REVIEW

Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress

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Drought and salinity (i.e. soil water stress) are the main environmental factors limiting photosynthesis and respiration and, consequently, plant growth. This review summarizes the current status of knowledge on photosynthesis and respiration under water stress. It is shown that diffusion limitations to photosynthesis under most water stress conditions are predominant, involving decreased mesophyll conductance to CO$_2$, an important but often neglected process. A general failure of photochemistry and biochemistry, by contrast, can occur only when daily maximum stomatal conductance ($g_s$) drops below 0.05–0.10 mol H$_2$O m$^{-2}$ s$^{-1}$. Because these changes are preceded by increased leaf antioxidant activities ($g_s$ below 0.15–0.20 mol H$_2$O m$^{-2}$ s$^{-1}$), it is suggested that metabolic responses to severe drought occur indirectly as a consequence of oxidative stress, rather than as a direct response to water shortage. As for respiration, it is remarkable that the electron partitioning towards the alternative respiration pathway sharply increases at the same $g_s$ threshold, although total respiration rates are less affected. Despite the considerable improvement in the understanding of plant responses to drought, several gaps of knowledge are highlighted which should become research priorities for the near future. These include how respiration and photosynthesis interact at severe stress, what are the boundaries and mechanisms of photosynthetic acclimation to water stress and what are the factors leading to different rates of recovery after a stress period.

Photosynthesis and respiration responses to drought and salinity

Both drought and salinity stresses reduce the capacity of plants to take up water from the soil (Munns 2002). At present, low water availability is the main environmental factor limiting plant growth and yield worldwide, and global change will likely make water scarcity an even greater limitation to plant productivity across an increasing amount of land (Chaves et al. 2003, Hamdy et al. 2003). The limitation of plant growth imposed by low water availability is mainly due to reductions in plant carbon balance, which is dependent on the balance between photosynthesis and respiration. Both processes are intimately linked. For instance, it has been shown that transgenic plants with modified respiration also alter their photosynthetic behaviour (Dutilleul et al. 2003, Nunes-Nesi et al. 2005), and an increased respiration rate seems necessary for photosynthesis recovery after a period of water stress (Kirschbaum 1988). Of the total

Abbreviations – ABA, abscisic acid; Altox, alternative oxidase; $A_N$, light-saturated net photosynthesis; APX, ascorbate peroxidase; ATP, adenosine triphosphate; $C_c$, chloroplast CO$_2$ concentration; $C_i$, sub-stomatal CO$_2$ concentration; ETR, electron transport rate; GR, glutathione reductase; $g_s$, maximum CO$_2$ stomatal conductance; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, Ribulose-1,5-bisphosphate; $V_{c,max}$, maximum velocity of carboxylation.
carbon assimilated in photosynthesis, usually more than half is lost in respiratory processes necessary for growth and maintenance, but this balance may change under water stress. For instance, although photosynthesis may decrease up to 100% becoming totally impaired under severe water shortage, the respiration rate may either increase or decrease under stress, but may never become totally impaired (Flexas et al. 2005). Therefore, it is imperative to increase our knowledge on the physiological bases of regulation of photosynthesis and respiration under water scarcity in order to be able to improve plant yield in semiarid regions and to face the climate change-driven increase in global aridity.

