Annals of Botany Page 1 of 10
doi:10.1093/aob/mcn125, available online at www.aob.oxfordjournals.org

OXFORD JOURNALS
OXFORD UNIVERSITY PRESS

REVIEW

Photosynthesis Under Drought and Salt Stress: Regulation Mechanisms from Whole Plant to Cell

M. M. CHAVES[1,2,*], J. FLEXAS[3] and C. PINHEIRO[2]

[1]*Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal,* [2]*Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. da República - EAN, 2780-157 Oeiras, Portugal and* [3]*Laboratori de Fisiologia Vegetal, Grup de Biologia de les Plantes en Condicions Mediterrànies, Universitat de les Illes Balears, Carretera de Valldemossa, Km 7·5, 07122 Palma de Mallorca, Balears, Spain*

Received: 6 March 2008 Returned for revision: 6 May 2008 Accepted: 12 June 2008

• *Background* Plants are often subjected to periods of soil and atmospheric water deficits during their life cycle as well as, in many areas of the globe, to high soil salinity. Understanding how plants respond to drought, salt and co-occurring stresses can play a major role in stabilizing crop performance under drought and saline conditions and in the protection of natural vegetation. Photosynthesis, together with cell growth, is among the primary processes to be affected by water or salt stress.
• *Scope* The effects of drought and salt stresses on photosynthesis are either direct (as the diffusion limitations through the stomata and the mesophyll and the alterations in photosynthetic metabolism) or secondary, such as the oxidative stress arising from the superimposition of multiple stresses. The carbon balance of a plant during a period of salt/water stress and recovery may depend as much on the velocity and degree of photosynthetic recovery, as it depends on the degree and velocity of photosynthesis decline during water depletion. Current knowledge about physiological limitations to photosynthetic recovery after different intensities of water and salt stress is still scarce. From the large amount of data available on transcript-profiling studies in plants subjected to drought and salt it is becoming apparent that plants perceive and respond to these stresses by quickly altering gene expression in parallel with physiological and biochemical alterations; this occurs even under mild to moderate stress conditions. From a recent comprehensive study that compared salt and drought stress it is apparent that both stresses led to down-regulation of some photosynthetic genes, with most of the changes being small (ratio threshold lower than 1) possibly reflecting the mild stress imposed. When compared with drought, salt stress affected more genes and more intensely, possibly reflecting the combined effects of dehydration and osmotic stress in salt-stressed plants.

Key words: Photosynthesis, stress, drought, salt, stomatal, mesophyll and biochemical limitations, gene expression, signalling.

INTRODUCTION

Plants are often subjected to periods of soil and atmospheric water deficits during their life cycle as well as, in many areas of the globe, to high soil salinity. It is estimated that >6 % of the world's land and 30 % of the world's irrigated areas already suffer from salinity problems (Unesco Water Portal, 2007). Expansion of agriculture to semi-arid and arid regions with the use of intensive irrigation will increase secondary salinization as a result of changes in the hydrologic balance of the soil between water applied (irrigation or rainfall) and water used by crops (transpiration). Moreover, the faster-than-predicted change in global climate (Intergovernmental Panel on Climate Change, 2007) and the different available scenarios for climate change suggest an increase in aridity for the semi-arid regions of the globe and the Mediterranean region in the near future. Together with overpopulation this will lead to an overexploitation of water resources for agriculture purposes, increased constraints to plant growth and survival and therefore to realizing crop yield potential (Chaves *et al.*, 2002, 2003; Passioura, 2007).

Understanding how plants respond to drought, salt and co-occurring stresses can play a major role in stabilizing crop performance under drought and saline conditions and in the protection of natural vegetation. Adequate management techniques and plant genetic breeding are the tools to improve resource use efficiency (including water) by plants.

Photosynthesis, together with cell growth, is among the primary processes to be affected by drought (Chaves, 1991) or by salinity (Munns *et al.*, 2006). The effects can be direct, as the decreased CO_2 availability caused by diffusion limitations through the stomata and the mesophyll (Flexas *et al.*, 2004, 2007) or the alterations of photosynthetic metabolism (Lawlor and Cornic, 2002) or they can arise as secondary effects, namely oxidative stress. The latter are mostly present under multiple stress conditions (Chaves and Oliveira, 2004) and can seriously affect leaf photosynthetic machinery (Ort, 2001).

Photosynthetic response to drought and salinity stress is highly complex. It involves the interplay of limitations taking place at different sites of the cell/leaf and at different time scales in relation to plant development. The intensity, duration and rate of progression of the stress will influence plant responses to water scarcity and salinity, because these factors will dictate whether mitigation processes associated with acclimation will occur or not. Acclimation responses under drought, which indirectly affect photosynthesis, include

* For correspondence. E-mail mchaves@isa.utl.pt

those related to growth inhibition or leaf shedding that, by restricting water expenditure by source tissues, will help to maintain plant water status and therefore plant carbon assimilation. Osmotic compounds that build up in response to a slowly imposed dehydration also have a function in sustaining tissue metabolic activity. Acclimation responses to salinity also include synthesis of compatible solutes as well as adjustments in ion transport (such as uptake, extrusion and sequestration of ions). These responses will eventually lead to restoration of cellular homeostasis, detoxification and therefore survival under stress.

In recent years, remarkable advances have taken place in various domains of stress physiology. A major one relates to the knowledge of long- and short-distance signalling, which plays a role in the feed-forward and the feed-back control of photosynthesis in response to drought and salt. It is acknowledged that signalling pathways leading to plants' stress responses are interconnected at many levels. On the other hand, recent results suggest that the molecular and metabolic responses observed under a combination of stresses (e.g. drought and heat) are unique and cannot be extrapolated from plant response to the individual stress (Mittler, 2006).

A large amount of data is now available on alterations occurring on gene expression in response to drought, salt and other stresses (e.g. Ma *et al.*, 2006; Shinozaki and Yamaguchi-Shinozaki, 2007). The main problem now is linking those changes with meaningful effects on physiological processes because many studies are not tissue-specific and the treatments artificial (Munns, 2005). In addition, when responses are multigenic it is hard to find out the controlling genes and key proteins. Identifying groups of genes by mapping regions of the genome responsive to the particular stress has been one of the approaches used, although necessarily slow (Tuberosa and Savi, 2006).

It is becoming apparent that stress responses involve the alterations of expression of a multitude of genes, a great diversity in the timing of those alterations, and the modulation of gene expression by the intensity, duration and rate of progression of imposed the stress. We seem to be facing a real 'reprogramming' of the whole plant to attain a new energetic and developmental equilibrium in order to cope with the upcoming stress, the products of stress-inducible genes functioning both in the initial stress responses and in establishing plant stress tolerance. A new paradigm is emerging – plant response to drought and salinity (even mild), and to stress in general, occurs via a series of physiological, cellular and molecular events developing in parallel and very rapidly. It mobilizes the whole metabolic machinery towards plant acclimation and survival. Attempting to identify the initial effects of drought on plant metabolism in general and in photosynthesis in particular is therefore losing relevance.

