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Maintenance of Photosynthesis at Low Leaf Water Potential in Wheat[1]

Role of Potassium Status and Irrigation History

Ashima Sen Gupta[2], Gerald A. Berkowitz*, and Paul A. Pier[3]

Department of Horticulture and Forestry, Cook College, Rutgers—The State University of New Jersey, New Brunswick, New Jersey 08903

ABSTRACT

The interaction of low water potential effects on photosynthesis, and leaf K^+ levels in wheat (*Triticum aestivum* L.) plants was studied. Plants were grown at three K^+ fertilization levels; 0.2, 2, and 6 millimolar. With well watered plants, 2 millimolar K^+ supported maximal photosynthetic rates; 0.2 millimolar K^+ was inhibitory, and 6 millimolar K^+ was superoptimal (*i.e.* rates were no greater than at 2 millimolar K^+). Photosynthesis was monitored at high (930 parts per million) and low (330 parts per million) external CO_2 throughout a series of water stress cycles. Plants subjected to one stress cycle were considered nonacclimated; plants subjected to two successive cycles were considered acclimated during the second cycle. Sensitivity of photosynthesis to declining leaf water potential was affected by K^+ status; 6 millimolar K^+ plants were less sensitive, and 0.2 millimolar K^+ plants were more sensitive than 2 millimolar K^+ plants to declining water potential. This occurred with nonacclimated and acclimated plants at both high and low assay CO_2. It was concluded that the K^+ effect on photosynthesis under stress was not mediated by treatment effects on stomatal resistance. Differences between the K^+ treatments were much less pronounced, however, when photosynthesis of nonacclimated and acclimated plants was plotted at a function of declining relative water content during the stress cycles. These results suggest that K^+ effects on the relationship between relative water content and water potential in stressed plants was primarily responsible for the bulk of the K^+-protective effect on photosynthesis in stressed plants. *In vitro* experiments with chloroplasts and protoplasts isolated from 2 millimolar K^+ and 6 millimolar K^+ plants indicated that upon dehydration, K^+ efflux from the chloroplast stroma into the cytoplasm is less pronounced in 6 millimolar K^+ protoplasts.

Previous work in this laboratory (6, 17) has indicated that leaf K^+ level modulates the severity of water stress effects on photosynthesis. Lowering cell K^+ increased the inhibition of nonstomatally controlled photosynthesis in wheat and spinach leaf slices vacuum infiltrated with low Ψ_s[4] solutions. It was also shown with this *in vitro* system that increasing cellular K^+ in wheat leaf tissue beyond that necessary to maintain photosynthesis at optimal levels in well-watered plants (*i.e.* superoptimal under control conditions), stimulated leaf slice photosynthesis under osmotic stress. Results of gas exchange experiments indicated that this effect was also evidenced in whole plants. At the end of a water stress cycle, wheat plants which were fertilized with above-normal K^+ had photosynthetic rates 70% higher than those under standard fertilization (17). Preliminary evidence suggested that altered K^+/H^+ exchange between the cytoplasm and chloroplast in leaves of high K^+-fertilized plants subjected to water deficits may be involved in maintaining photosynthetic capacity at low water potentials (17).

Rao *et al.* (18) have also investigated the interaction between leaf nutrient status and photosynthesis at low leaf Ψ_w. Working with sunflower, they found that decreasing cellular Mg^{2+} resulted in the enhancement of photosynthesis in water stressed plants. They attributed this partial reversal of water stress effects on photosynthetic capacity to a reduction in deleterious effects of high Mg^{2+} on photochemical processes in the dehydrated chloroplast. However, recent work by Ben *et al.* (1) and Conroy *et al.* (7) suggests that the mechanisms affected by chloroplast Mg^{2+} in water stressed leaves may not be responsible for inhibited chloroplast photosynthetic capacity under stress. Nonetheless, the work of Rao *et al.* is significant in that they demonstrated substantial enhancement of photosynthesis *in situ* at low leaf Ψ_w.

Rao *et al.* (18) also studied K^+ interaction with water stress effects on photosynthesis. In contrast to previous work in this laboratory (6, 17), they found no effect of altered K^+ status on CO_2- and light-saturated photosynthesis. However, Rao *et al.* indicated that due to variability in the leaf K^+ content in their experiments, they could not completely rule out a role of K^+ in alleviating stress effects on chloroplasts.

