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Chloroplast volume: cell water potential relationships and acclimation of photosynthesis to leaf water deficits

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Abstract

Studies were undertaken to determine if there is an association between nonstomatally-mediated acclimation of photosynthesis to low water potential (Ψw) and the maintenance of chloroplast volume during water stress. Spinach plants either kept well watered throughout their growth (non-acclimated), or subjected to water stress such that leaf Ψw dropped to -1.5 megapascals (MPa) and then were rewatered (acclimated) were subjected to drought episodes. During these stress periods, photosynthesis was maintained to a greater extent in acclimated plants as compared to non-acclimated plants at Ψw below -1 MPa.

Estimates of internal leaf $[CO_2]$ suggested that photosynthetic acclimation to low Ψw was not primarily due to altered stomatal response. As Ψw dropped from initial values, a decline in steady state levels of ribulose 1,5-bisphosphate (RuBP) occurred in both non-acclimated and acclimated plants. RuBP decline was less severe in acclimated plants.

Low Ψw effects on chloroplast volume in non-acclimated and acclimated plants were estimated by measuring the volume of intact chloroplasts isolated from plants in solutions which were made isotonic to declining leaf osmotic potential during the drought episodes. Chloroplast volume was maintained to a greater extent at low Ψw in acclimated, as compared with non-acclimated plants. Although substantial osmotic adjustment occurred in both non-acclimated and acclimated plants, the extent of osmotic adjustment was the same. These data were interpreted as supporting the hypothesis that cellular-level acclimation to low Ψw is associated with chloroplast volume maintenance, and this physiological acclimation is correlated with enhanced photosynthetic capacity of the leaf at low Ψw.

Abbreviations: internal leaf CO_2 concentration – $[CO_2]_i$; osmotic potential – Ψs; relative water content – RWC; ribulose 1,5-bisphosphate – RuBP; water potential – Ψw

Introduction

Prior exposure to water deficits results in acclimation to low Ψw in a range of crops species (Hanson and Hitz 1982). Acclimation to water stress can facilitate relatively greater photosynthesis at similar low water potentials during subsequent exposure of plants to periods of water deficit. In addition to altered stomatal response to low Ψw (Hanson and Hitz 1982), other physiological factors have been shown to contribute to the acclimation of photosynthesis to water stress in situ (Matthews and Boyer 1984). These 'cellular-level' acclimation mechanisms are sig-

nificant in that they lead to altered transpiration ratio in stressed plants, as carbon uptake is enhanced at any given stomatal conductance and transpiration rate (Jones and Rawson 1979). The increased rate of carbon gain per unit water lost facilitated by this type of adaptation to stress can be considered as leading to some degree of metabolic 'drought resistance'.

It is clear that inhibited chloroplast metabolism contributes to the overall reduction of photosynthesis in water stressed plants (Matthews and Boyer 1984). Cellular-level acclimation mechanisms have been shown to involve alterations in the chloroplast which lead to the maintenance of physiological function in the plastid at low leaf Ψw (Kaiser 1987, Rao et al. 1987). Previous work in this laboratory has focused on characterizing the specific physiological mechanisms associated with cellular-level acclimation to low Ψw. It has been shown that in corn (Berkowitz and Kroll 1988) and wheat (Sen Gupta and Berkowitz 1987), non-stomatally-controlled photosynthesis of leaf tissue prepared from water stressed plants is less sensitive to instantaneous exposure to low Ψw in vitro than tissue from control plants. This work indicated that cellular-level acclimation of photosynthesis to low Ψw was associated with an altered protoplast volume:Ψw relationship. As suggested in earlier work (Kaiser 1982), it was speculated in these studies that the chloroplast stromal volume tracked protoplast volume changes in the dehydrating leaf tissue. In other studies, the effect of low Ψw in vitro on photosynthesis and stromal volume of chloroplasts isolated from well-watered and water stressed plants, along with solute profiles of chloroplasts isolated from water stressed plants, suggested that the chloroplast may be capable of undergoing substantial osmotic adjustment (and resultant volume maintenance) in response to plant water deficits (Berkowitz 1987, Sen Gupta and Berkowitz 1988). Taken together, this work supports the hypothesis that low cell Ψw may not be directly injurious to the chloroplast's ability to assimilate CO_2 in water stressed leaves. Rather, it can be hypothesized that protoplast and/or chloroplast volume reduction in dehydrating leaf tissue is the biophysical mechanism which is associated with inhibited metabolism. In leaf tissue acclimated to low Ψw, maintenance of CO_2 assimilation by the chloroplast at low water potentials may, therefore, be facilitated by volume maintenance.

This hypothesis was addressed in the work described in this report. Experiments were undertaken to estimate the chloroplast volume changes which occurred as Ψw declined in leaves of spinach plants exposed to drought episodes. These measurements were made on control and stress acclimated plants. Photosynthesis was also monitored in these plants during the drought episodes. The objective of this work was to determine if cellular-level acclimation of photosynthesis is associated with the maintenance of chloroplast volume in leaves of water stressed spinach plants.

