Chapter 5
The Response of Photosynthesis to Soil Water Stress

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Abstract The physiological and molecular basis of photosynthetic responses to limited soil water availability (water stress) has been intensively examined over the last decade(s). Therefore, this chapter highlights the major achievements of the underlying processes of photosynthetic limitation under drought, an increasingly important issue within the context of climate change. Restricted CO_2 diffusion to the sites of carboxylation inside the chloroplast has been demonstrated to be the main limiting factor for photosynthesis, particularly during the early phases of stress. Stomatal (g_s) and mesophyll conductance (g_m), the two leaf diffusion components, contribute differently to this limitation, being largely influenced by the degree of water deficit. Thus, photosynthetic acclimation to drought and its recovery from drought depend primarily on the capacity to adjust g_m and g_s rapidly. The basis of g_m and g_s regulation is not fully understood, but several genetic, metabolic, and structural factors involved have been recently described. Secondary stress factors such as excessive light and elevated temperatures affect photosynthetic performance too, implying efficient photoprotection a necessary feature for stress-resistant plants.

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5.1 Introduction

Low water availability is considered as the main environmental factor limiting plant growth and yield worldwide, especially in semi-arid areas. It is well documented that one of the primary physiological impacts of drought is on photosynthesis (Flexas et al. 2004a). Presently, not only in the arid and semiarid zones drought is a constraint, but it is also increasingly affecting temperate regions occasionally subjected to severe drought events (Giorgi and Lionello 2008; Battisti and Naylor 2009). Improving the knowledge on photosynthesis responses to water stress is essential for the development of deficit irrigation programs, as well as for improving the accuracy of ecosystem productivity predictions from climate data.

There has been some controversy regarding the main physiological targets responsible for photosynthetic impairment under drought and/or salinity (Chaves 1991; Flexas and Medrano 2002; Lawlor and Cornic 2002). As a consequence of stomatal closure, CO_2 diffusion from the atmosphere to the site of carboxylation is reduced, and this is often regarded as the main cause for decreased photosynthesis under drought and salinity (Centritto et al. 2003; Loreto et al. 2003; Chaves and Oliveira 2004; Flexas et al. 2004a, b, 2009; Grassi and Magnani 2005; Chaves et al. 2009; Peeva and Cornic 2009). On the other hand, some authors have suggested that metabolic impairment due to water stressinduced decrease of leaf water content and increased ion concentration is more limiting for photosynthesis than stomatal closure (Tezara et al. 1999, 2002; Tang et al. 2002). More recently, decreased leaf internal diffusion of CO_2 (i.e., decreased mesophyll conductance, g_m) has been identified as another potential cause for photosynthesis impairment under drought (Flexas et al. 2002; Galmés et al. 2007a) and salinity (Centritto et al. 2003). No general consensus exists on the relative importance of each of these factors in limiting photosynthesis under drought, which could in fact depend on the prevailing light conditions (Zhou et al. 2007a; Flexas et al. 2009; Gallé et al. 2009), leaf and plant age (Varone et al. 2012), and differ between different drought intensities, as well as between drought imposition, acclimation, and recovery upon rewatering (Flexas et al. 2006a). The aim of this chapter is to review the current state-of-knowledge on photosynthetic responses of plants to water stress. We will focus mostly on C_3 plants, for which sufficient knowledge has been gained as to build up some generalizations. Studies are scarcer for C_4 plants (Ghannoum 2009) and, especially, for CAM plants, which should be an important research priority for the near future.

