

## Understanding down-regulation of photosynthesis under water stress: future prospects and searching for physiological tools for irrigation management

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### Abstract

Photosynthetic down-regulation and/or inhibition under water stress conditions are determinants for plant growth, survival and yield in drought-prone areas. Current knowledge about the sequence of metabolic events that leads to complete inhibition of photosynthesis under severe water stress is reviewed. An analysis of published data reveals that a key regulatory role for Rubisco in photosynthesis is improbable under water stress conditions. By contrast, the little data available for other Calvin cycle enzymes suggest the possibility of a key regulatory role for some enzymes involved in the regeneration of RuBP. There are insufficient data to determine the role of photophosphorylation.

Several important gaps in our knowledge of this field are highlighted. The most important is the remarkable scarcity of data about the regulation/inhibition of photosynthetic enzymes other than Rubisco under water stress. Consequently, new experiments are urgently needed to improve our current understanding of photosynthetic down-regulation under water stress. A second gap is the lack of knowledge of photosynthetic recovery after irrigation of plants which have been subjected to different stages of water stress. This knowledge is necessary in order to match physiological down-regulation by water stress with controlled irrigation programmes.

**Key words:** Drought, photosynthesis, stomatal limitations, metabolic limitations, C<sub>3</sub> plants, Rubisco

### Introduction

Water stress is considered to be the main environmental factor limiting plant growth and yield worldwide, especially in semi-arid areas (Boyer, 1982). It is well known that one of the primary physiological targets of water stress is photosynthesis (Chaves, 1991; Cornic, 1994; Lawlor, 1995). However, there is a long-standing controversy as to whether water stress mainly limits photosynthesis through stomatal closure (Sharkey, 1990; Chaves, 1991; Cornic, 1994; Ort *et al.*, 1994) or metabolic impairment (Boyer, 1976; Lawlor, 1995). The suggestion that impaired ATP synthesis is the main factor limiting photosynthesis even under mild water stress (Tezara *et al.*, 1999), has further stimulated debate in recent years (Cornic, 2000; Cornic & Fresneau, 2002; Flexas & Medrano, 2002; Lawlor & Cornic, 2002; Tezara *et al.*, 2002).

It has been argued that stomatal closure is the main limitation to photosynthesis, since maximum values can be recovered by supplying large amounts of CO<sub>2</sub> to the leaves (Cornic, 2000; Cornic & Fresneau, 2002). Other reports, however, have suggested that

maximum photosynthesis is not totally recovered by high CO<sub>2</sub> in water stressed plants (Graan & Boyer, 1990; Quick *et al.*, 1992). This has been attributed to the inhibition of key metabolic processes, such as photophosphorylation (Younis *et al.*, 1979; Tezara *et al.*, 1999), the capacity for ribulose-1,5-bisphosphate (RuBP) regeneration (Giménez *et al.*, 1992; Gunasekera & Berkowitz, 1993) and Rubisco activity (Medrano *et al.*, 1997; Maroco *et al.*, 2002; Parry *et al.*, 2002). Each of these processes has been proposed to be the main limitation to photosynthesis under water stress. Other authors, by contrast, have stated that photophosphorylation (Ortiz-López *et al.*, 1991), the capacity for RuBP regeneration (Lal *et al.*, 1996) or the activity of Rubisco (Lal *et al.*, 1996; Pankovic *et al.*, 1999; Delfine *et al.*, 2001; Pelloux *et al.*, 2001) remained unaffected during water stress.

Sometimes even the same authors have observed discrepancies among their own studies. For instance, Tezara *et al.* (1999) attributed water stress-induced decreases of photosynthesis to impaired photophosphorylation in sunflower, but they stated in a later study using the same species that "decrease in net photosynthesis with water deficiency was

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related to lower Rubisco activity rather than to ATP and RuBP contents" (Tezara *et al.*, 2002). Similarly, we have recently highlighted that decreased RuBP contents matched decreased photosynthetic capacity under water stress in field-grown grapevines (Medrano *et al.*, 2003), but we have also observed decreased photosynthetic capacity with constant RuBP contents under water stress in other experiments with the same species (Bota *et al.*, 2004). These apparent discrepancies could arise from the fact that different studies may have been made under different degrees of water stress, since the assessment of severity of water stress is a complex matter. The two most commonly used water status parameters to assess water stress intensity are leaf water potential ( $\Psi$ ) and relative water content (RWC). However, the precise response of stomatal conductance and photosynthesis to  $\Psi$  and RWC depends on the genotype (Tardieu & Simonneau, 1998), the environmental conditions during water stress (Schulze & Hall, 1982) and the velocity of water stress imposition (Flexas *et al.*, 1999), among other factors. Alternatively, to discriminate stress severities leading to metabolic limitations to photosynthesis, the water stress-induced variations of sub-stomatal  $\text{CO}_2$  concentration ( $C_i$ ) have been proposed as a reference parameter (Lawlor, 1995, 2002). However, it is now doubtful that this kind of analysis is reliable under drought. Two main problems have been described in relation to  $C_i$  calculations: patchy stomatal closure (Laisk, 1983; Buckley *et al.*, 1997) and changes in the cuticular conductance of water vapour (Boyer *et al.*, 1997). In addition, drought-induced changes in the mesophyll conductance of  $\text{CO}_2$  may also invalidate the interpretation of  $A_N$ - $C_i$  analysis (where  $A_N$  is net photosynthesis) (Flexas *et al.*, 2002 *a,b*; Centritto *et al.*, 2003).