Materials and methods

Plant material. Spinach seeds (*Spinacia oleracea* var. 'Melody') were sown (3/pot) in 4.3 dm^3 pots containing 1:1 peat:vermiculite placed in a growth chamber. Plants were irrigated with standard commercial (Peter's 'Geranium Special') fertilizer twice a week, and once with just water. The conditions in the growth chamber were 22°C and 50% RH constant, with an 11 h light period (250 $\mu E\ m^{-2}\ s^{-1}$). Plants were used 6 to 8 weeks after sowing. Fully expanded non-senescing leaves were used for all experiments. For the measurements of water relations parameters, photosynthesis, and RuBP as described below, the leaves used as treatment replicates were taken from three different plants growing in the same pot. We have found that soil Ψw variability in different pots causes more variability in our physiological measurements than the assay techniques themselves. Sampling three plants in one pot reduced considerably the variation in our measurements. After leaves were removed from a pot for a set of measurements on a given day, the plants in that pot were thrown away.

Drought episodes were initiated by withholding irrigation water from pots. Plants were considered 'non-acclimated' if they were kept well watered until the initiation of a water stress cycle during which data were recorded. Plants were considered acclimated after they had been subjected to an initial water stress cycle when leaf

Ψw was allowed to decline to −1.5 MPa. After this initial stress cycle, the pots were irrigated to runoff for 3 d. A second stress cycle was then initiated during which data were recorded on these acclimated plants. The water stress cycles typically lasted 8–12 d. Due to the time it took to undertake the various physiological measurements, and the destructive nature of many of the measurements, the data were recorded over a number of stress cycles on several sets of plants. Chloroplast volume, RWC, Ψs, and one of the two sets of presented photosynthesis data were recorded on the same set of plants. Leaf RuBP was measured on a second set of plants. Another set (i.e., measurements for acclimated and non-acclimated plants) of photosynthesis measurements was recorded on a third set of plants. Leaf Ψw was measured periodically on the sets of plants exposed to the different stress cycles. The data from the different stress cycles were normalized by expressing the results on a leaf Ψw basis.

Water relations. During the water stress cycles, leaf water status was monitored by taking measurements 5 to 6 h into the light period. Leaf Ψw was measured with a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, California). Leaves were enclosed in plastic wrap, and the pressure chamber walls were lined with wet paper towels during measurements. Leaf Ψs was determined by measuring the Ψw of frozen and thawed leaf discs (two/leaf) with a Wescor (Logan, Utah) HR33T microvoltmeter (operating in the hygrometric mode) and C-52 leaf chambers. RWC was ascertained by measuring the fresh, rehydrated (minimum of 4 h floating on distilled water at 4°C) and dry (80°C for a minimum of 2 d) weights of five 8-mm diameter discs cut from a leaf. All measurements were taken on the same leaf, and a minimum of three leaves were used as treatment replicates for all water status measurements. The extent of leaf osmotic adjustment during the water stress cycles was evaluated by examining double logarithmic plots of leaf RWC and Ψs as described previously (Sen Gupta and Berkowitz 1987).

Photosynthesis. Net photosynthesis and concomitant transpiration of attached leaves (three replicates/treatment) were monitored during water stress cycles using an ADC (P.K. Morgan Instr., Andover, MA) infrared gas analysis system. Saturating light was provided by a sodium vapor lamp (1600 $\mu E\, m^{-2} s^{-1}$) and air at 348 $\mu L/L$ [CO_2] was supplied to the illuminated leaf cuvette from compressed air cylinders. Immersion of the aluminum heat exchanger of the leaf cuvette in a water bath kept the cuvette and leaf temperature between 21 and 25°C during all measurements. Net photosynthesis and $[CO_2]_i$ were calculated using equations developed by von Caemmerer and Farquhar (1981).

Any current gas exchange studies which purport to evaluate the relative contributions of stomatal and nonstomatal effects of water stress on the inhibition of photosynthesis in situ using the analysis presented by Farquhar and Sharkey (1982) should acknowledge the important study recently published by Sharkey and Seemann (1989). They indicated that under water stress, leaves of *Phaseolus vulgaris* plants demonstrated 'patchy' or heterogenous stomatal closure. This phenomenon would lead to incorrect assumptions regarding the relationship between photosynthetic capacity and $[CO_2]_i$ in water stressed leaves. However, as part of an extensive study of patchy stomatal closure under water stress (D. Gunasekera, G. Berkowitz, manuscript in preparation) we have recently confirmed the characteristic patchy stomatal closure pattern in *Phaseolus vulgaris* plants found by Sharkey and Seemann, but in stark contrast, found homologous conductance patterns in spinach leaves at leaf water potentials down to −1.5 MPa. Therefore, we have confidence in the interpretation of our gas exchange data as presented in this report.