In this paper, we highlight the current state of the art on drought and salt effects on photosynthesis, covering the events occurring at various scales, evidencing the high complexity of the process and suggesting that different sensors perceive plant stress conditions simultaneously, resulting in improved stress tolerance.

WHAT IS SIMILAR, WHAT IS DIFFERENT IN DROUGHT AND SALINITY?

Early responses to water and salt stress have been considered mostly identical (Munns, 2002). Drought and salinity share a physiological water deficit that attains, more or less intensely, all plant organs (Fig. 1). However, under prolonged salt stress plants respond in addition to dehydration to hyper-ionic and hyper-osmotic stress. Leaf tissue water deficit *per se* can be triggered not only by low soil water content but also by high vapour pressure deficit of the atmosphere.

In addition to alterations in photosynthesis and cell growth, both stresses when slowly imposed, often induce osmotic adjustment which is considered an important mechanism to allow the maintenance of water uptake and cell turgor under stress conditions. The effects of drought and salinity on photosynthesis range from the restriction on CO_2 diffusion into the chloroplast, via limitations on stomatal opening mediated by shoot- and root-generated hormones, and on the mesophyll transport of CO_2, to alterations in leaf photochemistry and carbon metabolism. These effects vary according to the intensity and duration of the stress as well as with the leaf age (older leaves are more affected by drought and accumulate higher amounts of salt) and the plant species (Lawlor and Cornic, 2002; Munns, 2002; Chaves *et al.*, 2003; Flexas *et al.*, 2004; Galmés *et al.*, 2007*a*).

Under salinity, in addition to water deficits, plants endure salt-specific effects. Salt response follows a biphasic model, with current metabolic data indicating an early similarity with drought, whereas in the long-term plants are responding to ion toxicity. There are species-specific responses to salt. Some plants are able to prevent salt entry (salt exclusion at the whole-plant or the cellular level) or to minimize its concentration in the cytoplasm (by compartmentalizing salt in the vacuoles), thus avoiding toxic effects on photosynthesis and other key metabolic processes. When those processes do not exist or are insufficient, it was shown that Na^+ at a concentration above 100 mM severely inhibits many enzymes (including photosynthetic ones) (Munns *et al.*, 2006). The enzymes that require K^+ as a cofactor are particularly sensitive to high concentrations of Na^+ or high ratios of Na^+/K^+. In salt- acclimated plants, it was also shown that primary metabolites linked to amino acid and nitrogen or carbohydrate and polyol metabolism do increase; these compatible solutes play a role in osmotic adjustment, membrane and protein protection or scavenging of reactive oxygen species (ROS) and of excess accumulated ammonium ions. Interestingly, in those plants a depletion of organic acids is also observed following the decreased carbon assimilation as stomata close; this is contrary to what happens under drought. The reduced content of organic acids under salt stress may be involved in compensating for ionic imbalance (see the review by Sanchez *et al.*, 2007).

CO_2 diffusion through stomata and the mesophyll

Stomata close in response to leaf turgor decline, to high vapour pressure deficit in the atmosphere or to root-generated chemical signals, the latter being common to

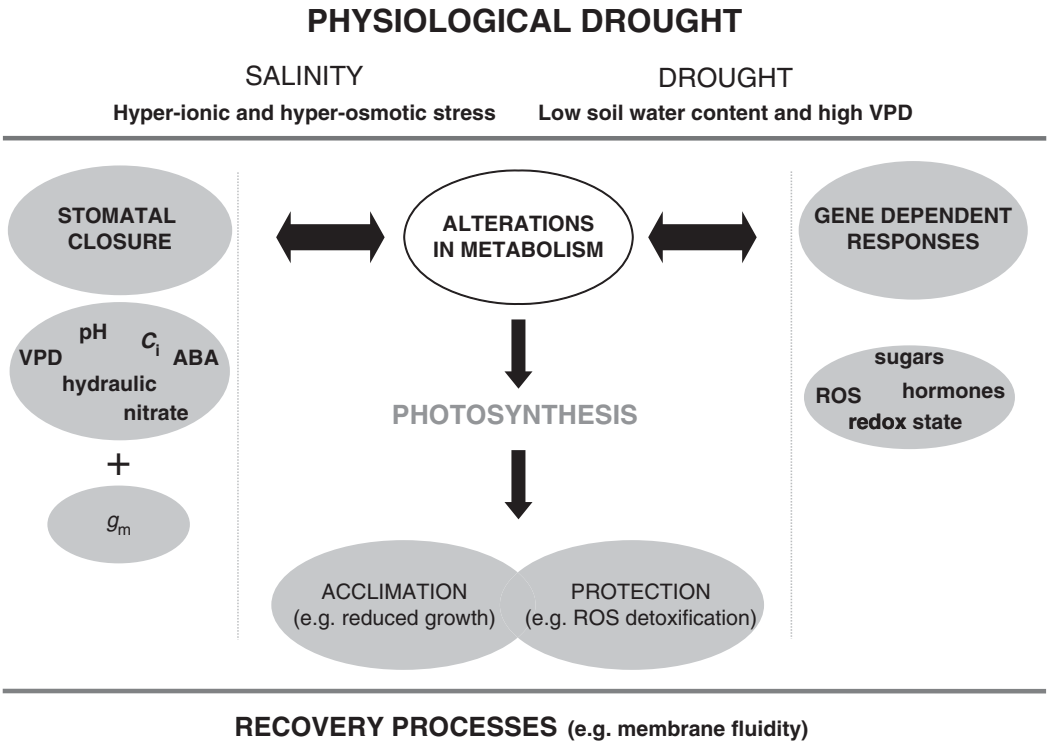

FIG. 1. Direct effects of drought and salinity on stomata and mesophyll (g_m) conductance as well as on gene expression, resulting in alterations of photosynthetic metabolism and ultimately on plant acclimation.

both drought and salinity. Supply of CO_2 to Rubisco is therefore impaired, what predisposes the photosynthetic apparatus to increased energy dissipation and down-regulation of photosynthesis when plants are subjected to high light and temperature. Under mild stress, a small decline in stomatal conductance may have protective effects against stress, by allowing plant water saving and improving plant water-use efficiency by the plant.