5.2 Diffusional and Biochemical Limitations During Drought Imposition in C_3 Plants

Because stomatal closure is among the earliest physiological events occurring in response to drought, and because stomatal conductance (g_s) often correlates strongly with net CO_2 assimilation (A_N), it has been frequently assumed that stomatal closure reduces CO_2 uptake in drought leaves. On the other hand, applying large CO_2 concentrations around leaves overcomes diffusion limitations to CO_2 and several reports have shown that very high CO_2 fully restores maximum photosynthesis in water stressed leaves (Kaiser 1987; Cornic et al. 1989). Moreover, by stripping the epidermis—where stomata reside—from leaves, stomatal limitation is removed, allowing CO_2 to freely diffuse into the leaf. Using this approach, some studies have shown that photosynthesis can be fully restored in water stressed leaves, supporting the idea of a stomatal limitation (Dietz and Heber 1983; Schwab et al. 1989). In other studies, however, it was not possible to restore A_N in water stressed leaves by either applying high CO_2 (Graan and Boyer 1990; Tezara et al. 1999) or stripping leaf epidermis (Tang et al. 2002). These discrepancies in the results obtained in similar experiments have fed the controversy as to whether stomatal or non-stomatal limitations—the latter often used as synonymous of biochemical limitations—are the main cause for decreased photosynthesis under water stress. One study by Tezara et al. (1999), in particular, had a strong influence in this debate. These authors suggested that the main limiting factor for photosynthesis under water stress was impaired photophosphorylation due to decreased chloroplast ATPase activity. Despite the important influence of this paper, the data in Tezara's study were relatively scarce and not fully conclusive. The experiment was performed by inducing a progressive water stress to sunflower plants, and leaf water potential was used as the indicator for water stress severity. While leaf ATP content decreased progressively along the entire gradient of water potential used during the experiment, this could be due to decreased ATP synthesis in the chloroplast or in other cell compartments. Data for chloroplast ATP content were available only at very low water potentials, when photosynthesis was already close to zero. Almost immediately after its publication, some authors claimed against the validity of the conclusions of this study (Cornic 2000), while others showed that impaired Rubisco activity, RuBP regeneration and even photoinhibition occur at similar levels of water stress as those causing impaired photophosphorylation (Flexas and Medrano 2002). Indeed, other reports have suggested that inactivation of Rubisco is the main cause for photosynthesis limitation under drought (Parry et al. 2002), including another study by Tezara et al. (2002) in drought stressed sunflower.

In an attempt to solve for the occurrence of this apparent controversy, Lawlor and Cornic (2002) compiled literature data and reanalyzed them using leaf relative water content as a common indicator for the intensity of water stress. Unfortunately, such analysis did not solve the controversy, as two clearly opposed patterns were found. The so-called 'Type I' response (see Fig. 5.1a) consisted in progressive decreases of A_N as RWC decreased, mainly as a consequence of stomatal closure, since the photosynthetic capacity (i.e., after overcoming stomatal limitations) was unaffected until very low RWC was reached. The 'Type II' response, in contrast, consisted in parallel decreases of A_N and photosynthetic capacity as RWC declined, supporting a predominant role for non-stomatal limitations (Fig. 5.1a). The occurrence of 'Type I' or 'Type II' responses was not found to be associated to specific genotypes or conditions during the experiments, so that even a single species could display one or another response depending on the study. In other words, there is no clear evidence on what does each type depends on, so that the 'types' may most likely reflect differences in the definition/determination method of photosynthetic capacity. In parallel, Flexas et al. (2002) showed in a study with field-grown grapevines that using daily maximum g_s as the common indicator for water stress intensity homogenized the responses between genotypes and growing conditions, which were different when using RWC as a reference. Moreover, it was shown that mesophyll conductance to CO_2 (g_m)—i.e., the facility for internal CO_2 diffusion inside leaves—, which had been typically considered large and constant, progressively decreased as water stress intensified, just as it does in salt-stressed plants (Centritto et al. 2003). This finding implied that not only stomatal closure and impaired photosynthetic capacity could be responsible for drought-induced photosynthesis, but also decreased g_m could play a major role. A limited leaf mesophyll conductance to CO_2 implies that removing leaf epidermis and/or increasing CO_2 concentration around leaves may not always result in fully saturating Rubisco. In this sense, many of the data used to conclude the occurrence of biochemical impairment of photosynthesis could not be valid.

Flexas and Medrano (2002) extended the use of g_s as the common basis for assessing the degree of drought to literature data on many species, and found general relationships between g_{s} and A_N regardless of the species and experimental conditions. Flexas et al. (2004b) specifically showed a common response between Type I, Type II, and even Type III (consisting in very isohydric plants, whose RWC does not change appreciably during drought imposition, see Fig. 5.1a), when using g_s instead of RWC as the reference parameter indicative of water stress. More recent studies have further re-analyzed data on a g_s basis, from which a large consensus has been reached as to how progressive water stress sequentially induces downregulation of the different components associated to photosynthesis (Flexas et al. 2004a,b, 2006a,b; de Souza et al. 2005; Grassi and Magnani 2005; Jiang et al. 2006; Galmés et al. 2007a; Zhou et al. 2007a; Liu et al. 2010). The common pattern consists in three phases, as follows (see Fig. 5.1b):