Chloroplast stromal volume. During water stress cycles, intact chloroplasts were isolated from leaves as described previously (Sen Gupta and Berkowitz 1988). Leaf Ψs was measured on these days 3 h into the light period, and chloroplasts were isolated 5 h into the light period (i.e., as soon as Ψs was measured and media prepared). In addition to sorbitol, the isolation media contained 50 mM Hepes–NaOH (pH 6.8), 2 mM Na_2EDTA, 1 mM $MgCl_2$, and 1 mM $MnCl_2$. The sorbitol concentration of the isola-

tion, resuspension, and assay media was adjusted so that the solution Ψs matched leaf Ψs. Approximately 5 g of deribbed leaves were homogenized twice for 3 s at 25 000 RPM in 50 mL of isolation medium. The homogenate was filtered through four layers of cheesecloth and two layers of Miracloth (Calbiochem), centrifuged at 750 g (SS34 rotor in RC5B Sorvall centrifuge) for 1 min, and resuspended in 15 mL isolation medium. This resuspension was layered onto 15 mL isolation medium which contained 40% (v/v) Percoll and 0.1% BSA, and then centrifuged at 2500 g for 1 min in an HB4 swinging bucket rotor. The intact (≥90%) chloroplasts pelleting through the Percoll cushion were resuspended in approximately 1.8 mL isolation medium.

The method of Heldt (1980) was used to ascertain the stromal volume of chloroplasts. Aliquots of the resuspended chloroplasts were mixed with 15 μCi/mL ^3H_2O and incubated for 5 min, and then 10 μCi/mL [^{14}C]-sorbitol was added (the [^{14}C]-sorbitol stock solution contained sufficient ^3H_2O to maintain a constant ^{3}H concentration). The solution Ψs, buffer, and ion concentrations were maintained constant throughout these steps. After [^{14}C]-sorbitol addition, 200 μL of the mixture (containing 40 μg Chl) was layered into 400 μL microfuge tubes which had 100 μL of 550 Dow Corning silicone oil layered on top of 20 μL of 14% $HClO_4$. The 550 oil had a specific density of 1.07 and viscosity of 125 centistokes. Tubes were then centrifuged in a Beckman Microfuge B for 30 s. For each volume measurement four microfuge tubes were used as replicates. After centrifugation, aliquots of the incubation media above the oil layer were sampled for radioactivity. The tubes were frozen and then cut in the oil layer. The microfuge tube tips containing $HClO_4$ with pelleted chloroplasts were then placed in 1.5 mL microfuge tubes with 380 μL H_2O, vortexed to resuspend the pellet and were centrifuged to pellet any oil carryover. Aliquots of the aqueous supernatant were sampled for radioactivity using a Beckman 3801 liquid scintillation counter. For calculation of chloroplast volumes, the stromal volume was calculated as the difference between the ^3H_2O (total) and [^{14}C]-sorbitol (extra-stromal) volumes associated with the chloroplast pellet. Chloroplast volume was corrected for percentage of plastids pelleting through the silicone oil, and expressed per unit Chl in the following manner. For each volume measurement, four additional microfuge tubes were prepared with 20 μL of 40% Percoll replacing the $HClO_4$ layer. The tubes were used in a fashion similar to the tubes used for volume measurements except that no radioactivity was added to the chloroplast suspension. These tubes were carefully cut in the Percoll layer to avoid oil carryover. The chloroplasts were resuspended in 1 mL of 80% acetone and, after centrifugation, Chl was read using the method of Arnon (1949). Previous studies (Sen Gupta and Berkowitz 1988) have indicated that Chl concentration/cell does not change substantially in spinach plants subjected to the severity of stress used in these experiments, and have validated the expression of chloroplast volume on this basis.

RuBP measurements. During the water stress cycles, leaves (three replications/treatment) were sampled for RuBP levels. The objective of this work was to ascertain leaf RuBP levels during steady-state photosynthesis under conditions identical to those used for the photosynthesis measurements. However, the ADC leaf chamber used for gas exchange studies allows for illumination of only a portion of a leaf. Because the leaves had to be rapidly frozen in liquid N_2 to accurately estimate leaf RuBP, it was impossible to separate the illuminated section from the rest of the leaf. Therefore, a specially-constructed leaf chamber was used for these studies. While still attached to the plant, leaves were sealed in a Plexiglass cuvette (20 × 15 × 10 cm), and illuminated at 1 600 μE m^{-2} s^{-1} by a 500 w incandescent flood light. Air at 800 mL/min was supplied to the cuvette. The Plexiglass chamber was constructed with an internal fan, and an aluminum-finned heat exchanger which had cool water circulating through it, allowing for the air in the chamber to be maintained at 22°C throughout the illumination period. Control experiments indicated that leaves of the spinach plants used in these experiments attained maximal rates of photosynthesis after 10–20 min, and maintained these steady-state rates for at least 30 min after the maximal rate had been ob-

tained. Also, under similar assay conditions, Perchorowicz and Jensen (1983) found RuBP levels in wheat leaves to be maximal, and maintained constant between 15 and 45 min. Therefore, spinach leaves were sampled for RuBP measurement after 30 min of illumination in the leaf chamber. The leaves were placed in liquid N_2 as quickly as possible (timed at 0.4 s) after removal from the leaf chamber. This point is critical to the validity of this assay. Other researchers (Sharkey and Seeman 1989) use a freeze-clamp procedure to instantaneously halt enzymic metabolism of RuBP in the leaf after removal from illumination. However, our brief post-illumination period likely did not influence our measurements. The standard error of our measurements was extremely small (compare with measurements generated using the freeze-clamp as shown in Sharkey and Seeman 1989), and when our procedures were used to determine RuBP in wheat leaves, they yielded values (310 ± 27 nmol mg Chl^{-1}) identical to those previously reported (Perchorowicz and Jensen 1983).