There has been some controversy regarding the main physiological targets responsible for photosynthetic impairment under drought and/or salinity (Flexas and Medrano 2002, Lawlor and Cornic 2002). However, there is now substantial consensus that reduced CO2 diffusion from the atmosphere to the site of carboxylation is the main cause for decreased photosynthesis under most water stress conditions (Chaves and Oliveira 2004, Flexas et al. 2004a). Such reduced leaf diffusive capacity is due to at least two components that are regulated almost simultaneously: stomatal closure and reduced mesophyll conductance. Although the former has been known for a long time to be one of the first responses of plants to soil water shortage, the latter has only recently been recognized as an equally important cause for reduced CO2 diffusion, under drought (Flexas et al. 2002, Warren et al. 2004) and salinity (Centritto et al. 2003). There are some observations suggesting that fine and rapid regulation of mesophyll conductance to CO2 in response to varying environmental conditions could be related to the expression and/or regulation of plasma membrane aquaporins (Flexas et al. unpublished data, Hanba et al. 2004, Uehlein et al. 2003). The variability of mesophyll conductance impairs the traditional use of photosynthesis responses to substomatal CO2 concentration (A\textsubscript{N–C\textsubscript{i}} curves) as a tool to evaluate the incidence of metabolic impairment under water stress conditions (Flexas et al. 2004a). An example of these limitations is shown with severely stressed grapevines in Fig. 1. Using a typical A\textsubscript{N–C\textsubscript{i}} analysis (Long and Bernacchi 2003), the maximum velocity of carboxylation (V\textsubscript{c, max}) appeared to be 167 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) in irrigated plants and only 9.5 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) in severely stressed plants showing no net photosynthesis at ambient CO2 and saturating light (Fig. 1A). These results suggest a 94% inhibition of initial Rubisco activity. However, when decreased mesophyll conductance to CO2 is taken into account, and the analysis is performed on a chloroplast CO2 (C\textsubscript{c}) concentration basis (Fig. 1B), then V\textsubscript{c, max} resulted 193 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) in control plants and 173 \(\mu\text{mol} \text{m}^{-2} \text{s}^{-1}\) in the stressed ones (i.e. only a 10% inhibition of Rubisco activity). Recently, alternative methods for A\textsubscript{N–C\textsubscript{i}} analysis taking into account variations in mesophyll conductance have been proposed (Ethier and Livingston 2004, Manter and Kerrigan 2004).

Although restricted CO2 diffusion across leaves is likely to be the most usual cause for decreased photosynthesis rates under water stress, metabolic impairment may also occur, particularly under severe stress. Combining data from our laboratory and from the literature, we have recently shown that metabolic impairment of photosynthesis only occurs when maximum
daily stomatal conductance drops below 0.05–0.10 mol H$_2$O m$^{-2}$ s$^{-1}$, regardless of the species analysed and of the water status of leaf tissues (Bota et al. 2004, Flexas et al. 2004a, 2004b). Such metabolic impairment is not orchestrated, but rather global. In this sense, it is remarkable that all the photochemical (chlorophyll content and fluorescence estimates of maximum photochemical efficiency) and biochemical (contents of ATP, RuBP and total soluble protein; activities of Rubisco, sucrose phosphate synthase, fructose bisphosphatase and nitrate reductase) components of photosynthesis analysed up to now share the same stomatal conductance threshold for their eventual impairment under water stress (Flexas et al. 2004a, 2004b). For Rubisco at least, the effects of severe water stress on its initial activity can be reproduced by inducing stomatal closure to levels below the indicated threshold trough addition of abscisic acid (ABA) to the nutrient solution of unstressed plants or by cutting the petiole in air to rapid dehydrate leaves (Flexas et al. unpublished results). Altogether, the results suggest that metabolic impairment of photosynthesis under severe stress is caused by reduced CO$_2$ availability in the mesophyll, rather than by leaf tissue dehydration.

By using a totally different approach, such as a photosynthesis limitation analysis, Grassi and Magnani (2005) have recently confirmed the same patterns of photosynthesis response to drought in a study during 3 consecutive years in field-grown ash and oak trees. In their results during summer stress development, whenever $g_s$ was higher than 0.1 mol H$_2$O m$^{-2}$ s$^{-1}$, biochemical limitations to photosynthesis were not detectable, and the sum of limitations imposed by stomatal closure and decreased mesophyll conductance accounted for the entire decrease in photosynthesis. Stomatal conductance accounted for approximately two-third of the observed decline, whereas mesophyll conductance accounted for the other one-third of decrease. At more severe stress conditions, when $g_s$ was below 0.1 mol H$_2$O m$^{-2}$ s$^{-1}$, biochemical limitations were detectable, although they never accounted for more than 15% of total photosynthetic limitations.

