smirnov, University of Exeter, UK, pers. com.) – in cellular metabolism under water deficit. As ROS generation is a general effect of many 'stresses', damage to ATP synthase from chloroplasts might be more widespread than appreciated. Understanding of the mechanisms and diversity of ATP synthase enzymes from different organelles and organisms is progressing rapidly (von Ballmoos *et al.*, 2008), so greater attention to the differences between chloroplast and mitochondrial enzymes (Atkin and Macherel, 2009) in higher plants may prove a useful line of study in understanding just what determines how plants respond to environment.

Avoidance of damage to ATP synthase would be a priority for plants, requiring maintenance of cell water content and regulation of ion concentration. Stomatal closure decreases water loss and maintains water content but at the risk of damaging photosynthetic metabolism. Slowly developing water stress in the field (Chaves *et al.*, 2009) is often more related to changes in leaf area than to stomatal closure (which may be partial and slow water loss by more than it decreases photosynthesis). The necessity to keep ATP synthase and the other components of metabolism functional may explain the multitude of water-conserving methods by modification of water loss, uptake and storage (Breckle, 2002; Schulze *et al.*, 2005) rather than adjustment of metabolism to a diminished water status.

The roles of respiration and the mitochondrial mechanisms in carbon metabolism and CO<sub>2</sub> production are much studied (Day *et al.*, 2004), although there is inadequate understanding of them in redox and energy homeostasis under water deficits. Atkin and Macherel (2009) extensively and critically review the topic. Although mitochondria may once have been regarded as only consumers of assimilates from the chloroplast, their many crucial roles in regulating photosynthesis and the responses to drought are becoming clear. Water deficit generally inhibits respiration in actively growing roots and whole plants: in mature leaves it decreases respiration in two-thirds of studies, with little indication of stimulation. Changes in respiration are small and relatively small compared with the large decreases in CO<sub>2</sub> assimilation with drought, suggesting a more stable system than in the chloroplast. But as CO<sub>2</sub> assimilation falls so respiration becomes proportionally more important, as Lawlor and Tezara (2009) show, increasing the internal CO<sub>2</sub> concentration. The characteristics of mitochondria, as Atkin and Macherel (2009) show, are consistent with a role as a 'safety valve' removing excess reductant (Rasmusson *et al.*, 2004). They are also a source of ATP, thus maintaining homeostasis of the redox and adenylate status of photosynthetic cells under water deficit. However, quantitative evidence of their role under adverse conditions is limited despite the advances in understanding of redox regulation within the context of metabolism (Buchanan and Balmer, 2005).

Impaired ATP supply is recognized as a basic cause of disruption to cellular homeostasis under other environmental conditions, such as flooding (Bailey-Serres and Chang, 2005), with anoxia decreasing electron transport (lack of O<sub>2</sub> acceptor) and slowing ATP generation, so cells become more reduced and pH falls (Felle, 2005). Mitochondria consume NAD(P)H under such conditions, as they appear to do under water deficit, and can also utilize nitrate as acceptor. However, ROS is generated, causing inhibition of cytochrome *c* (Igamberdiev and Hill, 2008). Anaerobic mitochondrial metabolism may have a more significant role than previously thought in alleviating the effects of anoxia on plant cells. It is interesting how the same basic mechanisms are affected (but in different ways) in very contrasting environmental conditions: water deficit and flooding.

Previous remarks refer to C<sub>3</sub> plants particularly, but C<sub>4</sub> photosynthesis is equally or even more sensitive to water deficits than C<sub>3</sub>, as Ghannoum (2009) concludes. This is surprising given that C<sub>4</sub>s are of considerable economic and ecological