Phase 1: Mild water stress ($g_s > 0.15$ mol H_2O m^{-2} s^{-1}). When g_s declines from a maximum to about 0.15 mol H_2O m^{-2} s^{-1}, as a consequence of progressively increasing water shortage, decreased g_s is the only cause for reduced A_N. The rate of linear electron transport (ETR), g_m, the activities of photosynthetic enzymes, the maximum quantum efficiency of PSII (Fv/Fm), the maximum velocity of carboxylation ($V_{\mathrm{c,max}}$), etc., remain constant during this phase. The rate of photorespiration (PR) progressively increases, as a consequence of decreased substomatal (C_i) and chloroplast (C_c) CO_2 concentrations. It is remarkable that this threshold is very common among plants, so that even plants whose maximum g_s is below 0.15 mol H_2O m^{-2} s^{-1}—for instance, slcerophyll species—accomplish this rule, being never found on phase 1 but starting at phase 2 from field capacity. This is recognizable by the fact that these plants show linear rather than curvilinear responses of ETR or g_m to g_s (Galmés et al. 2007a), i.e., these parameters do not remain constant at any gs, unlike what happens in phase 1.

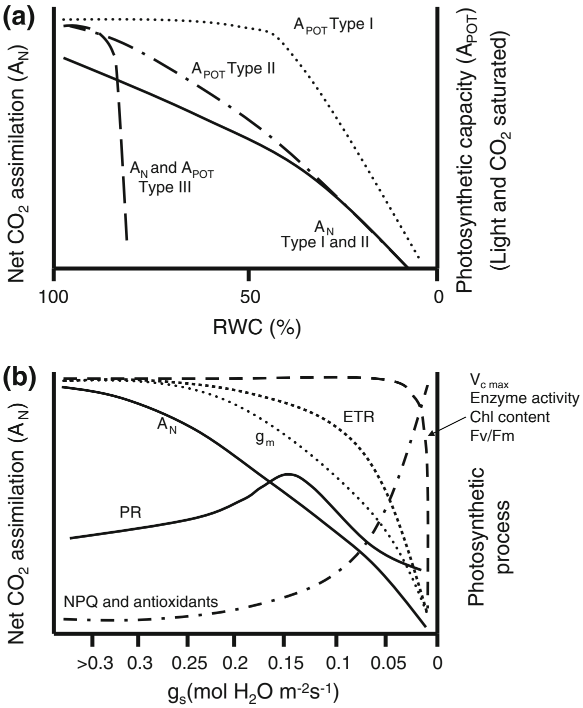

Fig. 5.1 **a** The response of net photosynthesis (A_N) and photosynthetic capacity (A_{POT}) to water stress when using leaf relative water (RWC) content as the reference for stress intensity. Three different Types of response appear, depending on the experiment. Modified after Lawlor and Cornic (2002) and Flexas et al. (2004b). **b** Generalized response of net photosynthesis (A_N) and several parameters related to photosynthetic capacity (see text for the meaning of abbreviations) to water stress when using daily maximum leaf stomatal conductance (g_s) content as the reference for stress intensity

Phase 2: Moderate water stress ($0.15 > g_s > 0.05$ mol H_2O m^{-2} s^{-1}). During this phase, further decreases of g_s are accompanied by large decreases of g_m. Some studies suggest that the decrease in g_m is associated with changes in the abundance of some aquaporins (Flexas et al. 2006c; Miyazawa et al. 2008), although further studies are required to fully elucidate how internal diffusion of CO_2 in leaves is regulated. Still during this phase, small but significant decreases of ETR occur, which are accompanied by subtle increases of non-photochemical quenching of chlorophyll fluorescence (NPQ), the activity of antioxidant enzymes, and the amounts of non-enzymatic antioxidants. These responses suggest that the leaves are preparing for conditions of severe stress, which can lead to secondary oxidative stress. The use of traditional gas exchange analysis (i.e., A_N-C_i curves) to evaluate the presence of non-stomatal limitations in this phase leads to the erroneous conclusion that $V_{c,max}$ is decreased. In fact, these apparent decreases in $V_{c,max}$ are simply an effect of decreased g_m, and the analysis of A_N -C_c curves shows almost constant $V_{c,max}$ during this phase. Neglecting g_m also results in erroneous estimates of the maximum rate of ETR (J_{max}, Centritto et al. 2003; Aganchich et al. 2009).