Studies examining the effects of water stress on respiration are scarcer than those analysing photosynthetic responses. Generally, the respiration rate decreases during water stress, due to reduced photosynthesize assimilation and growth needs. However, this behaviour may be somewhat species dependent, and respiration rate can also increase, particularly under severe water stress (Flexas et al. 2005, Ghashghaie et al. 2001). Nevertheless, the total respiration rates are usually kept within a narrower range than those of photosynthesis during water stress development, resulting in a progressively increased respiration to photosynthesis ratio, i.e. a decreased carbon balance (Flexas et al. 2005). Plants possess two respiratory pathways: the energy-conserving, cyanide-sensitive, cytochrome pathway and the energy-wasteful, cyanide-resistant, alternative pathway (Lambers et al. 2005). We have recently demonstrated in soybean that severe water stress induces a sharp decrease in the cytochrome respiration rate concomitant with a similar increase in the alternative respiration rate, so that total respiration rate remains quite constant (Ribas-Carbo et al. 2005). Moreover, these changes are not accompanied by increases in alternative oxidase protein content, indicating that these changes may be regarded as a biochemical regulation. The most surprising aspect is that the observed changes in mitochondrial electron partitioning during water stress only occur when stomatal conductance drops below 0.1 mol H$_2$O m$^{-2}$ s$^{-1}$, thus in coincidence with the observed threshold for photosynthesis metabolic impairment. The $g_s$ threshold coincidence for decreased leaf ATP contents and increased activity of ATP non-synthesizing alternative oxidase (see mirror patterns in Fig. 2A) suggests that increased alternative respiration pathway may account for, at least partly, the decrease in ATP contents. This view may be regarded as opposite to the drought-induced impairment of chloroplast photophosphorylation proposed by Tezara et al. (1999).

In sum, the response of photosynthesis to soil water shortage can be divided into two distinct phases: during the first stage, characterized by daily maximum stomatal conductances above 0.05–0.10 mol H$_2$O m$^{-2}$ s$^{-1}$, photosynthesis is mostly limited by restricted CO$_2$ diffusion (decreased stomatal conductance plus decreased mesophyll conductance); during the second stage, characterized by stomatal conductances below that threshold, a general metabolic impairment eventually occurs (i.e. depending on the species and conditions). Plant respiration rate, by contrast, remains within narrower ranges during stress, but the electron partitioning between the cytochrome and alternative pathways also changes in coincidence with the stomatal conductance threshold for photosynthetic impairment. Within this frame, some questions remain unanswered, which will be the focus of this article:

1. What are the causes for the simultaneous photochemical and biochemical dysfunction of photosynthesis under severe water stress? Moreover, as mentioned earlier, because respiratory metabolism can interfere with photosynthetic metabolism, and the change in electron...
partitioning during respiration occurs at the same conductance threshold where photosynthetic metabolism is impaired, can the cyanide-resistant alternative respiratory pathway play any role in the observed photosynthetic responses under severe stress?

(2) Provided the strong dependency of photosynthetic metabolism on stomatal conductance, is there any opportunity for photosynthesis acclimation to drought and/or salinity? And if so, which are the main physiological features leading to such acclimation?

Fig. 2. Literature survey on the relationship during drought or salinity between stomatal conductance and (A) electron partitioning during respiration (empty symbols) and ATP content (filled symbols) (B–E) the activity of several antioxidant enzymes in leaves (B, catalase; C, glutathione reductase; D, ascorbate peroxidase; E, superoxide dismutase), and (F) α-tocopherol content. Except for stomatal conductance (mol H₂O m⁻² s⁻¹), all parameters are expressed as percentage of maximum values to facilitate comparison, due to the large variability in the units given in the original references. Data for chloroplast and cytosol SOD are not distinguished in most of the original references. Data have been compiled from the following references. (A) electron partitioning from Ribas-Carbo et al. (2005), ATP content from Lawlor and Khanna-Chopra (1984) and Tzezara et al. (1999, 2002). (B) Mittler and Zilinskas (1994), Lima et al. (2002), Pinheiro et al. (2004), Jeyaramraja et al. (2005) and Sofo et al. (2005). (C) Loggini et al. (1999), Hernández et al. (1999), Van Heerden and Krüger 2002, Pinheiro et al. (2004) and Jeyaramraja et al. (2005). (D) Hernández et al. (1999), Lima et al. (2002), Van Heerden and Krüger (2002), Pinheiro et al. (2004), Jeyaramraja et al. (2005) and Sofo et al. (2005). (E) Mittler and Zilinskas (1994), Hernández et al. (1999), Lima et al. (2002), Pinheiro et al. (2004), Jeyaramraja et al. (2005) and Sofo et al. (2005). (F) Moran et al. (1994), Bartoli et al. (1999), Munne-Bosch et al. (1999, 2003), Munne-Bosch and Alegre (2000), Herbinger et al. (2002) and Ratnayaka et al. (2003). The species included in these studies are Triticum aestivum, Pisum sativum, Glycine max, Coffea canephora, Melissa officinalis, Cistus clusii, Cistus albidus, Rosmarinus officinalis, Melissa officinalis, Gossypium hirsutum, Olea europaea, Camellia sinensis and Helianthus annuus.
(3) Finally, because whole-life plant carbon balance depends on the balance between photosynthesis and respiration not only during the periods of stress imposition, but also during the periods after re-watering, how fast is photosynthetic recovery on re-watering? Does it depend on the severity of the previous stress and/or on acclimation factors?