importance in hot, dry conditions. When  $C_4$  metabolism was elucidated, evidence of large water use efficiency (related to small stomatal conductance and transpiration rates yet the maintenance of rapid rates of photosynthesis), led to the general view that  $C_4$  photosynthesis was insensitive to water deficits. However, such simplification was not justified and Ghannoum (2009) analyses the causes. The key feature of  $C_4$  photosynthesis is the  $CO_2$ -concentrating mechanism, which saturates photosynthesis within the bundle sheath and suppresses photorespiration by Rubisco.  $C_4$  photosynthesis is shown to be very sensitive to water deficit in three successive phases. Initially, stomatal closure may or may not decrease  $CO_2$  assimilation depending on conditions, because of the  $CO_2$ -concentrating mechanism and re-fixation of photorespired  $CO_2$ . Then stomatal and non-stomatal limitation occurs, with finally metabolic limitation, which includes reduced activity of photosynthetic enzymes and limited photorespiration or Mehler reaction, which may act as significant alternative electron sinks. Perhaps inadequate capacity of alternative pathways for use of energy and reductant (normally fully required for  $CO_2$  assimilation) renders  $C_4$ s more susceptible to photo-oxidative damage when metabolism is impaired, but information on these areas of metabolism in  $C_4$ s is not as advanced as in  $C_3$ s.

The other major form of photosynthesis is crassulacean acid metabolism (CAM). Herrera (2009) considers the role of facultative CAM in plant fitness under water deficit, and whether it is more important than for carbon accumulation. There are different forms of CAM but their roles are not well evaluated. In obligate CAM,  $CO_2$  assimilation is nocturnal when water loss is minimal, allowing long-term survival in severely water-limited environments, illustrating the points made earlier about adaptation to conditions. By comparison, Herrera (2009) shows in facultative CAM (also called inducible or  $C_3$ -CAM) and cycling CAM that drought-induced dark  $CO_2$  fixation is only a small proportion of  $C_3$   $CO_2$  assimilation and occurs for only a short period, so the adaptive advantages are small. Evidence is evaluated of the importance of facultative CAM on carbon and water balance, photo-protection of the photosynthetic apparatus, and on survival and reproductive effort. In some species facultative and cycling CAM contribute more to increased water-use efficiency, water absorption, prevention of photoinhibition and reproductive output, than to increased carbon balance. It is unclear if this represents an 'evolutionary stage' in CAM development of a particular ecological 'strategy'. This illustrates nicely the points made earlier about complexities of responses by different species to the environment, and warns against excessive simplification.

Ultimately, the aim of understanding plant responses to environment is to produce food, fibre and fuel. Selection of crops over a long period focussed on easily selectable traits, associated, for example in cereals, with seed size, number and retention on the plant, plus a restricted period for harvest and ease of harvesting (see Fuller, 2007, and other reviews in the *Annals of Botany* Special Issue on Crop Domestication). However, the plant characteristics altered in different crops are not identical. It is likely that characteristics associated with metabolic responses to water deficits, etc, have been co-selected with yield traits, not with 'tolerance' *per se*. Selection using molecular tools (e.g. QTL analysis) for more

specific metabolic characteristics is progressing rapidly (Vaughan *et al.*, 2007), with rice a major target (Cheng *et al.*, 2007; Sweeney and McCouch, 2007). Many of the improvements now required in crops for adaptation to environmental extremes are likely to come from more direct intervention in the genome to alter metabolism, or to provide greater genetic diversity. Regulation of gene expression in cells with imbalanced metabolism (i.e. stressed) is a topic of great current interest. In the relatively few years that molecular tools for transforming cells have been available, considerable effort (Arntzen *et al.*, 2003) has been devoted to modifying cell metabolism (including photosynthesis) by changing expression of single genes, or insertion of one or a few novel genes, with limited effect as far as improving 'stress' tolerance of yield. More recently, attention has been directed to more general regulation of gene expression by the operation of transcription factors (TFs). These may balance and regulate metabolism in co-ordinated ways to maintain homeostasis. Saibo *et al.* (2009) address this complex and rapidly changing field, showing that responses and adaptations require differential gene expression, which is regulated by several specific TFs involved in different response pathways. Many TFs, belonging to different families (e.g. MYB, bZIP and DREB), have been related to abiotic stress responses; however, only few are known to regulate the expression of photosynthesis-related genes in response to stress. Saibo *et al.* (2009) assess regulation of genes related to both stomatal and non-stomatal limitations to  $CO_2$  photosynthetic assimilation by TFs. An important aspect of response to water deficits is, as mentioned earlier, maintenance of water balance, which depends on water uptake relative to water loss. Uptake depends on rooting depth and density, etc. Loss depends on transpiration per unit leaf area, in large part dependent on stomatal characteristics (including number per unit area, size and aperture), and thus on stomatal development (Bergmann and Sack, 2007). Saibo *et al.* (2009) describe the role of several TFs of the MYB family in regulating stomatal numbers and sizes, and metabolic components, and assess their potential for modifying responses to drought. Thus there is a strong link to the review by Chaves *et al.* (2009). Interestingly, during domestication of barley (based on increasing the amount of harvestable seed) transcription factor genes were functionally impaired, rather than new ones being created (Pourkheirandish and Komatsuda, 2007) suggesting that naturally selected linkages of regulation with environment were disrupted, with the negative consequences compensated by human (agri)cultural changes. Potentially, such dissociation between metabolic processes might be required, although the consequences for homeostasis and system function and output may be much more severe than for 'end products'.