It is necessary to know the precise sequence of events leading to photosynthetic inhibition under progressive water stress because photosynthesis is one of the key determinants for plant productivity and survival, and water stress is thought to be an increasing problem for plant performance in the present climate change scenario (Chaves *et al.*, 2003). The response of respiration to water stress, the other basic component of plant productivity, is beyond the scope of the present review. However, the lack of knowledge about respiratory responses to water stress is remarkable, and this is an important issue that needs to be addressed in the near future (Flexas *et al.*, 2004). Regarding photosynthesis, the presence of non-stomatal limitations constrains the validity of canopy conductance models for the estimation of canopy photosynthesis under severe water stress conditions, thus reducing the accuracy of estimations of canopy productivity (Moriani *et al.*, 2002; Reichstein *et al.*, 2002). Moreover, the

appearance of non-stomatal limitations usually coincides with decreasing water-use-efficiency at the leaf level, which may have a repercussion for water-use-efficient irrigation programs (Flexas *et al.*, 2002*a,b*; Gulías *et al.*, 2002; Medrano *et al.*, 2002).

The aim of the present paper is to critically review the data in the literature and our own experimental results, in relation to the effects of water stress on photosynthesis. The present analysis supports the view that light saturated, daily maximum, stomatal conductance ( $g_s$ ) is a useful parameter for comparing the response of photosynthesis to water stress between different species and experiments. Water stress-induced depression of photosynthetic metabolism is reviewed using  $g_s$  as the reference parameter for water stress intensity. In all the experiments reviewed here, water stress was imposed gradually by withholding watering. Plants were grown at saturating or near-saturating light, and optimal temperatures (except in those experiments under field conditions, where plants could have eventually endured periods of excessive temperature). Measurements of photosynthesis were made at saturating light and atmospheric  $\text{CO}_2$  concentration.

### Looking for a Water Stress Reference Resulting in the Most Generalised Response Pattern

In an attempt to solve the observed discrepancies among different studies, Lawlor & Cornic (2002) have proposed that two different patterns or syndromes of photosynthetic responses to water stress could occur (Type I and Type II). Definitions of these syndromes use leaf relative water content (RWC) as the indicator of water stress intensity at the leaf level. In both Type I and Type II plants, net photosynthesis ( $A_N$ ) decreases as RWC becomes smaller due to increasing water stress. However, in Type I plants, the rate of photosynthesis under light- and  $\text{CO}_2$ -saturated conditions ( $A_{\text{SAT}}$ ) remains unaffected until RWC drops to very low values. By contrast, in Type II plants,  $A_{\text{SAT}}$  declines in parallel to net photosynthesis as RWC decreases.

Such classification of plant responses to water stress presents some limitations. First, the same species can behave as either Type I or Type II, depending on the experimental conditions (Lawlor & Cornic, 2002), thus indicating that the type of response to water stress is not fully determined by the genotype. Second, in some species like grapevines, it has been shown that  $A_{\text{SAT}}$  progressively declines during water stress with very low (if any) reductions in RWC. Such isohydric behaviour does not match either the Type I or the Type II response, so it could possibly be classified as a Type III (Fig. 1A).

We have shown that, among  $C_3$  plants, water

stress-induced changes in photosynthetic rate can be more generally related to variations in light-saturated stomatal conductance ( $g_s$ ) than to RWC or leaf water potential (Flexas & Medrano, 2002; Flexas *et al.*, 2002 *a,b*; Medrano *et al.*, 2002). The use of  $g_s$  (stomatal conductance) as an indicator of the intensity of water stress has revealed a more general pattern of photosynthetic responses to progressive water stress that is somewhat independent of a) the velocity of water stress imposition, b) the environmental conditions and c) the genotype. As shown in Figure 1B, examples of the three different response Types show a rather similar pattern of response of  $A_{SAT}$  to  $g_s$ . Even so, there is still some variability in the response of different photosynthetic parameters to  $g_s$ . This may come from other important factors affecting photosynthesis, such as life-form, leaf habit, canopy characteristics, intrinsic photosynthetic capacity of a given species, etc.

In spite of this, comparison of the response of photosynthesis to water stress on the basis of  $g_s$  seems to be more appropriate when comparing between different studies. In fact,  $g_s$  responds to many internal and external factors involved in plant water stress signalling (i.e. xylem and leaf water status, xylem pH and abscisic acid (ABA) content, and possibly others presently unknown), which makes  $g_s$  an integrative parameter of all the signals associated with the plant responding to water stress. In supporting of this idea, for instance, Correia *et al.* (1995) demonstrated that the maximum daily stomatal conductance, but not its diurnal fluctuations, was determined by the xylem ABA concentration in field-grown grapevines.

On the other hand, it remains to be elucidated whether the fact that relationships between  $g_s$  and photosynthetic parameters are largely independent of genotype and environmental conditions is due to (i) a direct effect of water stress-induced stomatal closure on down-regulation of photosynthetic metabolism, possibly through internal  $CO_2$  signalling, as already suggested (Sharkey, 1990; Ort *et al.*, 1994; Cornic & Fresneau, 2002), or (ii) a strong co-regulation of both stomatal closure and photosynthetic metabolism, possibly reflecting that both processes share one or several common agents that are elicited by water stress, as suggested by Cornic (1994). In view of the different patterns of relationship between RWC and photosynthesis observed, it is likely that decreased cell water content and/or increased concentration of certain ions is the general cause of metabolic impairment under natural conditions, as proposed by Lawlor (2002). Nevertheless, to fully understand the mechanism by which photosynthetic metabolism is impaired or down regulated at severe water stress would merit a detailed and profound analysis. For the moment, the robustness of the above mentioned relationships may

allow a direct comparison of the regulation of different metabolic processes in response to progressive water stress, even when data from different species are included. From here on,  $g_s$  will be used as the indicator of water stress severity in order to follow photosynthetic metabolic variations.