Phase 3: Severe water stress ($g_s < 0.05$ mol H_2O m^{-2} s^{-1}). When g_s drops below this threshold value the photosynthetic capacity is impaired in many studies, although not in others. Interestingly, the metabolic impairment generally consists of the simultaneous inhibition of all photosynthetic enzymes, decreases in chlorophyll and protein contents, and the appearance of permanent damage to the photosystems, which suggest that the leaves are enduring oxidative stress and/or inducing leaf senescence and remobilization of leaf nutrients (Munne-Bosch et al. 2001). In the studies where metabolic impairment is not apparent, it remains unclear whether it is indicative of highly resistant genotypes or because water stress was ameliorated by, e.g., moderate to low light and/or mild temperature. Metabolic impairment, when it occurs, follows a tightly regulated system, consisting in an impaired Rubisco activity at low g_s due to reduced C_c (Flexas et al. 2006b; Galmés et al. 2011a, b). However, whether the impairment was due to reduced activation state of Rubisco, reduced enzyme concentration and/or increased concentration of tightly bound inhibitors seems to be dependent on the species and the technique used to impose water stress (Bota et al. 2004; Flexas et al. 2006c). On the other hand, Zhou et al. (2007a) found that Rubisco impairment was strongly correlated to the presence of H_2O_2 regardless of the light conditions during water stress, suggesting either a role of H_2O_2 as a secondary messenger in the regulation of Rubisco activity or as causing factor of oxidative stress.

This response pattern to progressively declining g_s is similar to that often observed to progressively declining fraction of transpirable soil water (FTSW, e.g. Centritto et al. 2011), simply because g_s responds directly to FTSW rather than to leaf water potential or RWC (Turner et al. 1985). For this reason, the use of FTSW instead of RWC or even g_s as a tool to assess the general responses of photosynthetic capacity-related parameters seems an easy and promising way of comparing results from different experiments, species, etc.

A similar pattern of response has been described for C_4 plants (Hura et al. 2006, 2007; Ghannoun, 2009), except that leaf photosynthetic metabolism of C_4 plants—particularly Rubisco in bundle sheath cells—seems somewhat more sensitive to water stress than it is in C_3 plants (Carmo-Silva et al. 2007, 2008a,b). The response of photosynthesis to water stress in C_4 plants may be to some extent different to that of C_3 plants. Although generally stomatal limitations are also present, and may be dominant at the very early stages of water stress imposition (Marques da Silva and Arrabaça 2004), biochemical limitations often appear also at early stages of drought, immediately after stomatal limitations (Du et al. 1996; Lal and Edwards 1996; Saccardy et al. 1996; Ghannoun et al. 2002, 2003; Carmo-Silva et al. 2007, 2008a, b). In particular, Rubisco seems very sensitive to water stress, declining linearly with stress intensity in many C_4 species (Du et al. 1996; Carmo-Silva et al. 2007) but not in some others (Lal and Edwards 1996). By contrast, PEPC and C_4 acid decarboxylases are more resistant to water stress, declining only eventually under severe stress conditions (Saccardy et al. 1996; Carmo-Silva et al. 2008a). Although g_m is thought to be not limiting in C_4 plants because of their CO_2-concentrating mechanisms, it has been suggested that bundle sheath CO_2 leakiness could increase under water stress, leading to reduced CO_2 availability and a contributing to a less efficient fixation (Carmo-Silva et al. 2008b). Potential differences in the response to stress among different C_4 subtypes remain elusive (Carmo-Silva et al. 2007, 2008a, b).

In summary, there is a quite general response of photosynthesis to progressive water stress imposition across C_3 plants when g_s is used as the normalizing reference for the level of water stress intensity. Although g_s can respond very differently to leaf water potential, soil water content, etc., depending on the species or genotypes and on environmental conditions, the factors limiting photosynthesis at any given g_s can be accurately predicted. Despite the universality of the pattern, some of the limitations can differ depending on the prevailing light conditions during drought. For instance, under low light conditions g_m is not so highly depressed by water stress (Flexas et al. 2009, Galle et al. 2009). Moreover, the extent of the different limitations appear to vary during acclimation in some cases (Jiang et al. 2006), although not in others (Flexas et al. 2009), and they certainly vary significantly depending on the prevailing air humidity conditions (Perez-Martin et al. 2009) and on the age of leaves and plants subjected to water stress (Varone et al. 2012). Further studies are required in which photosynthesis responses to water stress are evaluated under different conditions, to improve our capacity for predicting photosynthesis limitations under water stress.