In addition to reduced CO_2 diffusion through the stomata, both stresses also result in an apparent reduced CO_2 diffusion through the leaf mesophyll, i.e. in a reduced mesophyll conductance to CO_2 (g_m; reviewed in Flexas *et al.*, 2004, 2007). Although not as straight forward as stomatal conductance measurements, estimations of g_m seem appropriate despite many assumptions involved in the most common methods used (Warren, 2006). This is supported by the fact that different methods involving totally different assumptions, result in very similar estimates, as demonstrated by Loreto *et al.* (1992) and Flexas *et al.* (2006*a*, 2007), among others. These changes in mesophyll conductance may be linked to physical alterations in the structure of the intercellular spaces due to leaf shrinkage (Lawlor and Cornic, 2002) or to alterations in the biochemistry (bicarbonate to CO_2 conversion) and/or membrane permeability (aquaporins). In an early work, Jones (1973) already suggested that leaf internal diffusion conductance was depressed under water-stress conditions. However, the model used by Jones assumed that CO_2 concentration in the chloroplast was close to zero or to the compensation point, which was later shown to be untrue (Farquhar and Sharkey, 1982). Comparison of chlorophyll fluorescence with gas exchange measurements also revealed that C_c was lower than C_i, and that the difference increased under conditions of water stress or salinity (Bongi and Loreto, 1989; Cornic *et al.*, 1989). That water stress specifically lowers C_c below C_i was independently confirmed by measuring leaf ^{18}O (Renou *et al.*, 1990) and ^{13}C discrimination (Brugnoli and Lauteri, 1991). Still, in these early works, it was assumed that g_m was largely unaffected by stress, and that discrepancies between C_c and C_i arose from invalid estimations of the latter due to either heterogeneous stomatal closure (Terashima, 1992; Buckley *et al.*, 1997) and/or interference of cuticular conductance (Boyer *et al.*, 1997). It was assumed that most of the mesophyll resistance to diffusion was caused by morphological and anatomical leaf traits, which are unlikely to change in response to stress, particularly in the short term. However, Genty *et al.* (1998) showed that most of the internal resistance to CO_2 diffusion was in the liquid phase inside cells instead of in intercellular air spaces, i.e. not so much dependent on leaf structure, and later studies specifically suggested that g_m was depressed under both salt (Delfine *et al.*, 1999; Centritto *et al.*, 2003) and water stress (Scartazza *et al.*, 1998; Flexas *et al.*, 2002; Galmés *et al.*, 2007*a*).

Flexas *et al.* (2006*b*) have shown that g_m responds very quickly (within a few minutes) to desiccation after cutting the leaf petiole in air, and that reduced g_m can be induced by exogenous application of abscisic acid (ABA) to well-watered plants. In addition, g_m also responds quickly to changes in temperature, light and CO_2 concentration (Flexas *et al.*, 2008). All together, these results strongly suggest that g_m is regulated biochemically, rather than simply by leaf anatomical traits. There is evidence for the

involvement of carbonic anhydrase (Gillon and Yakir, 2000) and aquaporins (Flexas *et al.*, 2006*a*) in the regulation of g_m, but the role of these metabolic components under conditions of drought and salinity remains unknown. Interestingly, different species exhibit different relative limitations to photosynthesis induced by stomata, mesophyll or the biochemistry, when water deficit progresses (Table 1).

Lateral diffusion of CO_2 in the mesophyll has been studied recently and was shown to induce important photosynthesis variations (Morison *et al.*, 2007). While no study is available on the magnitude and effects of such lateral diffusion under water or salt stress, it is likely that in cases of patchy stomatal closure, lateral diffusion from open stomata to mesophyll portions under closed stomata may support some photosynthesis and reduce possible photoinhibitory effects.

Biochemical and photochemical limitations to photosynthesis

Changes in leaf biochemistry that result in down-regulation of the photosynthetic metabolism may occur in response to lowered carbon substrate under prolonged stresses (Chaves and Oliveira, 2004; Flexas *et al.*, 2006*b*). For example, a de-activation of the carboxylating enzyme Rubisco by low intercellular CO_2 (C_i) has been observed (Meyer and Genty, 1998). Following stomatal closure and the fall in CO_2 concentration in the intercellular airspaces of leaves, other enzymes have been shown to decrease their activity (e.g. SPS or nitrate reductase); this change was quickly reversed when increasing CO_2 in the surrounding atmosphere (Sharkey *et al.*, 1990). Early biochemical effects of water deficits that involve alterations in photophosphorylation (a decrease in the amount of ATP leading to a decreased regeneration of RuBP) have also been described (Tezara *et al.*, 1999) and seem to be dependent on species showing different thresholds for metabolic down-regulation (Lawlor and Cornic, 2002). Under salt stress, metabolic limitations of photosynthesis resulting from increased concentrations of Na^+ and Cl^- in the leaf tissue (in general above 250 mM) do occur (Munns *et al.*, 2006). As previously pointed out the fast changes in gene expression following stress imposition that have been observed, suggest that alterations in metabolism start very early.

When, in addition to drought and salinity, plants are subjected to other environmental stresses such as high light and temperature either chronic (under high and/or persistent excess light) or dynamic (under moderate excess light) photoinhibition is likely to occur. In fact, under those conditions that limit CO_2 fixation, the rate of reducing power production is greater than the rate of its use by the Calvin cycle. Protection mechanisms against excess reducing power are thus an important strategy under water stress. These photoprotective mechanisms compete with photochemistry for the absorbed energy, leading to a decrease in quantum yield of PSII (Genty *et al.*, 1989). Such protection may be achieved by the regulated thermal dissipation in light-harvesting complexes, somehow involving the xanthophyll cycle (Demmig-Adams and Adams, 1996) and the lutein cycle (Matsubara *et al.*, 2001). Although its role is not totally clear yet, photorespiration may also be involved in protecting the photosynthetic apparatus against light damage as suggested by its increase under drought observed in several species (see the review by Chaves *et al.*, 2003). Photorespiratory-produced H_2O_2 may also be responsible for signalling and acclimation under restricted CO_2 availability (Noctor *et al.*, 2002). In addition to the photoprotective mechanisms that may use intercepted solar radiation that is not utilized by photochemistry, the avoidance mechanisms resulting from leaf or chloroplast movements known as 'paraheliotropism', that are very effective in reducing intercepted radiation, cannot be disregarded. The masking of chlorophyll by anthocyanins that prevent photo-oxidative damage to leaf cells is particularly important in senescing leaves since it allows an efficient nutrient retrieval from those leaves to the storage compartments of the plant (Feild *et al.*, 2001).

TABLE 1. *Relative limitations to photosynthesis (stomatal, mesophylic and biochemical) in three Mediterranean species subjected to mild, moderate, severe water deficit and recovery*

Treatment	Limitation			
	Total	Stomatal	Mesophyll conductance	Biochemical
Diplotaxis ibicensis				
Mild water deficit	17	7	9	1
Moderate water deficit	61	29	21	11
Severe water deficit	78	47	16	15
Recovery	47	13	26	8
Beta maritima ssp. *marcosii*				
Mild water deficit	0	0	0	0
Moderate water deficit	40	19	10	11
Severe water deficit	99	18	42	39
Recovery	36	6	26	4
Lavatera marítima				
Mild water deficit	24	18	6	0
Moderate water deficit	57	47	9	1
Severe water deficit	87	70	7	10
Recovery	31	22	8	0

Adapted from Galmés *et al.* (2007*a*).

REGULATION: WHAT DO WE KNOW?

Signalling

An efficient response to the environment is particularly important for plants, as sessile organisms. This means an ability of cells to quickly sense the surrounding environmental signals. Systemic signals generated by the tissue exposed to abiotic and biotic stress act in the co-ordination and execution of plant stress responses in terms of metabolic and developmental adjustments. Under salt and drought, these responses are triggered by primary osmotic stress signals (see Chaves *et al.*, 2003) or by secondary signal metabolites that generally increase or decrease in a transient mode. The latter include hormones (e.g. ABA, ethylene, cytokinins), ROS and intracellular second messengers (e.g. phospholipids, sugars, etc.).