### Down-Regulation of Photosynthetic Metabolism Along a Stomatal Conductance Gradient During Progressive Water Stress

An initial review of the literature, using  $g_s$  as the indicator of the severity of water stress, used chlorophyll fluorescence and gas exchange parameters to indicate key photosynthetic points (Flexas *et al.*, 2002 *a,b*; Medrano *et al.*, 2002). Such analysis suggested that photosynthetic metabolism is progressively down regulated as water stress intensifies, and three stages of inhibition of photosynthesis were described:

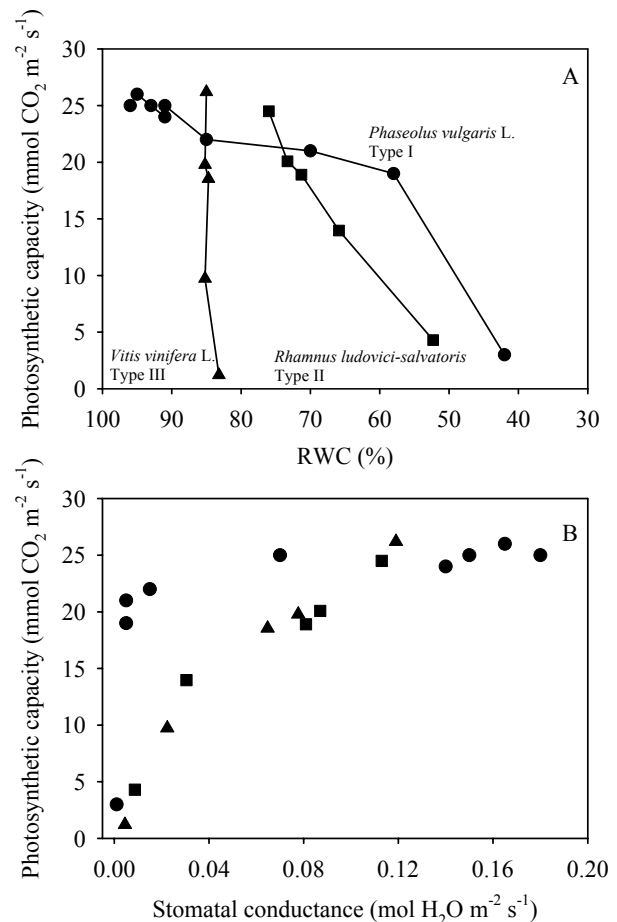


Fig. 1. (A) The dependence of photosynthetic capacity on RWC in three different species presenting three different syndromes: *Phaseolus vulgaris* L., Type I (circles); *Rhamnus ludovici-salvatoris* R. Chodat, Type II (squares) and *Vitis vinifera* L., Type III (triangles). (B) Response of photosynthetic capacity to stomatal conductance in these three species. Data on *Phaseolus* are from Brestic *et al.* (1995), and data on the other two species from J Bota *et al.* (unpublished results).

- Stage 1. As  $g_s$  decreases from its maximum value (remarkably, irrespective of the actual value, which was largely variable depending on the species) to about  $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , net photosynthesis ( $A_N$ ) decreased slowly with very small variations of  $A_{SAT}$  and no variation of the photosynthetic electron transport rate (ETR). Intrinsic water-use-efficiency ( $A_N/g_s$ ) progressively increased at this stage.

- Stage 2. When  $g_s$  drops between  $0.15$  and  $0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $A_N$  and  $A_{SAT}$  further decrease, and so does ETR, suggesting increased metabolic limitations. However, a continuous decline of the sub-stomatal  $\text{CO}_2$  concentration ( $C_i$ ) suggests that stomatal closure is still the dominant limitation to photosynthesis. The mesophyll conductance to  $\text{CO}_2$  is also suggested to start decreasing at this stage. Intrinsic water-use-efficiency still increased at this stage, reaching maximum values at  $g_s$  around  $0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ .

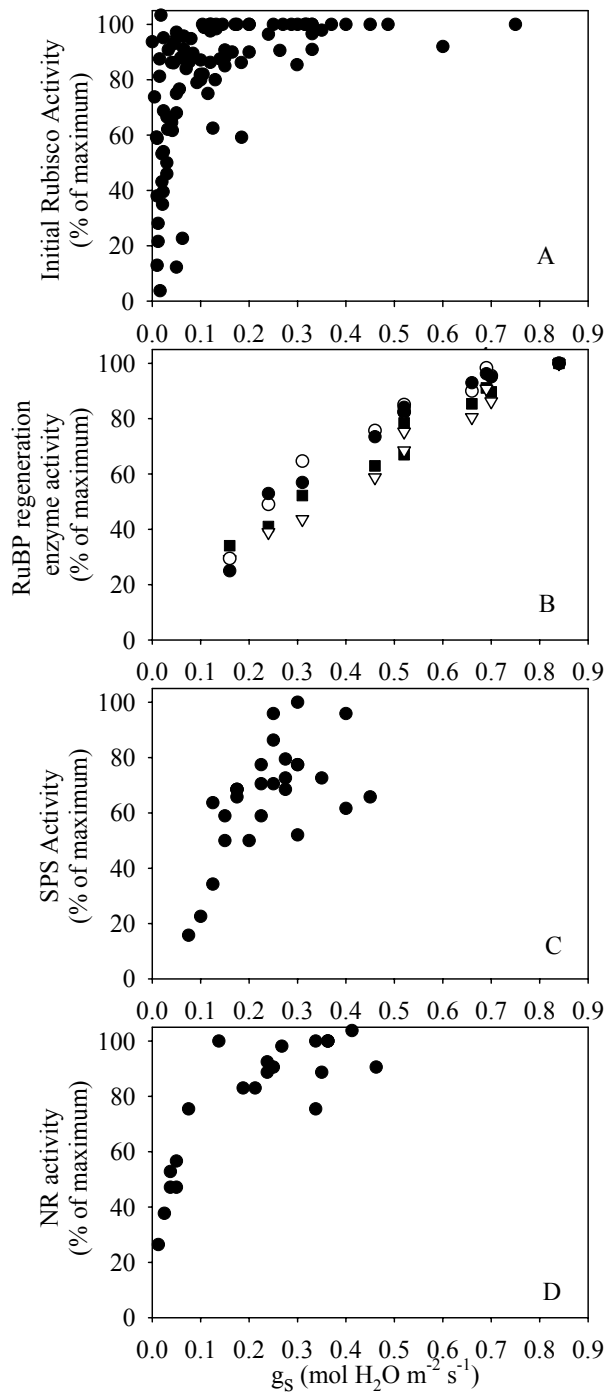
- Stage 3. When  $g_s$  declines below  $0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ,  $C_i$  commonly increases sharply, reflecting either erroneous estimations of  $C_i$  or impaired photosynthetic metabolism. Intrinsic water use efficiency usually decreases at this stage, and both  $A_{SAT}$  and ETR become very low.