**Causes for simultaneous photochemical and biochemical dysfunction of photosynthesis under severe water stress: downregulation or damage?**

This question is important, not only for the improvement of our basic understanding of why plants decrease photosynthesis under water stress, but also from a more practical perspective, because downregulation may be expected to reverse more rapidly than damage on re-watering. But, do we have at present any evidence to distinguish between metabolic downregulation and damage to the photosynthetic apparatus?

The fact that all photochemical and biochemical parameters analysed up to now share a common $g_s$ threshold for their inhibition under stress does not favour the idea of downregulation. For instance, if $CO_2$ was the most limiting factor for photosynthesis under severe stress, a downregulation of leaf photochemistry may be expected to balance the light and dark reactions of photosynthesis, but there may be no apparent reason to downregulate Rubisco activity or chlorophyll content as well. On the other hand, below the indicated $g_s$ threshold, the metabolic impairment may appear general but stochastic. Depending on the studies and species analysed, the impairment of a given metabolic process can vary between 0 and 100% (Flexas et al. 2004a, 2004b). This suggests that metabolic impairment may depend on the environmental conditions and/or on acclimation factors, but the number of data available is too scarce to define any clear pattern. A possible explanation, based on the fact that water stress is often accompanied by high light intensity, would be that general metabolic disruption occurs as a consequence of secondary oxidative stress developed under severe water stress and high light intensity. In this case, impairment may certainly be dependent on both environmental conditions (i.e. excess light) and acclimation factors (e.g. antioxidant capacity of leaves). The following paragraphs are devoted to analyse current evidences favouring that actually oxidative stress is occurring at severe water stress and is linked to the observed metabolic impairment.

First, the fact that mitochondrial electron partitioning towards the alternative pathway increases in coincidence with the $g_s$ threshold for photosynthetic impairment strongly suggests oxidative conditions. The involvement of increased alternative oxidase activity in the antioxidant defence of plants has been recognized in two ways (Lambers et al. 2005, Mittler 2002, Purvis 1997, Wagner and Krab 1995). First, it maintains mitochondrial electron transport, hence preventing the formation of reactive oxygen species. Second, it contributes to ascorbate synthesis. Ascorbate is an important molecule protecting plants from oxidative stress, and its synthesis is localized in the mitochondria. One of the enzymes involved in ascorbate synthesis, galactone-$\gamma$-lactone dehydrogenase, is an intrinsic component of mitochondrial complex I (Millar et al. 2003). Therefore, maintaining the activity of mitochondrial electron transport chain is essential for plant defence under oxidative stress, and alternative oxidase may well accomplish this function under severe water stress, where impaired growth makes cytochrome pathway-associated ATP synthesis not viable. Alternatively, it could be that stress-induced senescence and programmed cell death occur at the indicated $g_s$ threshold. It is also known that plants lacking the alternative oxidase are more susceptible to programmed cell death, since alternative oxidase induction prevents it (Vanlerberghe et al. 2002). Very recently, Bartoli et al. (2005) could demonstrate that increased alternative oxidase activity in drought-stressed plants resulted in enhanced photosynthetic electron transport under these conditions. The effect was more significant at high light intensities, which suggested it was actually due to reduced harmful effects of excess light due to the enhancement of the alternative pathway.

At the moment of writing previous reviews (Flexas et al. 2004a, 2004b), the studies available in which the response of typical antioxidant systems to water stress was assessed together with gas exchange analysis were insufficient to provide a clear picture regarding the $g_s$ threshold for their activation in response to stress. Since then, a few studies have appeared, which allow now plotting the response of such antioxidant systems to decreasing $g_s$ during water stress imposition (Fig. 2). As occurred with the components of photosynthetic metabolism components, a single pattern of response is revealed regardless of the species analysed and study conditions. Such a pattern consists in a lack of response of these processes when $g_s$ decreases from a maximum to about 0.15 mol H$_2$O m$^{-2}$ s$^{-1}$, followed by abrupt increases of the activity of all antioxidant systems below that threshold. The pattern is much more clear for those systems increasing five- to 10-fold in response to stress (ascorbate peroxidase activity,
superoxide dismutase activity and α-tocopherol content) than for those responding to a lesser extent (glutathione reductase and catalase activities). The fact that the \( g_s \) threshold for depressed photosynthetic metabolism is somewhat smaller than for increased antioxidant response strongly supports the idea that the former may be a result of oxidative stress occurring in severely stressed plants in which antioxidant capacity is not enough to cope with the generation of reactive oxygen species.