Drought and salinity trigger the production of ABA in roots which is transported to the shoots causing stomatal

closure and eventually restricting cellular growth. ABA can also be synthesized in leaf cells and translocated around the plant (Wilkinson and Davies, 2002). Recent evidence indicates that xylem/apoplastic pH influence ABA compartmentation and consequently the amount of ABA reaching the stomata. In droughted plants, for example, the more alkaline pH observed in xylem/apoplast leads to a decrease in the removal of ABA from xylem and leaf apoplast to the symplast (the so-called alkaline trapping of ABA), such that more ABA reaches the guard cells. A higher xylem sap pH can result from a variety of plant stresses in addition to soil drying – high light, salt and nitrate, for example (Jia and Davies, 2007), enabling the modulation of stomatal aperture in response to a variety of environmental variables. ABA also mediates effects in other physiological processes through alterations in gene expression. Finally, sugars travelling in the xylem of droughted plants or sugars that might increase in the apoplast of guard cells under high light are also likely to exert an important influence on stomatal sensitivity to ABA (Wilkinson and Davies, 2002).

Soluble sugars (namely sucrose, glucose and fructose) that are altered by water deficits and salinity, also act as signalling molecules under stress (Chaves *et al.*, 2004) and do interact with hormones as part of the sugar sensing and signalling network in plants (Rolland *et al.*, 2006). A major source for glucose signals is transitory starch breakdown from chloroplasts during the night. In general, under salt and drought stress soluble sugars tend to increase, while starch content decreases (Chaves, 1991). Under very severe dehydration soluble sugars may decrease (Pinheiro *et al.*, 2001). Sugars will modify gene expression and proteomic patterns, namely those governing photosynthetic metabolism. It is acknowledged that transcripts for genes involved in photosynthesis and other source activities (e.g. photoassimilate export and nutrient mobilization) decrease under high sugar content, whereas those involved in sink activities, like degradation of carbohydrates and the synthesis of storage polysaccharides, lipids and proteins are induced (for a review, see Stitt *et al.*, 2007). There is evidence that CO_2, light, water and other environmental signals can also be integrated and perceived as sugar signals (Pego *et al.*, 2000), suggesting that different signal types may be perceived by the same receptor or that the signal pathways converge downstream (Chaves *et al.*, 2003).

The redox-state of the photosynthetic electron components and the redox-active molecules also act as regulatory agents of metabolism (Foyer and Noctor, 2003). Redox signals are early warnings exerting control over the energy balance of a leaf. Alterations in the redox state of redox-active compounds regulate the expression of several genes linked to photosynthesis (both in the chloroplast and in the nucleus), thus providing the basis for the feedback response of photosynthesis to the environment or, in other words, the adjustment of energy production to consumption. Data available on the redox regulation of photosynthesis genes suggest a highly complex signalling network (see the reviews by Pfannschmidt, 2003; Pfannschmidt *et al.*, 2009). Redox signalling molecules include some key electron carriers, such as the plastoquinone pool, or electron acceptors (e.g. ferredoxin/thioredoxin system) as well as ROS (e.g. H_2O_2). H_2O_2 acts as a local or systemic signal for leaf stomata closure, leaf acclimation to high irradiance and the induction of heat shock proteins (Pastori and Foyer, 2002). Nitric oxide, a reactive nitrogen species, also acts as a signalling molecule, in particular by mediating the effects of hormones and other primary signalling molecules in response to environmental stimuli; it may act by increasing cell sensitivity to these molecules (Neill *et al.*, 2003). Recently, nitric oxide was shown to play a role as an intermediate of ABA effects on guard cells (Neill *et al.*, 2003).

Gene expression, proteomics, metabolomics

To study the dynamics of plant metabolism under stress and unravel regulatory mechanisms in place, it is important to combine the traditional more descriptive physiological approaches with the techniques of functional genomics, namely the high throughput methods for transcriptomic, proteomic, metabolomic and ionomic analysis. Using this integrated analysis it would be possible to study the dynamics of plant metabolism in the context of the plant system as a whole.

In Table 2, some recent work (transcriptomics and proteomics) on model plants, crops and a tree subjected to different salinity and drought conditions is summarized. This table clearly shows that the number of stress responsive genes or proteins is related to the stress intensity (dramatically demonstrated by Cramer *et al.*, 2007) and with tissue origin (Zhou *et al.*, 2007). Table 2 also reflects the usual trend of larger alterations found at transcriptomic level (5–10 %) than at protein level (usually <1 %), the exception being the findings of Bogeat-Triboulot *et al.* (2007). In fact, these authors found that as the result of the stress, protein concentration can be affected without alterations in the expression of the corresponding genes.

Genes or proteins associated with photosynthetic pathways were in general not among the most altered by the stress. For example, in *Thellungiella* (a stress-tolerant plant), photosynthesis genes correspond to 15 % of all genes down-regulated (Wong *et al.*, 2006), while in rice alterations in photosynthesis-related genes are mostly associated with stress recovery (Zhou *et al.*, 2007). As a result of being relatively unaffected by salinity and drought, photosynthesis related genes and proteins have not been deeply analysed so far. However, the work by Kilian *et al.* (2007) made available extensive data. A gene was therefore made by gene analysis using the tool Genevestigator (www.genevestigator.ethz.ch; Zimmermann *et al.*, 2005). The effect of salinity and drought on genes associated with photosynthesis (AraCyc Pathways, www.arabidopsis.org/tools/aracyc) was checked in arabidopsis seedlings using the expression profiles available (generated with the Affymetrix ATH1 chip; Kilian *et al.*, 2007). The imposed stresses were evaluated for 24 h. Although no information on the physiological status of the seedlings was given, the imposed stresses may be considered as mild. Of the 139 photosynthesis-related genes (AraCyc Pathways) the AtGenExpress salt and drought microarray has information for 102 genes. As a general trend, both stresses led to gene down-regulation, most of the changes being small (ratio threshold lower than 1; Fig. 2), possibly

TABLE 2. *Salt and drought effects on the expression of gene and proteins in some model plants and crops: some examples from recent literature*