Therefore, the analysis of gas exchange and chlorophyll fluorescence variations along a  $g_s$  gradient suggests that photosynthetic metabolism is not impaired under water stress until  $g_s$  is quite low, and  $A_N$  is already severely reduced, as already stated (Cornic & Fresneau, 2002). However, as mentioned previously there are questions about whether assessments of metabolic limitations based on  $C_i$  analysis are reliable under drought. Two main problems have been described related to  $C_i$  calculations in stressed leaves: patchy stomatal closure (Laisk, 1983; Buckley *et al.*, 1997) and the increase of the relative importance of cuticular transpiration when stomata are closing in drying leaves (Boyer *et al.*, 1997). Moreover, even if correctly estimated,  $C_i$  may not represent the actual  $\text{CO}_2$  concentration in the chloroplasts ( $C_c$ ), since the mesophyll conductance to  $\text{CO}_2$  is finite and decreases in response to water stress (Flexas *et al.*, 2002a; Centritto *et al.*, 2003). Therefore, the previous analysis based on *in vivo* measurements is questionable and, to assess metabolic limitations to photosynthesis, it would be better to rely on biochemical analysis. In fact, Flexas & Medrano (2002) and Medrano *et al.* (2002), have already highlighted that biochemical evidence does not always match gas exchange and fluorescence data. Flexas & Medrano (2002) presented a preliminary analysis of some biochemical data regarding photosynthetic metabolism, using discrete  $g_s$  intervals as a reference of water stress intensity. In the present review, we analyse only metabolic parameters for which a sufficient amount of data

including simultaneous  $g_s$  measurements is available, so that a clear picture of variations along an entire  $g_s$  gradient can be drawn.

A large number of simultaneous measurements of  $g_s$  and Rubisco activity under water stress conditions has accumulated during the last 20 yr or so, which has been enlarged with recent data by Castrillo *et al.* (2001), Delfine *et al.* (2001), Maroco *et al.* (2002) and Tezara *et al.* (2002), as well as from unpublished results from our group in up to eleven new species (Bota *et al.*, 2004; J Galmés, unpublished). Figure 2A shows a pool of all these results. It is very clear that Rubisco activity remains largely unaffected by water stress at  $g_s$  higher than  $0.10 - 0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , and this is irrespective of the maximum  $g_s$  attained by a given species. Below that threshold, Rubisco activity steeply declines, as stomata close further. The strength of the observed  $g_s$  threshold is remarkable, since the plot includes data from a large number of different species that present substantially different ecological strategies and water stress resistance. Therefore, the relationship presented strongly supports the notion that Rubisco activity is highly stable and resistant to water stress.

By contrast, the data on water stress-induced regulation of the activity of photosynthetic enzymes other than Rubisco are scarce. To the best of our knowledge there is only one study where the activity of several enzymes involved in RuBP regeneration has been measured concurrently with  $g_s$  under more than two different water stress intensities (Thimmanaik *et al.*, 2002, see Fig. 2B). The same applies for the activity of sucrose phosphate synthase (SPS), which was analysed in maize (a  $C_4$  plant) by Pelleschi *et al.* (1997, see Fig. 2C), and nitrate reductase (NR), which was analysed in *Ziziphus rotundifolia* by Arndt *et al.* (2001, see Fig. 2D). Thimmanaik *et al.* (2002) studied the activity of several photosynthetic enzymes under progressive water stress in two different cultivars of *Morus alba* (Fig. 2B). Unlike Rubisco, the activity of all those enzymes started declining at early steps of water stress, when  $g_s$  was still high. By contrast, the activities of SPS (Fig. 2C) and NR (Fig. 2D) remained substantially unaffected at  $g_s$  higher than  $0.10-0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , as did Rubisco. The high stability of the activity of the latter two enzymes under water stress is remarkable, since both have been considered to be down regulated by low  $\text{CO}_2$  availability under mild water stress (Kaiser & Förster, 1989; Vasey *et al.*, 1991). Therefore, it seems that the activities of many enzymes related to photosynthesis remain unaffected by moderate water stress, being impaired only when  $g_s$  is lower than  $0.10-0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , whereas some enzymes involved in the regeneration of RuBP are progressively impaired or down regulated from very early stages of water stress (Fig. 2). Thus, these



results present the possibility that some enzymes involved in the regeneration of RuBP could play a key regulatory role in photosynthesis under water stress, although this role should be confirmed in a larger number of species. Also, photosynthesis could be impaired by reduced photophosphorylation under water stress (Tezara *et al.*, 1999; Lawlor, 2002). Although this possibility remains open as well, a recent report by Tezara *et al.* (2002) has shown that when  $g_s$  was strongly reduced under water stress, from 0.8 to 0.1 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, the observed decrease in leaf ATP content was only marginally significant. Further studies are needed to clarify whether photophosphorylation is generally impaired