Although the link between photosynthesis and conditions in the chloroplast and the rest of the cell may appear to be very distant from the evolution of symbiosis between the oxygen-evolving, free-living, blue-green algal precursor of the current higher plant chloroplast and the eukaryotic 'mother-cell', recent evidence suggests that it is close. One of the larger questions concerning the current chloroplast is how has the distribution of genes between it and the nucleus arisen? It is part of the general question of why organelle-genes have migrated to the nucleus despite apparent disadvantages, which must be

less than the advantages (Blanchard and Lynch, 2000). Compared to current, putative free-living progenitor algae, chloroplasts have lost many genes that have migrated (and continue to do so) to the nucleus, as the review by Cullis *et al.* (2009) clearly describes and relates to conditions. There are active processes by which the nuclear genome might be acquiring or removing DNA sequences from the chloroplast genome and, of particular interest, there are possible effects of 'stressful' environments on the process. Given the sensitivity of ATP synthase to ROS, it is likely that transferring all but essential components would have selective advantages. Transfer of most of the protein-encoding functions for chloroplast-located proteins to the nuclear genome probably improves control of gene expression and integration of the chloroplast into the cell, plus enabling new proteins to be made and moved into the organelle when required for repair. Continual transfer of DNA, including complete functional genes, has been observed, but the mechanisms are unclear. The study of the effects of the cell and organelle conditions on gene expression, and the long-term effects, is still in its infancy and this review, considered in conjunction with the others, provides much food for thought.

Biotic factors that impair photosynthesis are addressed by Nability *et al.* (2009), who discuss how photosynthesis is affected by arthropod herbivores, and the importance of indirect suppression of the surviving mechanisms compared to the loss of photosynthetic capacity from reduced leaf area (see Núñez-Farfán *et al.*, 2007). Application of thermal and fluorescent imaging shows that surviving tissue is adversely affected. Four mechanisms contribute to this: severed vasculature that affects water supply, altered sink demand, defence-induced autotoxicity, and defence-induced down-regulation of photosynthesis, including herbivore-induced gene-regulating mechanisms that modulate photosynthesis. Thus, from an ecological (and crop production) perspective, ignoring indirect suppression of photosynthesis by arthropods may under-estimate its importance, and the review illustrates the mechanisms and highlights the need for further studies. Again, the role of gene expression in determining responses is emphasized.

In summary, the reviews in this Special Issue have been selected to illustrate different aspects of the matrix discussed in the opening section of this Preface, with a focus on water and photosynthesis. The aim is to understand both qualitative and (with even greater difficulty) quantitative effects on photosynthetic mechanisms and the implications for plants. I believe the reviews provide critical analyses relating photosynthesis and associated metabolism to the environment, with a balanced assessment of the current state of the science (or is it 'of play' or 'of the art'?), helping to indicate ways forward in concepts and experiments. I sincerely thank the authors for their considerable effort in writing these reviews and for their patience with my editorial and organizational shortcomings. Thanks also to *Annals of Botany*, which has generously supported the efforts to advance scientific understanding, specifically to Mike Jackson who supported the Special Issue when Chief Editor, and to the current Chief Editor, Pat Heslop-Harrison, for his continuing support and advice. Particular thanks are due to David Frost, Managing Editor, for his outstanding help, encouragement and patience.

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