5.3 Secondary Oxidative Stress Effects on Photosynthesis Under Combined Excess Light and Water Stress

When CO_2 availability in the chloroplasts is drastically reduced under drought, the use of electrons for CO_2 assimilation in the Calvin cycle decreases. Excess of electrons can be diverted to other processes, such as PR or thermal dissipation; the latter being considered a major process in plant photoprotection under stress conditions (Demmig-Adams and Adams 2006). However, when these processes are saturated, ETR components become overreduced, resulting in electrons being transferred to oxygen at PSI or via the Mehler reaction. This generates reactive oxygen species (ROS), such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical that can cause oxidative damage to the photosynthetic apparatus if the plant is not efficient in scavenging these molecules.

Depending on the duration and severity of the stress, the plant species, the specific tissue/organ and the phase of development, ROS can elicit antioxidant responses, typically observed under mild stress, or can lead to accelerated senescence, programmed cell death or necrosis (Levine 1999; Munne-Bosch and Alegre 2002). Acclimation to stress is generally associated with enhanced activity of the antioxidant molecules, which are able to remove oxygen radicals or repair the damage, thus keeping ROS concentration relatively low (Smirnoff 1998; Dat et al. 2000). Antioxidant activity include enzymatic and non-enzymatic mechanisms such as (a) the superoxide dismutases (SODs), which catalyze the dismutation of O_2^- to H_2O_2, (b) the catalases (CATs), which are responsible for the removal of H_2O_2, and (c) the enzymes and metabolites of the ascorbate-glutathione cycle, which are also involved in the removal of H_2O_2 (Foyer and Noctor 2003). Increased activities of plant antioxidant systems under stress have been considered characteristic of drought-resistant species (e.g., Gao et al. 2009). In a recent work by Rivero et al. (2007), the expression of isopentenyltransferase (IPT) that catalyzes the rate-limiting step in cytokinin (CK) synthesis led to increased concentrations of enzymes associated with the glutathione-ascorbate cycle in transgenic plants, and resulted in the suppression of drought-induced leaf senescence and in an outstanding drought-tolerance. In transgenic plants with elevated CK production that were subjected to drought, a 20 % of the upregulated transcripts were related to ROS metabolism. The efficient scavenging of ROS protects the photosynthetic apparatus during drought stress, leading to improved water use efficiency of the transgenic plants during and after stress. The presence of high light intensity and high ROS levels during water stress have been shown to exacerbate drought effects on g_m (Flexas et al. 2009; Galle et al. 2009), leaf photochemistry and Rubisco activity (Zhou et al. 2007a). It has been also hypothesized that oxidative damage to the chloroplast ATPase, which was caused by ROS under conditions of low CO_2 and excess light, resulted in a water stress-induced decrease of the photosynthetic capacity (Lawlor and Tezara 2009).

In addition to the deleterious effects, ROS can also serve as secondary messengers in the signaling for the activation of defence responses (Dat et al. 2000). This dual function of ROS, first described in responses to pathogens and later demonstrated in response to several abiotic stresses, presumably plays an important role on the acclimation processes (Dat et al. 2000), which may deserve better attention in future studies.