Fig. 2 (left). (A) Relationship between initial Rubisco activity and  $g_s$ . We selected data on Rubisco activity from the available literature in which  $g_s$  was given. Data are average values for three to 12 replicates, depending on the reference (standard errors have been removed to facilitate visualising general trends). These data are from the following species and references: *Capsicum annuum* L. (Delfine *et al.*, 2001), *Casuarina equisetifolia* L. (Sánchez-Rodríguez *et al.*, 1999); *Cistus albidus* L. (J Galmés, unpublished results); *Helianthus annuus* L. (Pancovic *et al.*, 1999; Tezara *et al.*, 1999, 2002), *Hordeum vulgare* L. (Lal *et al.*, 1996; Wingler *et al.*, 1999, 2000); *Hypericum balearicum* L. (J Galmés, unpublished results); *Lycopersicon esculentum* Mill. (Castrillo *et al.*, 2001); *Lysimachia minoricensis* J. J. Rodr. (J Galmés, unpublished results); *Medicago sativa* L. (Antolín & Sánchez-Díaz, 1993), *Mentha aquatica* L. (J Galmés, unpublished results); *Nicotiana sylvestris* L. (Bota *et al.*, 2004); *Phaseolus vulgaris* L. (Bota *et al.*, 2004; Brestic *et al.*, 1995; Castrillo *et al.*, 2001); *Phlomis italica* L. (J Galmés, unpublished results); *Pistacia lentiscus* L. (J Galmés, unpublished results); *Rhamnus alaternus* L. (Bota *et al.*, 2004); *Rhamnus ludovicisalvatoris* R. Chodat (Bota *et al.*, 2004); *Trifolium subterraneum* L. (Medrano *et al.*, 1997), *Triticum aestivum* L. (Holaday *et al.*, 1992), *Vicia faba* L. (Lal *et al.*, 1996) and *Vitis vinifera* L. (Bota *et al.*, 2004; Maroco *et al.*, 2002). (B) Effect of progressive water stress on the activity of several photosynthetic enzymes: RuBP kinase (filled circles), 3-PGA kinase (empty circles), NAD dehydrogenase (filled triangles) and NADP dehydrogenase (filled squares) in two different cultivars of *Morus alba* L., studied by Thimmanaik *et al.* (2002). (C) Effect of progressive water stress on SPS activity in maize studied by Pelleschi *et al.* (1997). (D) Effect of progressive water stress on NR activity, analysed in *Ziziphus rotundifolia* Lam. by Arndt *et al.* (2001). Data are expressed as % of maximum values for comparison.

under water stress and at what  $g_s$  value.

Summarising, both gas-exchange/chlorophyll fluorescence data and biochemical data, analysed using  $g_s$  as a reference for water stress intensity, strongly support the idea that photosynthetic metabolism is quite resistant to water stress, and does not limit photosynthesis until the water stress is severe (i.e.  $g_s$  below 0.10 - 0.15 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>). However, there is still an important lack of knowledge about the variations in many metabolic components affecting photosynthesis along a  $g_s$  gradient during water stress. Among these, the following must be considered: (1) all the enzymes involved in the regeneration of RuBP in the Calvin cycle; (2) water stress-induced oxidative stress and protective antioxidant responses; (3) photophosphorylation; and (4) agents possibly involved in CO<sub>2</sub> diffusion inside leaves (carbonic anhydrase, aquaporins). Therefore, it would be necessary to study these aspects properly for a better

understanding of the metabolic impairment under severe water stress. Moreover, even if  $0.10 - 0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  is assumed to be the  $g_s$  threshold below which non-stomatal limitations to photosynthesis appear, two important questions need to be addressed:

1. How frequent is the occurrence of low stomatal conductance under semi-arid conditions, such as those in the Mediterranean area?
2. How fast is photosynthesis in recovering from low  $g_s$  after re-watering?

### How Frequent is the Occurrence of Low Stomatal Conductance under Semi-Arid Conditions?

It has been shown that photosynthetic metabolism seems little impaired under water stress whenever  $g_s$  is higher than  $0.10-0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . This could lead to the misinterpretation that water stress-induced metabolic impairment is rare under natural conditions. However,  $g_s$  values lower than  $0.10-0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  are often met by plants living in semi-arid areas, like the Mediterranean.

To illustrate the variability that can be found on seasonal patterns of  $g_s$  under Mediterranean conditions, two examples are given in Fig. 3. Fig. 3A shows the seasonal variation of  $g_s$  in natural stands of holm oak (*Quercus ilex*) during 2000 in two localities in Mallorca, which are separated by less than 50 Km (J Gulías *et al.*, unpublished results). While in Binifaldó  $g_s$  was below  $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  only during a few months in summer, in Puigpunyent  $g_s$  was below this value during most of the year. Similarly, important differences can be observed among years, caused by the large inter-annual precipitation variability usually observed under Mediterranean conditions. For instance, field-grown *Vitis vinifera* cv. Tempranillo showed  $g_s$  values lower than  $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  during most of the summer in 1999, but these values were never reached during the rainy summer of 2002, where  $g_s$  progressively increased to atypically high values along the summer (Fig. 3B, J Flexas *et al.* unpublished results). Also in 1999, at the same vineyard, *Vitis vinifera* cv. Manto Negro was able to maintain  $g_s$  values above  $0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  for 2-3 wk more than Tempranillo (Fig. 3B).