After immersion in liquid N_2, leaves were ground and Chl extracted twice with 80% acetone at 0°C. Chl extraction involved resuspension in acetone, and centrifugation for 5 min at 4000 g. The supernatant was discarded, and the pellet was resuspended in 1 mL 5% $HClO_4$ along with 10 mg BSA, extracted in a glass homogenizer, and centrifuged at 4000 g for 5 min. The supernatant was removed and saved, and the pellet extracted twice more in 5% $HClO_4$. The three $HClO_4$ supernatants from each leaf extract were pooled, and stored at −85°C prior to RuBP analysis using the protocol described by Perchorowicz and Jensen (1983), except that after extract neutralization with MOPS and KOH, the solution was centrifuged at 4000 g for 10 min to precipitate the $KClO_4$. Also, 4×10^{-3} units of commercial purified RuBP carboxylase were used in each assay instead of 0.1 mg of enzyme extracted from tobacco.

General. Unless otherwise noted, all reagents were purchased from Sigma (St. Louis, MO). Silicone oils were from William Nye Co., New Bedford, MA. ^3H_2O and [^{14}C]-sorbitol were obtained from ICN Radiochemicals, Chicago, IL. Data are presented in all figures as means ± the standard error. In many cases, one or both sets of error bars for a given data point are not visible because they are covered by the symbol representing the mean value for that treatment.

Results

Water deficit effects on photosynthesis. During an in situ drought episode, photosynthesis was inhibited as leaf Ψw declined in both non-acclimated and acclimated spinach plants (Fig. 1). However, at water potentials below −1 MPa, photosynthesis was maintained to a greater extent in acclimated, as compared to non-acclimated plants. The difference in photosynthesis between acclimated and non-acclimated plants at low Ψw was substantial. For example, at leaf Ψw of −1.2 MPa, photosynthesis in non-acclimated and acclimated plants was 10.2 and 18.3 μmol $CO_2\ m^{-2}\ s^{-1}$, respectively. Acclimation allowed for an 80% stimulation in photosynthesis at this Ψw. This experiment was repeated on a different set of plants; the Ψw:photosynthesis profile of non-acclimated and acclimated plants was essentially similar to that shown in Fig. 1. For example, at a leaf Ψw of −1.5 MPa, the photosynthetic rate in non-acclimated plants was inhibited by 68% from that found under well-watered conditions in the second experiment. At −1.5 MPa leaf Ψw, photosynthesis of acclimated plants was inhibited by only 38.5% from optimal rates with the second set of plants.

Calculations of $[CO_2]_i$ at high and low Ψw for the plants used in the two photosynthesis experiments are shown in Table 1. These data indicate that non-stomatal factors contributed to the acclimation of photosynthesis to low Ψw. For the plants used in the experiment shown in Fig. 1, at high Ψw, $[CO_2]_i$ was similar in non-acclimated and acclimated plants (Table 1, Experiment #1). At low Ψw, $[CO_2]_i$ was significantly higher in non-acclimated plants (Table 1), even though photosynthesis was 75% higher in acclimated plants (Fig. 1). In the second experiment, some reduction in $[CO_2]_i$ was noted in both non-acclimated and acclimated plants at the end of the drought episode (Table 1). Significantly, however, $[CO_2]_i$ was similar (210 μL/L) in both acclimated and non-acclimated plants when leaf

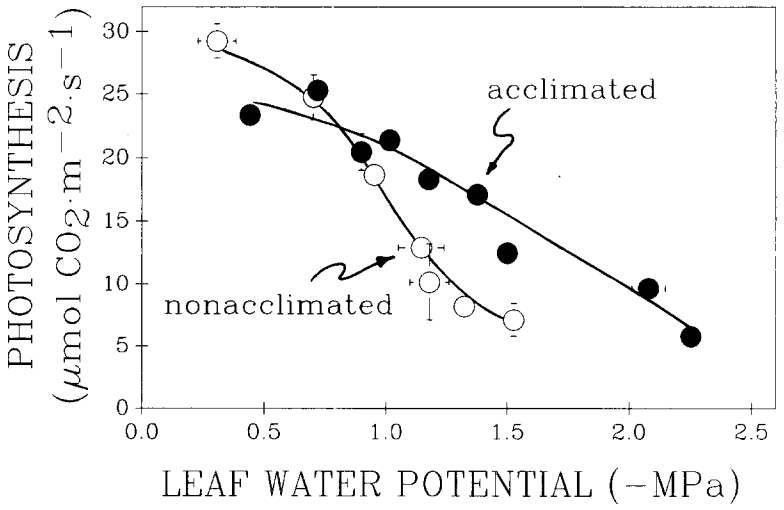

Fig. 1. The effect of decreasing leaf Ψw during an in situ drought episode on photosynthesis of non-acclimated, and acclimated plants. In this, and all other figures except Fig. 6, data are presented as means ± S.E. for both the ordinate and abscissa values. In many cases, the S.E. bars are covered by the symbols.