Species	Stress analysed	Stress intensity	Type of analysis	Chip/proteome dimension	Organ	Affected genes/proteins				Reference
						Drought		Salinity		
						Up	Down	Up	Down	
O. sativa	Salinity and drought	Mild/late	Transcript	36926	Leaf	582	795	1676	1270	Zhou *et al.* (2007)
					Shoot	1257	646	817	1323	
					Panicle	614	1305	1310	2284	
	Salinity and drought	Early	Transcript	1720	Shoot	62	nt	57	nt	Rabbani *et al.* (2003)
	Salinity	Early	Protein	2500	Leaf	na	na	7	2	Parker *et al.* (2006)
		Late				na	na	21	12	
Thellungiella	Salinity and drought	Mild	Transcript	3628	Rosette	47	31	0	3	Wong *et al.* (2006)
P. euphratica	Drought	Early	Transcript	6340	Leaf	8	3	na	na	Bogeat-Triboulot *et al.* (2007)
					Root	7	7	na	na	
		Late			Leaf	22	14	na	na	
					Root	6	16	na	na	
		Early	Protein	nt	Leaf	95	122	na	na	
		Late			Leaf	82	74	na	na	
Arabidopsis	Salinity and drought	Early	Transcript	24000	Rosette	60	14	735	494	Kilian *et al.* (2007)
					Root	6	12	1310	666	
	Salinity	Early	Protein	2949	Cell suspension	na	na	266		Ndimba *et al.* (2005)
Vitis vinifera	Salinity and drought	Mild/late	Protein	758	Shoot	5	17	12	16	Vincent *et al.* (2007)
	Salinity and drought	Early	Transcript	14000	Shoot	6	8	0	0	Cramer *et al.* (2007)
		Severe/late				5383	5558	5031	5323	

nt, Not available; na, not applicable.

reflecting the mild stress. When compared with drought, salt stress affects a higher number of genes and a more intense effect is observed, reflecting the combined effects of salinity (dehydration and osmotic stress). Within a multigenic family, the same stress can have distinct effects on the several genes, not only quantitative but also qualitative (Fig. 3). While both genes of fructose-bisphosphatase are similarly down-regulated by salt stress, the genes of other enzymes of the Calvin cycle and photorespiration (fructose-bisphosphate aldolase, phosphoribulokinase, transketolase, ribose-5-phosphate isomerase, phosphoglycolate phosphatase and strikingly glycine hydroxymethyltransferase) are differently affected (Fig. 3). This points to the plant capacity to react and respond differently to distinct growth conditions and to the need to address the question of metabolic compartmentation. This may also explain some inconsistent data in the literature. What are the physiological consequences of such disparities? How can we decipher such responses? Since biological activity results from the active protein pool in a given compartment, the effect of a stress on a metabolic pathway is the result of their integration/complementation. Therefore, it is necessary to link genes with gene products and their biological function, hopefully provided in the near future by metabolomics studies using mass spectroscopy-based metabolite profiling. In a recent paper by Stitt *et al.* (2007), a multilevel genomics analysis was used to unravel plant response to low carbon availability, a situation common to drought and salt stress. A large number of sugar-responsive genes showed diurnal changes in transcript levels in response to low carbon stress. Among the hundreds of genes whose transcript levels were altered by carbon starvation, a co-ordinated repression of genes involved in photosynthesis and chloroplast protein synthesis and folding was observed. Interestingly, the changes in transcript levels did not lead to immediate changes in enzyme activities. It was concluded that the diurnal changes in transcripts integrated over the days are the signal for alterations in enzyme activities that follow and will allow plant acclimation to the new condition.

Bogeat-Triboulot *et al.* (2007) combined the analysis of gene expression, protein profiles and ecophysiological performance of *Populus euphratica* subjected to gradual soil water depletion. They observed that acclimation to water deficits involved the regulation of different networks of genes, linked to protection and function maintenance of roots and shoots. Drought successively induced shoot growth cessation, stomatal closure, moderate increases in ROS, decreases in photosynthesis and in root growth. These alterations were paralleled by transcriptional changes in 1·2 % of the genes on the array that were fully reversible upon rewatering. However, no correlation was observed between the abundance of transcripts and proteins.

RECOVERY AFTER STRESS DICTATES SURVIVAL

The carbon balance of a plant during a period of salt/water stress and recovery may depend as much on the velocity and degree of photosynthetic recovery, as it depends on the degree and velocity of photosynthesis decline during water depletion. Surprisingly, since early studies by Kirschbaum (1987, 1988), photosynthesis recovery after stress has been scarcely studied. In general, plants subjected to mild stress recover fast (within 1 or 2 d) after stress is alleviated, but plants subjected to severe water stress recover only 40–60 % of the maximum photosynthesis rate during the day after re-watering, and recovery continues during the next days, but maximum photosynthesis rates are not always recovered (Kirschbaum, 1987, 1988; Delfine *et al.*, 1999; Sofo *et al.*, 2004; Grzesiak *et al.*, 2006; Bogeat-Triboulot *et al.*, 2007; Gallé *et al.*, 2007).

Kirschbaum (1987, 1988) showed that recovery after a severe drought was a two-stage process: a first stage occurred during the first days upon re-watering, and consisted

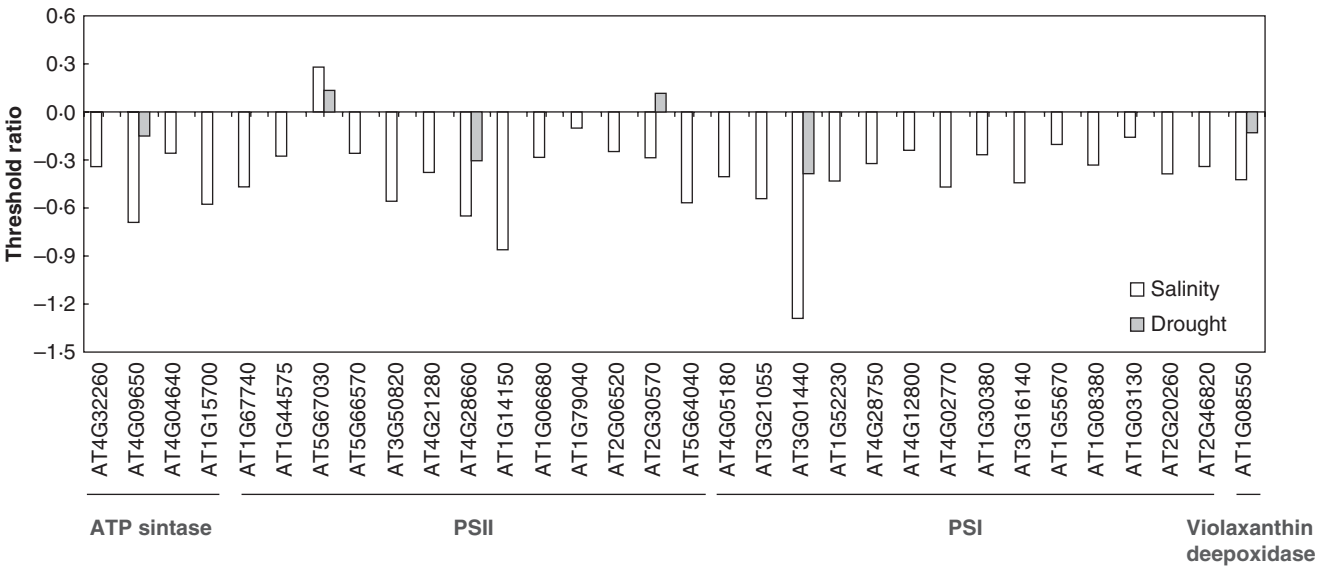

FIG. 2. AtGenExpress: salt and drought effects on several photosynthesis-related genes (ATP synthesis coupled to proton transport, light reaction and xanthophyll cycle). Based on Kilian *et al.* (2007) and workout with www.genevestigator.ethz.ch.