Therefore, it seems that  $g_s$  lower than  $0.10-0.15 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  is a condition which is often met and consequently plants frequently endure metabolic impairment, at least in semi-arid areas. These conditions may impose restrictions to photosynthetic recovery by leaves after rainfall or irrigation, with its consequent losses for plant productivity. However, do we really know how rapidly photosynthesis recovers from low  $g_s$  after re-watering?

### How Fast does Photosynthesis Recover from Low $g_s$ After Re-watering?

Several studies have analysed photosynthetic recovery upon re-watering after a water stress period (Larcher *et al.*, 1981; Castrillo & Calcagno, 1989; van Rensburg & Krüger, 1993; Giardi *et al.*, 1996; Flexas *et al.*, 1999; Marron *et al.*, 2002; Thimmanaik *et al.*, 2002). However, a systematic analysis, including amount and velocity of recovery starting at different water stress intensities and in different plant species, is still lacking.

It is usually assumed that the presence of non-stomatal limitations or metabolic impairment imposes restrictions to photosynthetic recovery by leaves after rainfall or irrigation (Quick *et al.*, 1992). However, this is not always the case. In grapevines, a complete recovery of the maximum  $A_N$  occurred after just one night upon irrigation, even though previous  $g_s$  was below  $0.1 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$  and some metabolic down-regulation was evidenced by decreased ETR (Flexas *et al.*, 1999). In the same species under severe water stress, however, when  $g_s$  was lower than  $0.05 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ , photosynthesis did not reverse one day after irrigation (Quick *et al.*, 1992). Therefore, under mild to moderate water stress, photosynthetic recovery after re-watering is quite fast, at least in a well-adapted species such as grapevines. However, how rapid is recovery from severe stress? Figure 4 shows a time course for photosynthetic recovery upon irrigation of grapevine plants growing outdoors during summer in the Mediterranean, and subjected to very severe water stress. Clearly, there was about a 60% recovery only one night after irrigation, but it took up to 4 days to reach almost full recovery (prior to water stress imposition,  $A_N$  values were  $11 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , not shown). The extent of recovery of  $A_N$ ,  $g_s$  and ETR was similar (Fig. 4), although this could be species-dependent. These results are similar to those obtained by Marron *et al.* (2002) in two different poplar clones subjected to similar water stress intensity and also presenting very low  $g_s$ . The poplars showed about 50% recovery of  $g_s$  during the first day upon re-watering, and they took 4 more days to achieve full recovery. Intervals of several days (usually less than one week) for full photosynthesis recovery after very low  $g_s$  would also be consistent with the present evidence about the recovery of different metabolic processes. Castrillo & Calcagno (1989), for instance, showed in two cultivars of tomato that Rubisco activity was recovered from 50-60% of controls to 100% 4 days after re-watering. Similarly, Thimmanaik *et al.* (2002) showed an almost complete recovery of several Calvin cycle enzymes only 2 days after re-watering, even when their activities were strongly depressed (see Fig. 2). However, these authors considered as 'severe water

stress' a situation where  $g_s$  was still higher than  $0.2 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ . Finally, Mittler *et al.* (2001) have described that photosynthetic metabolism can be recovered within 24 h in *Retama raetam*, a desert plant that maintains very low levels of most photosynthetic and photosynthesis-related enzymes during the summer, which has been called a 'dormant' canopy. The reason for such a rapid capacity to recover seems to be due to the continuous presence during the summer of the RNA transcripts encoding for the abolished proteins (Mittler *et al.*, 2001).

By contrast, we observed in field-grown *Rhamnus ludovici-salvatoris* plants, an evergreen sclerophyll shrub endemic of the Balearic Islands, that almost no photosynthetic recovery occurred 1 wk after frequent irrigation in mid summer, even when leaf RWC was fully recovered (J Gulias and J Flexas, unpublished). Frequently, field-grown woody plants from arid and semi-arid areas do not improve