Ψw declined to −1.5 MPa. However, photosynthesis was much less inhibited in the acclimated plants subjected to stress (as noted previously in the text). The similarity of $[CO_2]_i$ under stress indicates that also in this second experiment, non-stomatal factors contributed to the maintenance of photosynthesis at low Ψw in acclimated plants. As discussed in the Materials and Methods section, preliminary analysis of the extent of heterogenous stomatal conductance in water stressed spinach plants indicates that 'patchy' stomatal closure does not occur (data not shown). Therefore, we believe that our analysis of the relationship between photosynthesis and $[CO_2]_i$ in spinach plants at low Ψw as shown in Fig. 1 and Table 1 is valid.

Table 1. Internal leaf CO_2 concentrations calculated for the plants used in the experiment shown in Fig. 1 (listed here as Experiment #1) and for plants used in a second, similar experiment (Experiment #2). For the non-acclimated and acclimated plants used in each experiment, $[CO_2]_i$ values are given only for well watered plants prior to the initiation of the drought episode, and for plants stressed to a leaf Ψw of approximately −1.5 MPa. $[CO_2]_i$ values are presented as the mean ($n = 3$) ± the standard error. Data in parentheses next to each $[CO_2]_i$ are the mean leaf Ψw expressed as −MPa ($n = 3$) for that treatment

	$[CO_2]_i$ (μL/L)	
	Non-acclimated	Acclimated
Expt. #1		
Prior to stress	240 ± 4 (0.31)	256 ± 4 (0.44)
Stressed	302 ± 6 (1.53)	260 ± 12 (1.50)
Expt. #2		
Prior to stress	246 ± 4 (0.33)	244 ± 6 (0.51)
Stressed	211 ± 15 (1.50)	210 ± 11 (1.53)

RuBP levels were monitored in non-acclimated and acclimated plants as leaf Ψw declined during drought episodes (Fig. 2). Trends in the data suggest that as leaf Ψw declined, acclimated plants maintained greater steady-state [RuBP] than non-acclimated plants. The maintenance of higher [RuBP] during steady-state photosynthesis can be due to one of two possibilities: either the rate of RuBP removal (i.e., RuBP carboxylation, or photosynthetic rate) is decreased; or the rate of RuBP regeneration from photosynthetic cycle intermediates is increased. From the data shown in Fig. 1, it is clear that as leaf Ψw declines, photosynthesis is higher, not lower, in acclimated as compared to non-acclimated plants. Therefore, it can be speculated that at low Ψw, increased steady-state [RuBP] in the chloroplast of acclimated plants could possibly be due to the acclimation of chloroplast metabolism to low Ψw, such that the rate of RuBP regeneration is maintained to a greater extent than occurs in non-acclimated plants.

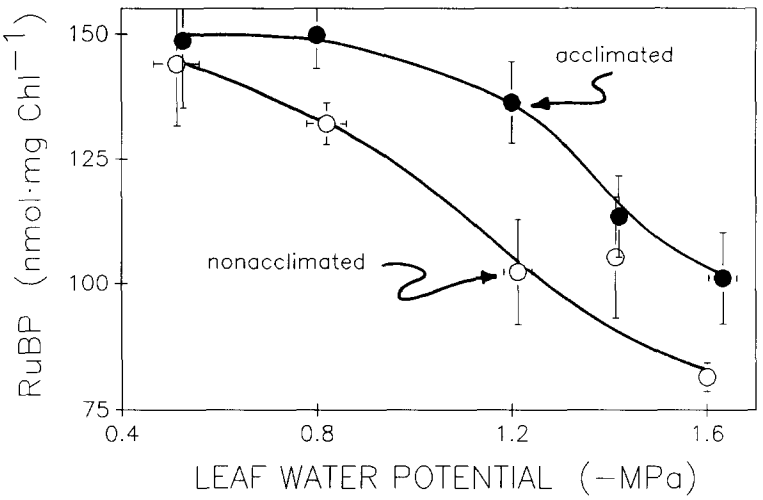

Fig. 2. The effect of decreasing leaf Ψw on leaf RuBP levels in non-acclimated and acclimated plants.

The Ψw : chloroplast volume relationship and acclimation to stress. Estimation of the effect of leaf Ψw decline on in situ chloroplast volume was made by measuring the volume of intact chloroplasts isolated from plants exposed to drought episodes. The chloroplasts were isolated, and their volume measured, in media which was made isotonic to the leaf Ψs. Although the volume measurements were not made on the chloroplast in the intact leaf, the procedure of isolating plastids in isotonic medium should theoretically prevent shrinking or swelling during the isolation procedure (Nobel 1983).