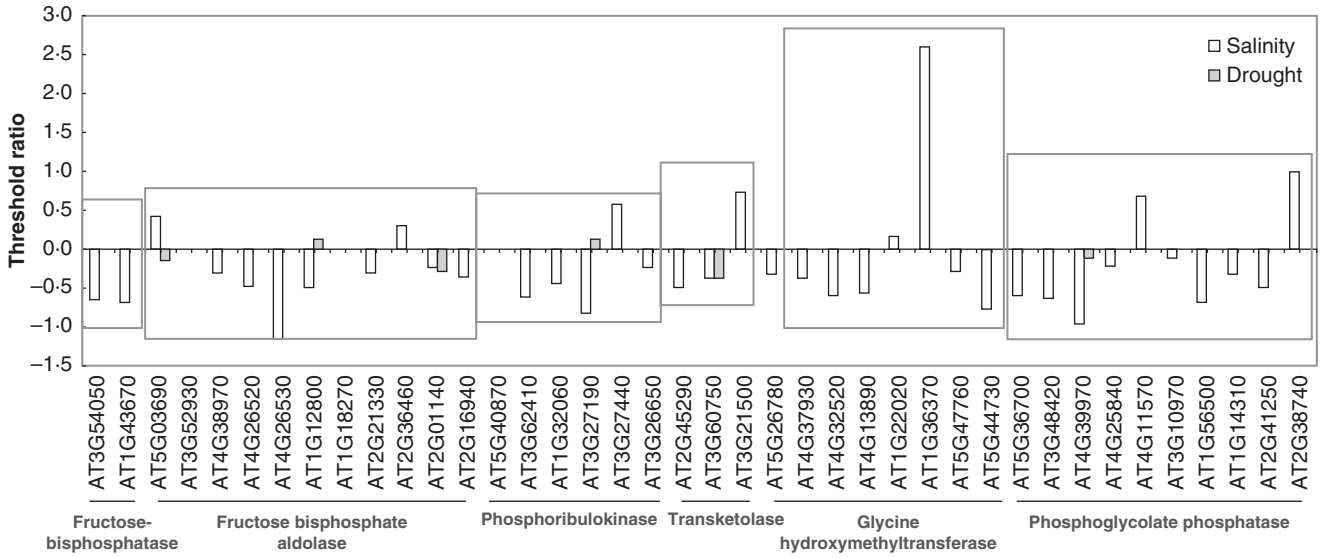

FIG. 3. Salt and drought effect (AtGenExpress) on the genes of some multigenic families (Calvin cycle and photorespiration). Rectangles represent genes coding for the same enzyme. Based on Kilian *et al.* (2007) and workout with www.genevestigator.ethz.ch.

basically in leaf re-watering and stomata re-opening; and a second stage lasted several days and was supposed to require *de novo* synthesis of photosynthetic proteins. Recently, Bogeat-Triboulot *et al.* (2007) have shown that recovery after water stress, determined 10 d after re-watering, was accompanied by increases in some photosynthetic proteins, particularly Rubisco activase and proteins of the water splitting complex, although increased proteins transcripts were not detected. In the cases where photosynthesis recovery is slow and/or incomplete, sustained photoprotection and/or oxidative stress have been suggested as possible causes (Sofo *et al.*, 2004; Gallé *et al.*, 2007). The influence of previous water stress severity on the velocity and extent of photosynthesis recovery has been illustrated in kidney bean by Miyashita *et al.* (2005) and Grzesiak *et al.* (2006). On the other hand, Pérez-Pérez *et al.* (2007) have shown that the interaction of salt and water stress strongly reduces plant's capacity to recover photosynthesis after stress alleviation as compared with plants subjected to a single stress. However, in these studies, the physiological mechanisms limiting recovery were not assessed. Therefore, current knowledge about physiological limitations to photosynthetic recovery after different water- and salt-stress intensities, as well as under different environmental conditions, is scarce.

In some species, a sustained down-regulation of stomatal conductance after re-watering imposes a substantial limitation to photosynthesis recovery, at the time that it increases the intrinsic water-use efficiency (Bogeat-Triboulot *et al.*, 2007; Gallé *et al.*, 2007; Galmés *et al.*, 2007*a*). In these studies, however, the causes for sustained stomatal closure were not investigated. It has been observed that, in some species, limited recovery of leaf-specific hydraulic conductivity is the apparent cause for down-regulation of stomatal conductance after re-watering (Galmés *et al.*, 2007*b*), and it has recently been shown that aquaporins play a dominant role in the regulation of dynamic changes in variable hydraulic conductance of leaves (Cochard *et al.*, 2007). However, in other species like the *Vitis* hybrid R-110 (*Vitis berlandieri* × *rupestris*), sustained stomatal closure lasts for weeks after re-watering but it does not seem to be related to root or stem hydraulic conductivity, ABA, or to the expression of aquaporins in roots or leaves (Galmés *et al.*, 2007*c*; J. Flexas *et al.*, unpubl. res.). Recently, it has been shown that electrical rather than hydraulic signals may play the main role in regulating stomatal re-opening after a water-stress period in maize (Grams *et al.*, 2007).

In other species, notably in many Mediterranean species belonging to different growth forms and functional groups, a slowly reversible mesophyll conductance to CO_2 has been shown to be an important factor limiting photosynthesis recovery after severe water stress (Galmés *et al.*, 2007*a*). Finally, in some studies such as that of Ennahli and Earl (2005) in cotton, impaired photosynthetic biochemistry was shown to be the main cause for limited photosynthesis recovery. In summary, the factors limiting photosynthesis recovery after water and salt stress seem to be multiple, and to strongly depend on the species and conditions analysed. This important aspect of plant physiology deserves further studies in the near future.

FUTURE PERSPECTIVES

The accumulated knowledge on physiological, cellular and molecular responses of plants to drought and salinity, including the signalling events occurring under both stresses, is already permitting great progress in crop management and breeding. Some improvement in plant stress tolerance has been achieved by introducing of stress-inducible genes into some model plants (see review by Shinozaki and Yamaguchi-Shinozaki, 2007). To further understand the complexity of plant response to drought and salt, including the effects on photosynthesis, we have to strengthen multilevel genomics and physiological studies, covering different intensity and timing of imposition of the stresses in

genotypes with different sensitivity to stress. It is already apparent that a significant number of genes related to photosynthetic metabolism is quickly down- or up-regulated in response to drought and salt, even under mild to moderate stress conditions. The differential expression patterns observed in multi-gene families responding to drought and salinity are a clue for understanding plant plasticity regarding the multitude of abiotic and biotic constraints to which plants are exposed. On the physiological side, studies on how photosynthesis recovers following stress suggest a high plant specificity that would be interesting to explore in order to increase plant potential to tolerate stress.

ACKNOWLEDGEMENTS

M. M. Chaves and C. Pinheiro dedicate this review paper to their former supervisor Cândido Pereira Pinto Ricardo on his retirement, recognizing his valuable input for plant biochemistry in Portugal.