5.4 Photosynthesis Limitations During Rewatering After Drought Stress

The carbon balance of a plant following a complete period of water stress and recovery may depend as much on the velocity and degree of the recovery of photosynthesis after stress relief, as it certainly depends on the degree and velocity of photosynthesis decline during stress imposition (Flexas et al. 2006a; Centritto et al. 2011). In general, plants subject to severe water stress recover only 40–60 % of the maximum photosynthesis rate during the day after rewatering, and recovery continues during the next few days, although maximum photosynthesis rates are not fully recovered in some cases (Kirschbaum 1988; Sofo et al. 2004; Grzesiak et al. 2006; Bogeat-Triboulot et al. 2007; Gallé et al. 2007). The extent and velocity of recovery may depend on the severity of the stress endured prior to rewatering. The influence of previous water stress episodes was illustrated by Miyashita et al. (2005) and Grzesiak et al. (2006). Here, we grouped data available from the literature in three intensity levels of previous stress episodes to obtain a more general picture on how this affects the velocity of recovery after rewatering (Table 5.1). For the three groups, average g_s prior to rewatering was $\leq$ 0.05 mol H_2O m^{-2} s^{-1}, i.e., they were all at Phase 3 described in Sect. 5.2. However, the three groups differed in the endured inhibition of photosynthesis. When A_N was, on average, 36 % of maximum values observed in control plants, total recovery of A_N occurred in 4 days. When A_N was only 23 % of control, then full recovery took up

Table 5.1 Time required for achieving full recovery of photosynthesis upon rewatering after water stress. Water stress intensities (reflected by g_s and A_N before rewatering) were classified into 3 different ranges

g_s before re-watering (mol H_2O m^{-2} s^{-1})	A_N before re-watering (% of control values)	Time to achieve full recovery of A_N (days)
0.018 ± 0.004	3.0 ± 1.2	18.2 ± 6.7
0.046 ± 0.010	22.9 ± 3.2	6.2 ± 2.5
0.048 ± 0.012	36.1 ± 1.8	4.3 ± 1.0

Data from: de Souza et al. (2004), Miyashita et al. (2005), dos Santos et al. (2006), Hura et al. (2006), Bougeat-Triboulot et al. (2007), Cai et al. (2007), Galle et al. (2007), Montanaro et al. (2007), Perez-Perez et al. (2007), Pou et al. (2008) and Galle et al. (2009)

to 6 days, and when it was as low as 3 % of controls full recovery took up to 18 days (Table 5.1).

Concerning the physiological mechanisms limiting recovery, Kirschbaum (1988) showed that recovery after a severe dehydration was a two-stage process: the first stage occurs during the first days upon rewatering, and is associated to recovery of water status and stomata reopening; the second stage lasts several days and likely requires de novo synthesis of photosynthetic proteins. Concerning the first of these two phases, however, in some species a sustained downregulation of g_s after rewatering imposes substantial limitations to photosynthesis, while increasing intrinsic water-use efficiency (Bogeat-Triboulot et al. 2007; Gallé and Feller 2007; Gallé et al. 2007, 2009; Galmés et al. 2007a; Flexas et al. 2009; Xu et al. 2009). Orange trees that endured severe water stress do not fully recover g_s even after two months of rewatering (Fereres et al. 1979). In some of these cases, limited recovery of leaf-specific hydraulic conductivity is the likely cause for the long-term downregulation of g_s after rewatering (Galmés et al. 2007b; Pou et al. 2008). Resco et al. (2009) have demonstrated specifically that the number of days necessary to reach maximum photosynthesis after a pulse of rain (i.e., the inverse of velocity of recovery) depends on antecedent, drought-induced percentage loss of hydraulic conductance, and Brodribb and Cochard (2009) reached a similar conclusion in conifers. Alternatively, in beech stomatal occlusions formed during water stress restrained recovery of g_s after rewatering (Gallé and Feller 2007). During the first days after rewatering, g_m remains low in some species (Galmés et al. 2007a) but it reverses fast in many others (Flexas et al. 2009; Galle et al. 2009, 2011; Varone et al. 2012), for which it is not regarded as a major limiting factor for photosynthesis recovery.

As for the second phase, Bogeat-Triboulot et al. (2007) showed recently that recovery after water stress, determined 10 days after rewatering, 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, photoinhibition and/or oxidative stress have been suggested as possible causes (Sofo et al. 2004; Gallé et al. 2007).

In summary, restricted velocity of photosynthesis recovery after rewatering causes significant losses of carbon gains in plants, and the precise understanding of the mechanisms leading to such slow recovery requires further investigation.