As shown in Fig. 3, low Ψw caused less of a decline in chloroplast volume in acclimated, as compared with non-acclimated plants. Volume was maintained essentially constant in both acclimated and non-acclimated plants at leaf Ψw greater than −0.8 MPa. As leaf Ψw declined below this point, chloroplast volume was apparently reduced to a substantial extent in the leaves of non-acclimated plants. Especially at water

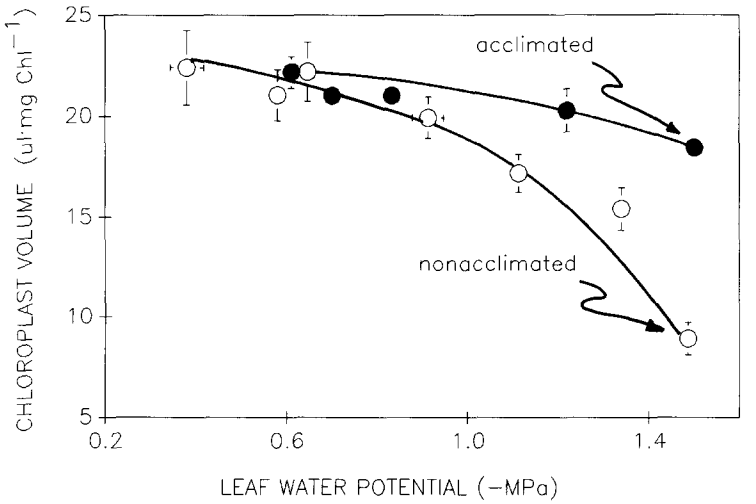

Fig. 3. The relationship chloroplast volume and declining leaf Ψw during in situ drought episode in non-acclimated and acclimated plants.

potentials below −1.2 MPa, chloroplast volume was maintained to a much greater extent in acclimated, as compared with non-acclimated plants. It should be noted that the method used to isolate intact chloroplasts from spinach leaves necessarily involves the recovery of a small proportion of the total amount of chloroplasts in the parent leaf tissue. The volume measurement, therefore, may not be ascertained on a sample of chloroplasts which correctly represents the total chloroplast population of the leaf. However, we believe that this possible technical problem with the assay procedure did not influence the results shown in Fig. 3. The volume of the chloroplasts in the leaves of acclimated plants had already been reduced during the initial stress cycle. However, the measurement technique indicated that upon rewatering, their volume returned to the same absolute value as was found in unstressed plants (Fig. 3). The data in Fig. 3 also indicate that as leaf Ψw dropped in acclimated and non-acclimated plants, measurements demonstrated the maintenance of a constant volume initially, and then a progressive drop in the absolute chloroplast volume.

Previous research (Flower and Ludlow 1986, Sen Gupta et al. 1989) has indicated that alteration in the RWC:Ψw relationship can result in acclimation of photosynthesis to low Ψw. It was hypothesized that the chloroplast volume maintenance at low Ψw which occurred in acclimated plants may be due to an altered Ψw:RWC relationship, and possibly not be associated with a specifically chloroplast-localized acclimation mechanism. The analysis presented in Fig. 4 discounts this possibility. When the chloroplast volume during the stress cycles was plotted as a function of declining RWC, there still appeared to be a substantial difference in the extent of volume maintenance occurring in acclimated, as compared with non-acclimated plants.

Previous research (Santakumari and Berkowitz 1990) has indicated that water stress-induced leaf osmotic adjustment can alter the leaf Ψw:symplast volume relationship in crop plants. It was hypothesized, therefore, that the difference in chloroplast volume maintenance at low Ψw between acclimated and non-acclimated plants could be due to differential extent of osmotic adjustment which occurred in the cells of leaves during the imposed drought episodes. Extent of osmotic adjustment which occurred in the spinach plants during the drought episodes was evaluated using the analysis shown in Fig. 5. As described by Morgan (1980) Ψs at partial cell hydration follows the Van't Hoff relationship: in a double logarithmic plot, Ψs will change linearly with RWC change in the absence of osmotic adjustment. Osmotic adjustment (i.e., net solute accumulation and/or production in leaf cells) during a water stress cycle will be evidenced by deviations from the predicted linear relationship

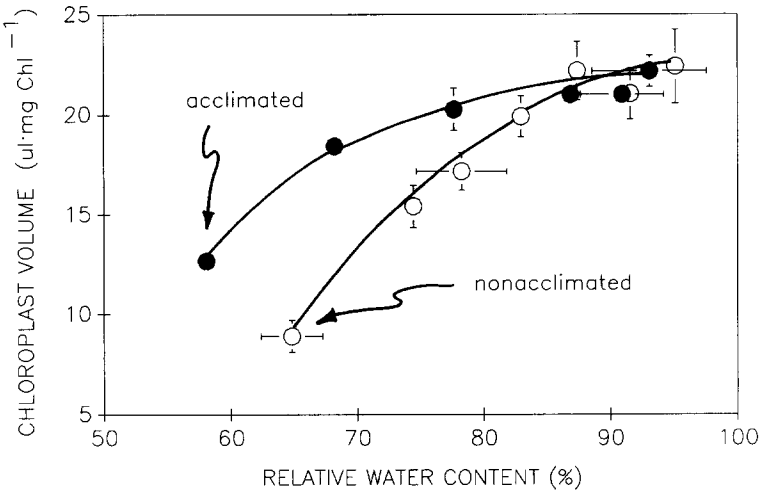

Fig. 4. Chloroplast volume in leaves of non-acclimated and acclimated plants exposed to in situ water stress cycles plotted as a function of declining RWC during the drought episode.