REFERENCES

Bogeat-Triboulot MB, Brosche M, Renaut J, Jouve L, Le Thiec D, Fayyaz P, *et al*. 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiology* **143**: 876–892.

Bongi G, Loreto F. 1989. Gas-exchange properties of salt-stressed olive (*Olea europaea* L.) leaves. *Plant Physiology* **90**: 1408–1416.

Boyer JS, Wong SC, Farquhar GD. 1997. CO_2 and water vapour exchange across leaf cuticle (epidermis) at various water potentials. *Plant Physiology* **114**: 185–191.

Brugnoli E, Lauteri M. 1991. Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant (*Gossypium hirsutum* L.) and salt-sensitive (*Phaseolus vulgaris* L.) C_3 non-halophytes. *Plant Physiology* **95**: 628–635.

Buckley TN, Farquhar GD, Mott KA. 1997. Qualitative effects of patchy stomatal conductance distribution features on gas-exchange calculations. *Plant, Cell & Environment* **20**: 867–880.

Centritto M, Loreto F, Chartzoulakis K. 2003. The use of low [CO_2] to estimate diffusional and non-diffusional limitations of photosynthetic capacity of salt-stressed olive saplings. *Plant, Cell & Environment* **26**: 585–594.

Chaves MM. 1991. Effects of water deficits on carbon assimilation. *Journal of Experimental Botany* **42**: 1–16.

Chaves MM, Oliveira MM. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany* **55**: 2365–2384.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, *et al*. 2002. How plants cope with water stress in the field: photosynthesis and growth. *Annals of Botany* **89**: 907–916.

Chaves MM, Maroco JP, Pereira JS. 2003. Understanding plant responses to drought – from genes to the whole plant. *Functional Plant Biology* **30**: 239–264.

Cochard H, Venisse J-S, Barigah TS, Brunel N, Herbette S, Guilliot A, Tyree MT, Sakr S. 2007. Putative role of aquaporins in variable hydraulic conductance of leaves in response to light. *Plant Physiology* **143**: 122–133.

Cornic G, Le Gouallec JL, Briantais JM, Hodges M. 1989. Effect of dehydration and high light on photosynthesis of two C_3 plants: *Phaseolus vulgaris* L. and *Elastostema repens* (Lour.) Hall F. *Planta* **177**: 84–90.

Cramer GR, Ergül A, Grimplet J, Tillett RL, Tattersall EAR, Bohlman MC, *et al*. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. *Functional & Integrative Genomics* **7**: 111–134.

Delfine S, Alvino A, Villani MC, Loreto F. 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach leaves recovering from salt stress. *Plant Physiology* **119**: 1101–1106.

Demmig-Adams B, Adams III WW. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. *Trends in Plant science* **1**: 21–26.

Ennahli S, Earl HJ. 2005. Physiological limitations to photosynthetic carbon assimilation in cotton under water stress. *Crop Science* **45**: 2374–2382.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* **33**: 317–345.

Feild TS, Lee DW, Holbrook NM. 2001. Why leaves turn red in autumn: the role of anthocyanins in senescing leaves of red-osier dogwood. *Plant Physiology* **127**: 566–574.

Flexas J, Bota J, Escalona JM, Sampol B, Medrano H. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology* **29**: 461–471.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. *Plant Biology* **6**: 269–279.

Flexas J, Ribas-Carbó M, Hanson DT, Bota J, Otto B, Cifre J, *et al*. 2006*a*. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 *in vivo*. *The Plant Journal* **48**: 427–439.

Flexas J, Ribas-Carbó M, Bota J, Galmés J, Henkle M, Martínez-Cañellas S, *et al*. 2006*b*. Decreased Rubisco activity during water stress is not induced by decreased relative water content but related to conditions of low stomatal conductance and chloroplast CO_2 concentration. *New Phytologist* **172**: 73–82.

Flexas J, Diaz-Espejo A, Galmés J, Kaldenhoff R, Medrano H, Ribas-Carbo M. 2007. Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant, Cell & Environment* **30**: 1284–1298.

Flexas J, Ribas-Carbo M, Diaz-Espejo A, Galmés J, Medrano H. 2008. Mesophyll conductance to CO_2: current knowledge and future prospects. *Plant, Cell & Environment* **31**: 602–612.

Foyer CH, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiologia Plantarum* **119**: 355–364.

Gallé A, Haldimann P, Feller U. 2007. Photosynthetic performance and water relations in young pubescent oak (*Quercus pubescens*) trees during drought stress and recovery. *New Phytologist* **174**: 799–810.

Galmés J, Medrano H, Flexas J. 2007*a*. Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytologist* **175**: 81–93.

Galmés J, Flexas J, Savé R, Medrano H. 2007*b*. Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant and Soil* **290**: 139–155.

Galmés J, Pou A, Alsina MM, Tomàs M, Medrano H, Flexas J. 2007*c*. Aquaporin expression in response to different water stress intensities and recovery in Richter-110 *Vitis* sp.: relationship with ecophysiological status. *Planta* **226**: 671–681.

Genty B, Briantais JM, Baker NR. 1989. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**: 87–92.

Genty B, Meyer S, Piel C, Badeck F, Liozon R. 1998. CO_2 diffusion inside leaf mesophyll of ligneous plants. In: Garab Ged. *Photosynthesis: mechanisms and effects*. Dordrecht, The Netherlands: Kluwer Academic Publishers, 3961–3967.

Gillon JS, Yakir D. 2000. Internal conductance to CO_2 diffusion and $C^{18}OO$ discrimination in C_3 leaves. *Plant Physiology* **123**: 201–213.

Grams TEE, Koziolek C, Lautner S, Matyssek R, Fromm J. 2007. Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in *Zea mays* L. *Plant, Cell & Environment* **30**: 79–84.

Grzesiak MT, Grzesiak S, Skoczowski A. 2006. Changes of leaf water potential and gas exchange during and after drought in triticale and maize genotypes differing in drought tolerance. *Photosynthetica* **44**: 561–568.

Intergovernmental Panel on Climate Change. 2007. http://www.ipcc.ch. Accessed 25 October 2007.

Jia W, Davies WJ. 2007. Modification of leaf apoplastic pH in relation to stomatal sensitivity to root-sourced abscisic acid signals. *Plant Physiology* **143**: 68–77.

Jones HG. 1973. Moderate-term water stresses and associated changes in some photosynthetic parameters in cotton. *New Phytologist* **72**: 1095–1105.

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S, Batistic O, *et al*. 2007. The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. *The Plant Journal* **50**: 347–363.

Kirschbaum MUF. 1987. Water-stress in *Eucalyptus pauciflora* – comparison of effects on stomatal conductance with effects on the mesophyll capacity for photosynthesis, and investigation of a possible involvement of photoinhibition. *Planta* **171**: 466–473.

Kirschbaum MUF. 1988. Recovery of photosynthesis from water stress in *Eucalyptus pauciflora* – a process in two stages. *Plant, Cell & Environment* **11**: 685–694.