Altogether, there are some evidences supporting the idea that drought- and/or salt-induced depressions of the photosynthetic capacity are associated with secondary oxidative stress developing as a result of combined water stress and excess light. Further work will be needed to confirm this hypothesis, including the comparison of plants stressed at different light intensities and the analysis of the response of transgenic plants differing in the expression of mitochondrial alternative oxidase or antioxidant enzymes.

**Acclimation of photosynthesis to long-term drought or salinity: can plants maintain a positive carbon gain without water?**

The photosynthetic responses described above come, in general, from studies in which water stress was applied to plants over relatively short periods. Therefore, most of the observed patterns may correspond to immediate responses to water stress, which correspond with functional reductions. However, under natural conditions, water stress develops much more gradually, over periods comprising weeks or months. Acclimation to water stress comprises all those responses, involving gene expression and modification of plant physiology and morphology, and taking place in days to weeks, which leads to a homeostatic compensation for the initial negative effects of water stress. Acclimated plants would improve their water relations and photosynthesis in respect to non-acclimated plants, which may lead to lower decreases in carbon gain and growth losses (Fig. 3A). Despite the obvious importance of the acclimation processes, very few studies have addressed the question, and hence, the physiological mechanisms involved in acclimation are mostly unknown.

Responses to prolonged soil water stress include increases in stomatal conductance and photosynthetic capacity (Stewart et al. 1994), increased concentration of several photosynthetic enzymes along with a decrease in their specific activity (Büssis et al. 1998, Pankovic et al. 1999) and the maintenance of a high thylakoid electron transport rate (Pankovic et al. 1999). However, none of those studies undertook the comparison, under similar stress conditions, of plants and/or leaves acclimated and not acclimated to water stress.

To the best of our knowledge, only in two studies this analysis was provided, and in both, it was suggested that higher photosynthesis rates in acclimated leaves was associated with the maintenance of higher electron transport rates as compared with non-acclimated leaves (Kitao et al. 2003, Maury et al. 1996). We have observed, in tobacco plants maintained for 3 weeks at three different levels of constant soil water deficit, that the thylakoid electron transport rate was also higher in comparison.
Theoretical diagram showing how photosynthetic recovery after concentration (Fig. 3B). This response may be unexpected, because photosynthesis under water stress is generally limited by carboxylation, not by electron transport or RuBP regeneration. On the other hand, it has been shown that the transcription of several genes associated with the structure and function of photosystems is progressively depressed during water stress, and it increases again on re-watering (Chaves et al. 2003, Collett et al. 2003).

Such a discrepancy between physiological studies and gene expression analysis is also apparent regarding acclimation of Rubisco to water stress. Although physiological determinations in soybean suggest that Rubisco content in leaves increases with acclimation to stress (Pankovic et al. 1999), other authors have observed that under stress imposition there is a decrease in the transcription of the large subunit of Rubisco in pines (Pelloux et al. 2001) and of the small subunit in tobacco (Kawaguchi et al. 2003). Clearly, further studies are needed in which detailed physiological analysis is combined with multiple gene expression using, for instance, DNA microarrays. Up to date, only one study has analysed the relationship between photosynthetic acclimation and multiple gene expression in *Pinus taeda* (Watkinson et al. 2003). The results of this study showed that there are different patterns of gene expression depending on the intensity of water stress, as well as on the number of consecutive cycles of stress and recovery. Therefore, the acclimation patterns may respond to a complexity of different conditionings, whose better understanding may be key to increase the current knowledge about the limits and mechanisms for photosynthesis acclimation to water stress.