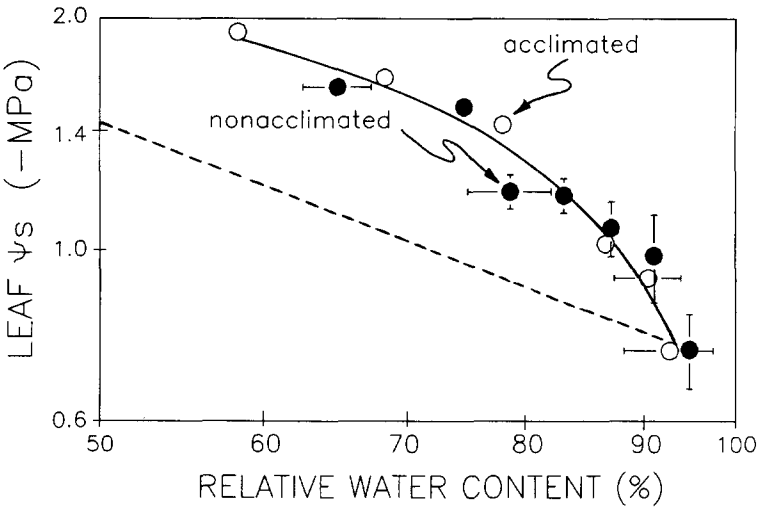

Fig. 5. Evaluation of degree of osmotic adjustment in non-acclimated and acclimated plants during in situ water stress. The relationship between declining RWC and Ψs in stressed plants is presented in a double logarithmic plot. The broken line represents the theoretical decline in Ψs from initial values caused by the solute-concentrating effects of cell dehydration. Data values falling above the line denote a degree of osmotic adjustment.

in such a plot. As shown in Fig. 5, substantial osmotic adjustment occurred in both non-acclimated and acclimated plants as RWC declined during the drought episodes. However, the extent of osmotic adjustment was identical in both non-acclimated and acclimated plants. The extent of osmotic adjustment which occurred during the stress cycles appears biphasic (Fig. 5). During initial RWC decline (down to 80%), the leaf tissue displayed extensive osmotic adjustment. Below 80% RWC, the slope of the Ψw:RWC relationship paralleled the slope of the broken line in Fig. 5; indicating an absence of further osmotic adjustment during the latter portion of the stress cycles. From the data presented in Fig. 5 it can be concluded that the difference in chloroplast volume maintenance at low Ψw between acclimated and non-acclimated plants (Fig. 3) could not be due to differences in the extent of bulk leaf osmotic adjustment.

Discussion

Data presented in this report document the differential response of photosynthesis to low Ψw in control and stress adapted plants. Estimations of $[CO_2]_i$ indicate that maintenance of higher photosynthetic rates at the same low Ψw in acclimated, as compared to non-acclimated plants was likely due to altered chloroplast response. It should be noted, however, that this assertion does not preclude an altered stomatal response to low Ψw in acclimated plants.

Chloroplast-localized differences were noted in stress adapted plants. The measurement of higher steady-state RuBP levels in acclimated, as compared to non-acclimated plants as Ψw declined suggests that one component of stress acclimation may involve altered chloroplast metabolism during the imposed drought episode. The data presented in this report, however, should not be interpreted as proving a causal relationship between the enhanced capability for RuBP regeneration and higher photosynthetic rates at low Ψw in stress adapted plants. In order to determine whether changes in stromal RuBP would impact photosynthetic rate, the level of RuBP must be expressed as a molar ratio to the available RuBP carboxylase binding sites (Seemann and Sharkey 1986). This was not done in our study. It cannot be determined, therefore, if, and at what point during the drought episodes RuBP level declined below the concentration of carboxylase binding sites and rate-limited photosynthesis.

Although the measurement technique by its nature was only an estimation of in situ stromal

volumes, it appears that chloroplast stromal volume is maintained at low Ψw to a greater extent in acclimated plants. It should be noted that previous work has identified a potential artifact in the dual label technique of chloroplast volume measurement used in these experiments. When isolated chloroplasts are exposed to hypertonic Ψs in vitro, they become transiently permeable to sorbitol, and the calculated stromal volume is likely not correct (Robinson 1985). However, chloroplasts were only incubated in isotonic solutions during the isolation and measurement steps used in our technique. Therefore, we do not believe that this potential artifact is pertinent to our investigation. Although the chloroplast volume measurement technique used in this study was an in vitro assay which only estimated the in situ volume, there is at present no better method developed to estimate the absolute in situ volume of a large sample of chloroplasts. Electron micrographs have an obvious sampling size limitation. Evaluation of the proton NMR spectra of compartmentalized water (McCain and Markley 1985) are limited to plant leaves with uniformly oriented chloroplasts, and yield only qualitative, relative volume measurements.