Lawlor DW, Cornic G. 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell & Environment* **25**: 275–294.

Loreto F, Harley PC, Di Marco G, Sharkey TD. 1992. Estimation of mesophyll conductance to CO_2 flux by three different methods. *Plant Physiology* **98**: 1437–1443.

Ma SS, Gong QQ, Bohnert HJ. 2006. Dissecting salt stress pathways. *Journal of Experimental Botany* **57**: 1097–1107.

Matsubara S, Gilmore AM, Osmond CB. 2001. Diurnal and acclimatory responses of violaxanthin and lutein epoxide in the Australian mistletoe *Amyema miquelii*. *Australian Journal of Plant Physiology* **28**: 793–800.

Meyer S, Genty B. 1998. Mapping intercellular CO_2 mole fraction (Ci) in *Rosa rubiginosa* leaves fed with abscisic acid by using chlorophyll fluorescence imaging: significance of Ci estimated from leaf gas exchange. *Plant Physiology* **116**: 947–957.

Mittler R. 2006. Abiotic stress, the field environment and stress combination. *Trends in Plant Science* **11**: 15–19.

Miyashita K, Tanakamaru S, Maitani T, Kimura K. 2005. Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. *Environmental and Experimental Botany* **53**: 205–214.

Morison JIL, Lawson T, Cornic G. 2007. Lateral CO_2 diffusion inside dicotyledonous leaves can be substantial: quantification in different light intensities. *Plant Physiology* **145**: 680–690.

Munns R. 2002. Comparative physiology of salt and water stress. *Plant, Cell & Environment* **25**: 239–250.

Munns R. 2005. Genes and salt tolerance: bringing them together. *New Phytologist* **167**: 645–663.

Munns R, James RA, Läuchli A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany* **57**: 1025–1043.

Ndimba BK, Chivasa S, Simon WJ, Slabas AR. 2005. Identification of Arabidopsis salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics* **5**: 4185–4196.

Neill SJ, Desikan R, Hancock JT. 2003. Nitric oxide signalling in plants. *New Phytologist* **159**: 11–35.

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH. 2002. Drought and oxidative load in the leaves of C-3 plants: a predominant role for photorespiration? *Annals of Botany* **89**: 841–850.

Ort DR. 2001. When there is too much light. *Plant Physiology* **125**: 29–32.

Parker R, Flowers TJ, Moore AL, Harpham NVJ. 2006. An accurate and reproducible method for proteome profiling of the effects of salt stress in the rice leaf lamina. *Journal of Experimental Botany* **57**: 1109–1118.

Passioura J. 2007. The drought environment: physical, biological and agricultural perspectives. *Journal of Experimental Botany* **58**: 113–117.

Pastori GM, Foyer CH. 2002. Common components, networks, and pathways of cross-tolerance to stress: the central role of 'redox' and abscisic acid-mediated controls. *Plant Physiology* **129**: 460–468.

Pego JV, Kortstee AJ, Huijser C, Smeekens SCM. 2000. Photosynthesis, sugars and the regulation of gene expression. *Journal of Experimental Botany* **51**: 407–416.

Pérez-Pérez JG, Syvertsen JP, Botía P, García-Sánchez F. 2007. Leaf water relations and net gas exchange responses of salinized Carrizo citrange seedlings during drought stress and recovery. *Annals of Botany* **100**: 335–345.

Pfannschmidt T. 2003. Chloroplast redox signals: how photosynthesis controls its own genes. *Trends in Plant Science* **8**: 33–41.

Pfannschmidt T, Bräutigam K, Wagner R, Dietzel L, Schröter Y, Steiner S, Nykytenko A. 2009. Potential regulation of gene expression in photosynthetic cells by redox and energy state: approaches towards better understanding. *Annals of Botany* doi:10.1093/aob./mcn081.

Pinheiro C, Chaves MM, Ricardo CP. 2001. Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of *Lupinus albus* L. *Journal of Experimental Botany* **52**: 1063–1070.

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, *et al*. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA get-blot analyses. *Plant Physiology* **133**: 1755–1767.

Renou J-L, Gerbaud A, Just D, André M. 1990. Differing substomatal and chloroplastic concentrations in water-stressed wheat. *Planta* **182**: 415–419.

Rolland F, Baena-Gonzalez E, Sheen J. 2006. Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**: 675–709.

Sanchez DH, Siahpoosh MR, Roessner U, Udvardi M, Kopka J. 2007. Plant metabolomics reveals conserved and divergent metabolic responses to salinity. *Physiologia Plantarum* **132**: 209–219.

Scartazza A, Lauteri M, Guido MC, Brugnoli E. 1998. Carbon isotope discrimination in leaf and stem sugars, water-use efficiency and mesophyll conductance during different developmental stages in rice subjected to drought. *Australian Journal of Plant Physiology* **25**: 489–498.

Sharkey TD. 1990. Water stress effects on photosynthesis. *Photosynthetica* **24**: 651–656.

Shinozaki K, Yamaguchi-Shinozaki K. 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany* **58**: 221–227.

Sofo A, Dichio B, Xiloyannis C, Masia A. 2004. Effects of different irradiance levels on some antioxidant enzymes and on malondialdehyde content during rewatering in olive tree. *Plant Science* **166**: 293–302.

Stitt M, Gibon Y, Lunn JE, Piques M. 2007. Multilevel genomics analysis of carbon signalling during low carbon availability: coordinating the supply and utilisation of carbon in a fluctuating environment. *Functional Plant Biology* **34**: 526–549.

Terashima I. 1992. Anatomy of non-uniform leaf photosynthesis. *Photosynthesis Research* **31**: 195–212.

Tezara W, Mitchell VJ, Driscoll SD, Lawlor DW. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature* **401**: 914–917.

Tuberosa R, Salvi S. 2006. Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science* **11**: 405–412.

Unesco Water Portal. 2007. http://www.unesco.org/water. Accessed 25 October 2007.

Vincent D, Ergül A, Bohlman MC, Tattersall EAR, Tillett RL, Wheatley MD, *et al*. 2007. Proteomic analysis reveals differences between *Vitis vinifera* L. cv. Chardonnay and cv. Cabernet Sauvignon and their responses to water deficit and salinity. *Journal of Experimental Botany* **58**: 1873–1892.

Warren CR. 2006. Estimating the internal conductance to CO_2 movement. *Functional Plant Biology* **33**: 431–442.

Wilkinson S, Davies WJ. 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant, Cell & Environment* **25**: 195–210.

Wong CE, Li Y, Labbe A, Guevara D, Nuin P, Whitty B, *et al*. 2006. Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of *Arabidopsis*. *Plant Physiology* **140**: 1437–1450.

Zhou J, Wang X, Jiao Y, Qin Y, Liu X, He K, *et al*. 2007. Global genome expression analysis of rice in response to drought and high-salinity stresses in shoot, flag leaf, and panicle. *Plant Molecular Biology* **63**: 591–608.

Zimmermann P, Hennig L, Gruissem W. 2005. Gene expression analysis and network discovery using Genevestigator. *Trends in Plant Science* **10**: 407–409.