![Theoretical diagram showing how photosynthetic recovery after water stress may strongly impact plant carbon gain (arrows). Three different possibilities, of increasing impact on plant carbon gain, are considered: rapid and total recovery (long dashed arrow), slow but total recovery (shorted dashed arrow) and slow and incomplete recovery (dotted arrow). For each treatment, total carbon lost is represented by the areas formed between the initial solid arrow and the corresponding recovery arrows. The initial carbon loss during water stress imposition is equal for all treatments (dotted area); the carbon loss during recovery depends on its velocity and extent (dark grey – fast recovery; pale grey – slow recovery). The carbon loss area for plants with incomplete recovery is not represented, because it depends on the length of the crop cycle and the percentage of recovery.](image)

**The other side of carbon balance under semiarid conditions: how fast does photosynthesis recover after a water stress period?**

The carbon balance of a plant during a period of 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 (Fig. 4). Surprisingly, since early studies by Kirschbaum (1987), there is a scarcity of studies analysing the capacity of recovery from different water stress intensities, as well as evaluating the physiological features limiting recovery. There are some indications suggesting that previous water stress intensity is a crucial factor affecting both the velocity and the extent of recovery after re-watering. For instance, grapevines subjected to mild water stress (i.e. maximum stomatal conductance above 0.1 mol H₂O m⁻² s⁻¹) recover completely during the day after re-watering, whereas more severely stressed plants recovered slowly during the next week and did not reach the maximum photosynthesis rates presented before water stress (Flexas et al. 2004b). In general, 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 (De Souza et al. 2004, Flexas et al. 2004b, Kirschbaum 1987). As suggested for the different responses observed at severe water stress, secondary oxidative stress could be also affecting the velocity and extent of recovery, because it has been shown that antioxidant mechanisms raise their concentration and activities on re-watering, and plants presenting higher activities and/or contents of antioxidants recover maximum photosynthetic rates more rapidly (Mittler and Zilinskas 1994, Yan et al. 2003).

Recently, the strong influence of previous water stress severity on the velocity and extent of photosynthesis recovery has been excellently illustrated in kidney bean by Miyashita et al. (2005), although the physiological mechanisms limiting recovery were not assessed. Therefore, current knowledge about physiological limitations to photosynthetic recovery after different water
stress intensities and under different environmental conditions is scarce. Acquisition of this knowledge would be necessary to improve the understanding of plant responses to drought and salinity, and on the applied aspect, it could be crucial for the development of water-saving irrigation schedules in agriculture.

Concluding remarks and future prospects

The current state of the art on the regulation of photosynthesis and respiration in response to water stress has been summarized. It is suggested that in all but very severe stress situations, diffusional limitations to CO₂ (stomatal plus mesophyll) account for most of the observed decreases in photosynthesis during water stress development. At severe stress, metabolic impairment of photosynthesis also occurs in some cases, probably due to secondary oxidative stress. The response of respiration to severe water stress consists in increased electron partitioning to alternative oxidase and decreased cytochrome pathway, with little effect on total respiration rates.

Besides, a number of important gaps of knowledge have been highlighted. Here, we propose several research priorities that, in our opinion, would help advance in the understanding of photosynthetic response to drought and salinity:

1. Understanding why metabolic impairment at severe water stress is global rather than orchestrated and why it is eventual (i.e. occurring in some studies but not in others). Although a role of secondary oxidative stress has been hypothesized, it would be necessary to prove it, ideally by comparing the response of transgenic plants differing in their antioxidant capacities. It would also be necessary to analyse the role of respiration (particularly of alternative respiration) in the maintenance/regulation of photosynthesis under water stress. Again, transgenic plants differing in the expression of alternative oxidase would be a key instrument to achieve this goal.

2. Combining detailed physiological analysis with multiple gene expression during water stress imposition at different intensities, acclimation and recovery periods. Increased availability of DNA microarray techniques may provide tools for this kind of analysis. A multiplicity of such studies under different conditions and using different species may help unravelling the limits and the physiological mechanisms involved in photosynthesis acclimation of plants to water stress situations differing in intensity, duration and so on.

3. Analysing the recovery of different photosynthetic components on re-watering from different water stress intensities in different plants and conditions. This knowledge would be of importance for the development of deficit irrigation programs, as well as for improving the accuracy of ecosystem productivity predictions from climate data.

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References


Ethier GH, Livingston NJ (2004) On the need to incorporate sensitivity to CO₂ transfer conductance into the Farquhar–


Mittler R, Zilinskas B (1994) Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. Plant J 5: 397–405
Purvis AC (1997) Role of the alternative oxidase in limiting superoxide production by plant mitochondria. Physiol Plant 100: 165–170

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