In previous studies from this laboratory, exhaustive analyses of solute accumulation specifically in the chloroplast of water stressed spinach plants were undertaken (Sen Gupta and Berkowitz 1988). These studies indicated that net solute accumulation can occur specifically in the chloroplast during leaf water deficits. We interpret the data from these previous experiments (Sen Gupta and Berkowitz 1988) as suggesting that enhanced chloroplast osmotic adjustment could be responsible for the greater chloroplast volume maintenance which occurred in acclimated plants at low Ψw (Fig. 3). This speculation seems reasonable in light of the data shown in Fig. 5. Even though osmotic adjustment was evident in non-acclimated and acclimated plants during the imposed drought episodes, the extent of bulk leaf osmotic adjustment was identical in control and stress adapted plants.

The differences in extent of low Ψw-induced chloroplast volume reduction in acclimated and non-acclimated plants (Fig. 3) appear correlated with the differences in photosynthesis evident during the latter part of the drought episodes (Fig. 1). This correlative relationship is more clearly illustrated by the analysis presented in Fig. 6. In this figure, the extent of photosynthetic inhibition which occurred at a given low Ψw

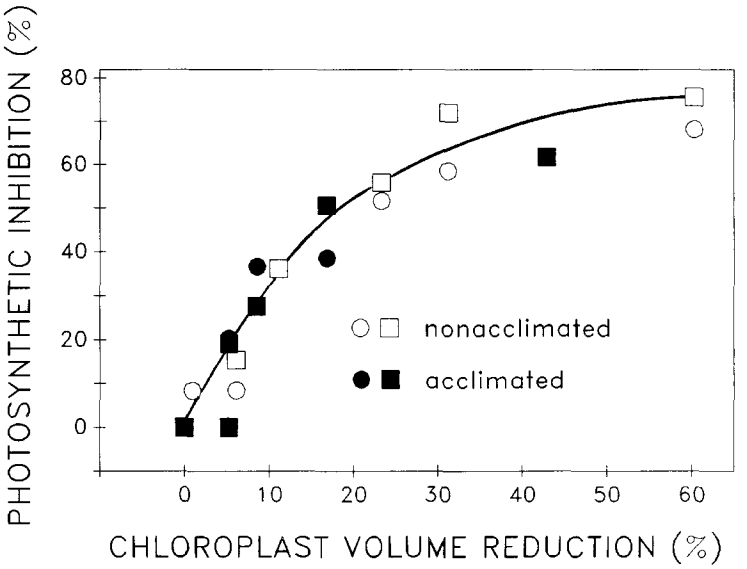

Fig. 6. Inhibition of photosynthesis during in situ drought episodes plotted as a function of change in chloroplast volume. Data are presented for acclimated (closed symbols) and non-acclimated (open symbols) plants. When chloroplast volume and photosynthesis measurements were made at similar water potentials, the values were used in the presentation shown in this figure. The results of two separate photosynthesis experiments (experiment #1 from Table 1 and Fig. 1 (■, □) and experiment #2 from Table 1 (●, ○) are shown.

during the drought episodes from two separate photosynthesis experiments was plotted as a function of the chloroplast volume at that Ψw. It appears that the relationship between extent of low Ψw-induced chloroplast volume reduction, and low Ψw inhibition of photosynthesis is similar in both non-acclimated and acclimated plants. The physiological mechanism of acclimation appears to be a maintenance of the chloroplast stromal volume at low Ψw. Non-acclimated and acclimated plants do not differ in terms of how much inhibition of photosynthesis is associated with a given change in chloroplast volume, but rather how much chloroplast volume reduction occurs as Ψw declines in water stressed plants.

Conclusion

To our knowledge, this report is the first investigation where in situ chloroplast volume changes were estimated in non-acclimated and acclimated plants exposed to drought episodes. Clearly, it appears that in spinach, the chloroplast can acclimate to low cell Ψw by undergoing osmotic adjustment (Sen Gupta and Berkowitz 1988). Chloroplast osmotic adjustment likely allows for the maintenance of stromal volume in the face of declining Ψw to an extent beyond that displayed by the cell as a whole when pre-stressed plants are subjected to subsequent drought episodes. The results presented here suggest that this chloroplast volume maintenance may be an important physiological mechanism which facilitates a degree of 'drought resistance', in that it is correlated with an enhanced ability of leaves to photosynthesize at low Ψw in drought-acclimated plants. In previous studies from several research groups, it has been hypothesized that there may be a fundamental relationship between chloroplast volume changes and photosynthetic inhibition at low Ψw (Berkowitz 1987, Berkowitz and Gibbs 1983, Berkowitz and Kroll 1988, Conroy et al. 1988, Kaiser 1987, Matthews and Boyer 1984, Rao et al. 1987, Santakumari and Berkowitz 1990, Sen Gupta and Berkowitz 1987, Sen Gupta and Berkowitz 1988). This is the first report where photosynthetic acclimation to low Ψw has been associated with maintenance of chloroplast volume by estimating these parameters in control and stress adapted plants subjected to water stress in situ.

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