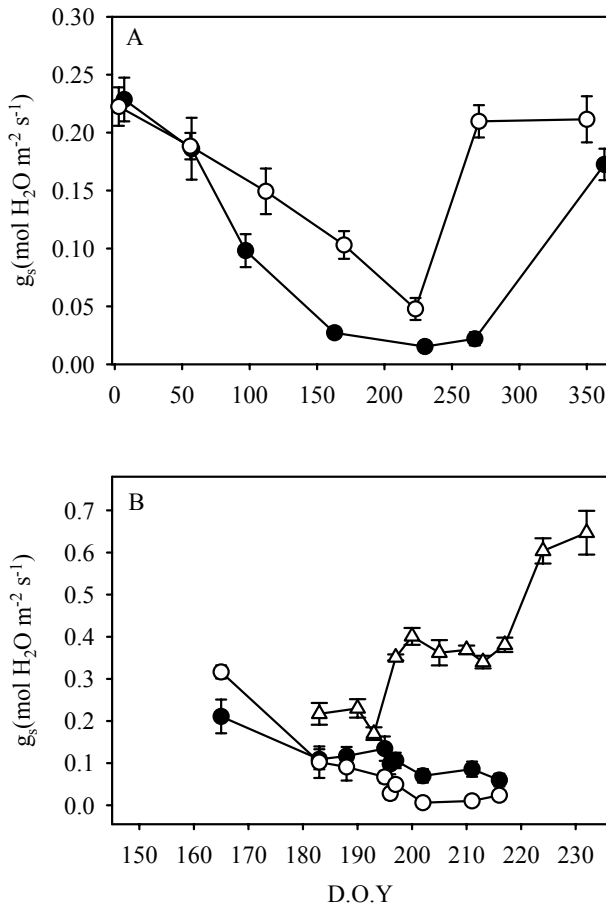


Fig. 3. Evolution of  $g_s$  along the year in different species and studies: (A) *Quercus ilex* L. in two different localities in Mallorca, Binifaldó (open circles) and Puigpunyent (closed circles) during 2000 (J Gulias, unpublished results). (B) Two cultivars of field grown grapevine (*Vitis vinifera* L.): Tempranillo during summer 1999 (open circles) and summer 2002 (open triangles), and Manto Negro during summer 1999 (closed circles) (J Flexas, unpublished results).

photosynthesis during summer even when supplemental water is added (Nogués & Alegre, 2002), and some have been shown to require several weeks or months for complete photosynthetic recovery after re-hydration (Harley *et al.*, 1987; Munné-Bosch & Alegre, 2000).

In addition, van Rensburg & Krüger (1993), using four different tobacco cultivars, clearly showed that the time required for full recovery was strongly dependent upon the different water stress-tolerance capacity. Moreover, Marron *et al.* (2002) stated that

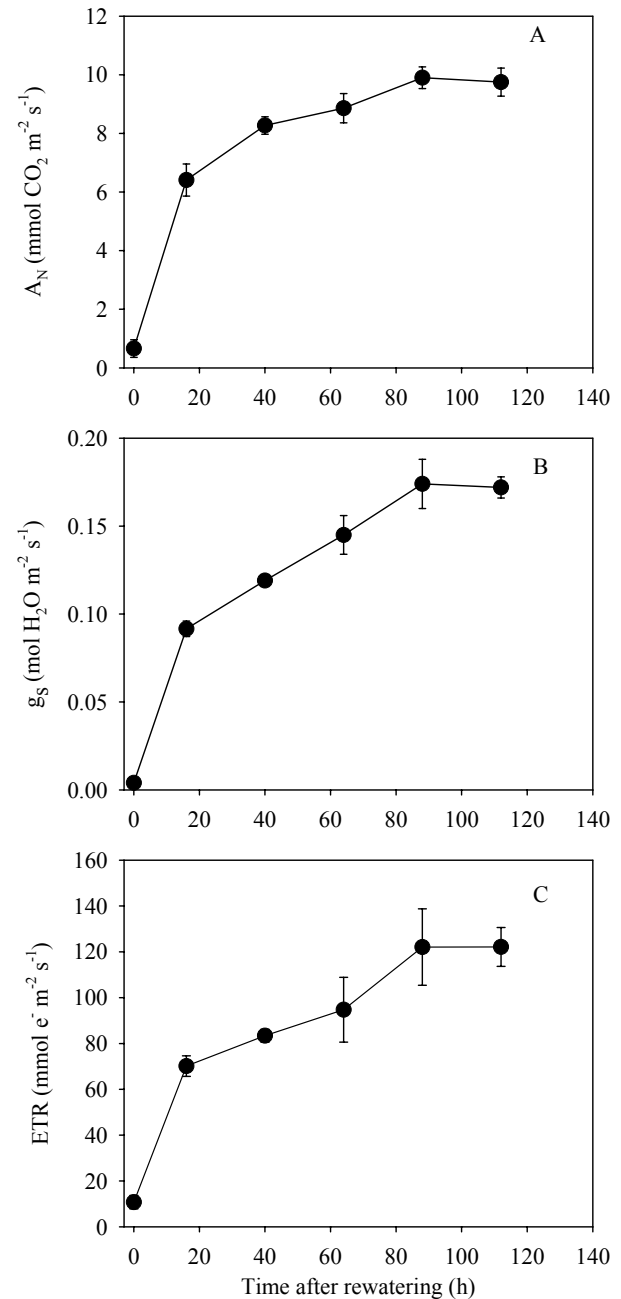


Fig. 4. Effect of re-watering on photosynthetic parameters,  $A_N$  (A),  $g_s$  (B) and ETR (C) in grapevine plants (*Vitis vinifera* L.) growing under outdoors conditions during summer 1999 in Mallorca. Results are means  $\pm$  SE of six replicates.

in their study the recovery was only observed in young but fully matured leaves, while older leaves did not recover upon re-watering and entered a senescence and oxidative process. Also, Giardi *et al.* (1996) showed that photosystem II structure and function recovered by only 50% two days after irrigation in severely water stress stressed *Pisum* plants, but no recovery was observed when water stress was accompanied by a high light treatment. Unfortunately, these authors did not provide  $g_s$  values. Finally, it has also been shown that recovery is quicker and higher after a first drying cycle than after subsequent cycles (Larcher *et al.*, 1981; Flexas *et al.*, 1999).

Therefore, recovery may depend not only on the severity of stress and the species analysed, but also on a complex interaction with plant or leaf age, light intensity, number of consecutive drying cycles, and many other possible factors. In summary, current knowledge about the implication of stomatal and non-stomatal limitations in recovery of photosynthesis upon re-watering after different water stress intensities imposed in different plants and conditions is extremely scarce. This knowledge would be of importance for the development of irrigation programmes that maximise water use efficiency, as well as for improving the accuracy of the predictions of ecosystem productivity from climate data.