5.5 Photosynthesis Acclimation: Genomics and Proteomics

The response of photosynthesis to water stress may differ between acclimated and non-acclimated plants. Acclimation to water stress may lead to homeostatic compensation for the initial negative effects of water stress on photosynthesis, and involves gene expression and modification of plant physiology and morphology, taking place in days to weeks. Osmotic adjustment is perhaps the best example of physiological acclimation to water stress (Chaves and Oliveira 2004; see also Chap. 7). Very few evidences for photosynthetic acclimation to water stress have been described. For instance, in some studies, leaves unfolded under water stress show somewhat higher photosynthesis rates than non-acclimated leaves, associated with morphological adaptations and higher ETR rates (Maury et al. 1996; Kitao et al. 2003; Galmés et al. 2006). Recently, Galle et al. (2011) observed a permanent downregulation of g_s in *Cistus* plants subject to periodical cycles of water stress and recovery, appearing after the first cycle, which can be considered an acclimation response. However, *Quercus* plants showed almost identical photosynthetic responses to each repeated drought cycle, i.e., showing no evidence for acclimation. Similarly, in many studies with different species in which a constant level of water stress was kept for a week or more, in order to allow for acclimation, no clear evidence for photosynthetic acclimation was found (Flexas et al. 2009; Galle et al. 2009; Varone et al. 2012).

Because acclimation involves gene expression, an alternative approach to evaluate the mechanisms leading to acclimation may be looking at changes in gene expression and protein contents, i.e., by genomic and proteomic approaches. In such studies, photosynthetic pathways are in general not among the most altered by the stress (Killian et al. 2007; Chaves et al. 2009). For example, in *Thellungiella* (a stress tolerant relative of *Arabidopsis*), only 15 % of all genes downregulated are involved in photosynthesis (Wong et al. 2006). In rice, alterations in photosynthesis related genes are mostly associated with stress recovery but not with stress imposition (Zhou et al. 2007b). Even in those photosynthetic genes responding to stress, the most common trend is a downregulation, i.e., they would not contribute to acclimation of photosynthesis, but rather to its further decline. In addition, the alterations found at transcriptomic level are larger (5–10 %) than at protein level (usually less than 1 %). However, transcriptomic analysis also in *Vitis* have shown that some photosynthetic genes, like those of the Rubisco activase, some Calvin cycle enzymes and some PSI- and PSII-related genes are instead upregulated during acclimation to water stress (Cramer et al. 2007). Although proteomic analysis showed that some photosynthetic proteins were downregulated during water stress, it also confirmed that some—notably

Rubisco and sedoheptulose-1,5-bisphosphatase— were indeed upregulated (Vincent et al. 2007). Moreover, the number of stress responsive genes or proteins seems to be related with the stress intensity (Cramer et al. 2007) and with tissue origin (Zhou et al. 2007b). On the other hand, a recent proteomics study in peanut cultivars has suggested that the response can also differ in drought-sensitive and drought-tolerant genotypes (Kottapalli et al. 2009). For instance, while Rubisco large and small subunits were decreased in both types of cultivars, some PSII proteins were decreased and ATP synthase increased only in the tolerant genotype (Kottapalli et al. 2009). Still, precaution has to be taken when analyzing gene expression data, since they may not necessarily have a reflection in the protein contents and physiological properties, due to post-transcriptional regulation. For instance, Bogeat-Triboulot et al. (2007) did not observe any correlation between the abundance of transcripts and proteins in an experiment with *Populus euphratica* subjected to gradual soil water depletion.

In summary, there is little evidence for acclimation of photosynthesis to water stress conditions in the short term, while the genetic basis of photosynthetic acclimation needs still to be determined.

5.6 Concluding Remarks

Major progress in the understanding of the physiological and molecular limitations and the signaling events underlying photosynthetic responses to drought has been achieved over the last decade(s), which is reviewed in the present chapter. The available evidence suggests that restricted CO_2 diffusion to the carboxylation sites is the main limiting factor for photosynthesis, especially during the early phases of stress. This is initially due to reduced stomatal conductance (g_s), but it also involves reduced mesophyll conductance (g_m). Under more severe drought conditions, which occur mostly under high irradiance, metabolic impairment (a consequence of oxidative stress) corresponds invariably to, and seems to be caused by, low g_s. Both physiological and molecular approaches suggest that very little acclimation of photosynthesis to prolonged water stress occurs among most plants. The extent and velocity of photosynthesis recovery upon rewatering depends on the intensity of water stress endured, and specifically on the degree of photosynthetic inhibition. The mechanisms limiting rapid recovery appear to diverge between plants and environmental conditions, but slow recovery of stomatal conductance seems to be the most common cause.

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