### Concluding Remarks and Future Prospect

In summary, using light-saturated, daily maximum stomatal conductance ( $g_s$ ) as the indicator for the intensity of water stress reveals a pattern of photosynthetic response to water stress that is common to all  $C_3$  species analysed. Analysing different components of photosynthesis along a  $g_s$  range suggests that photosynthetic metabolism is substantially resistant to water stress until  $g_s$  is below 0.10–0.15 mol  $H_2O$   $m^{-2}$   $s^{-1}$ . Nevertheless, these low  $g_s$  values are usually observed under natural conditions in semi-arid areas, suggesting that non-stomatal limitations are an important component of over-season photosynthetic inhibition in these areas at the global scale. The  $g_s$  threshold usually coincides with a changing pattern of variation of intrinsic water use efficiency ( $A_N/g_s$ ) in response to water stress, so that maximum  $A_N/g_s$  is achieved at the limit between diffusional and metabolic limitations to photosynthesis (Fig. 5). This should be taken into account when developing water use efficient irrigation programmes.

Besides these findings, a number of important gaps of knowledge have been highlighted in the present review. Here we propose what, to our point of view, should be the research priorities in order to advance in the understanding of photosynthesis response to

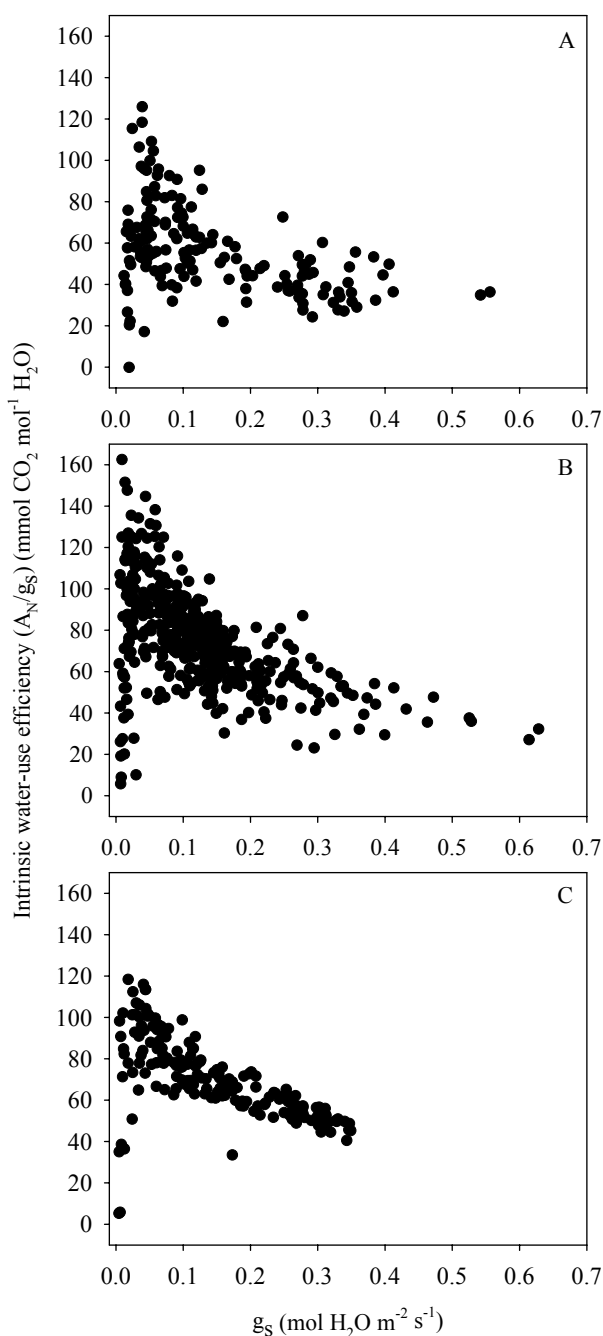


Fig. 5. Relationship between the intrinsic water use efficiency ( $A_N/g_s$ ) and the stomatal conductance ( $g_s$ ) in three different studies: (A) Study including endemic (*Hypericum balearicum* L., *Lysimachia minoricensis* J. J. Rodr., *Phlomis italica* L.) and non-endemic (*Cistus albidus* L., *Mentha aquatica* L., *Pistacia lentiscus* L.) species from the Balearic Islands by J Galmés (unpublished results). (B) Study of 13 Mediterranean sclerophyll woody species: *Arbutus unedo* L., *Cistus albidus* L., *Cistus monspeliensis* L., *Cistus salvifolius* L., *Hypericum balearicum* L., *Cneorum tricoccon* L., *Olea europaea* L., *Phyllirea latifolia* L., *Pistacia lentiscus* L., *Quercus coccifera* L., *Quercus ilex* L., *Rhamnus alaternus* L., *Rhamnus ludovici-salvatoris* R. Chodat, by J Gulías (unpublished results). (C) Study in *Vitis vinifera* L. plants by J Flexas (unpublished results).

water stress and irrigation scheduling:

1. The variations along a g<sub>s</sub> gradient during water stress of many metabolic components affecting photosynthesis are presently unknown, and should be evaluated. Among these, the most important would be: (1) all the enzymes involved in regeneration of RuBP in the Calvin cycle; (2) water stress-induced oxidative stress and protective antioxidant responses; (3) photophosphorylation; and (4) agents possibly involved in CO<sub>2</sub> diffusion inside leaves (carbonic anhydrase, aquaporins). Similarly, the responses of plant respiration and respiratory components should also be evaluated, provided that respiration is, together with photosynthesis, the other important component of plant production.

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

3. From a more practical point of view, the improved physiological knowledge should induce the development of physiologically based indicators to improve irrigation programs in semi-arid areas, which should be tested extensively under real crop conditions. In this sense, scaling up from physiological to more agronomical programs would be required.

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