In a recent review of water stress effects on photosynthesis, Kaiser (12) has suggested that K^+ enhancement of nonstomatally mediated photosynthesis under stress as shown by Berkowitz and Whalen (6) may be due to an altered Ψ_w/RWC

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[2] Present address: Boyce Thompson Plant Research Institute, Tower Road, Cornell University, Ithaca, NY 14850.

[3] Present address: Tennessee Valley Authority, Chemical Engineering Building, Air Quality Branch, Muscle Shoals, AL 35660.

[4] Abbreviations: Ψ_s, osmotic potential; RWC, relative water content; Ψ_w water potential.

relationship in high K^+ leaves. We have found that osmotic adjustment in response to water stress alters the Ψ_w/RWC in leaves, and that this effect can result in substantial acclimation of chloroplast metabolism to low leaf Ψ_w (21, 22). Berkowitz and Kroll (5) have shown that subjecting plants to repeated cycles of *in situ* water deficits results in cellular-level acclimation of nonstomatally mediated photosynthesis to low leaf Ψ_w due to an altered protoplast volume/Ψ_w relationship.

In view of the recent contributions to the pertinent literature, specifically Rao *et al.*'s conclusion that K^+ does not modulate stress effects on photosynthesis (18), the work in this laboratory (5, 21, 22) and others (7) which indicates that chloroplast sensitivity to leaf water deficits may be mediated by volume changes, and Kaiser's suggestion that leaf K^+ status may influence the volume/Ψ_w relationship (12), we reinvestigated the relationship between K^+ and chloroplast sensitivity to leaf water deficits. In these studies, our objectives were to (a) measure photosynthesis at varying cell K^+ through the course of *in situ* drought cycles, (b) partition K^+-induced cellular-level acclimation of photosynthesis to leaf water deficits caused by altered volume/RWC and other mechanisms, and (c) determine if repeated stress cycling of plants influences the relationships outlined in objectives (a) and (b).

MATERIALS AND METHODS

Plant Material

Wheat seeds (*Triticum aestivum* L. cv 'Tyler') were germinated in flats of 1:1 peat/vermiculite and transplanted (3/pot) after 10 d into pots containing approximately 800 cm^3 1:2 milled peat/acid washed sand. Pots were watered as described previously (17) with complete fertilizer containing either 0.2, 2, or 6 mM K^+ supplied as K_2SO_4. These three fertilizer regimes were the treatments used in all studies. Plants were grown in a growth chamber at 21°C and 50% RH with an 11 h light (250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) period. Mature (*i.e.* auricles exposed on the culm) nonsenescing leaves were used for all measurements which were generally taken 3 to 4 h into the light period. Plants were subjected to periods of water stress by withholding water. Nonacclimated plants were kept well-watered until they were exposed to a water stress cycle over a 12 d period during which data were recorded. Acclimated plants were subjected to a similar 12 d stress cycle, irrigated, and then immediately exposed to a second stress cycle. Measurements were taken on these acclimated plants during the second stress cycle.

Water Relations

Leaf Ψ_w and Ψ_s were measured using a pressure chamber, and thermocouple psychrometers, respectively, as described previously (17). RWC was determined by measuring the fresh, rehydrated (turgid), and dry weights of leaf discs (21). All water relations measurements are presented as the means of three replications ± the standard error, except where indicated.

Gas Exchange

Transpiration and net photosynthesis *in situ* were measured using an ADC (P. K. Morgan Instr. Inc., Andover, MA) IR gas analysis system. Measurements were made at varying external CO_2 in air as described previously (22). Light was provided by a sodium vapor lamp (2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR), and cuvette temperature was maintained between 26 and 29°C. Transpiration, photosynthesis, and temperature measurements were used to calculate internal leaf [CO_2] and stomatal resistance (9, 17). These measurements were taken at two external CO_2 concentrations; 330 (*i.e.* near ambient) and 930 ppm. Measurement of photosynthesis at a range of external (and calculated internal) [CO_2] suggested that an external [CO_2] of 930 ppm was saturating, or near-saturating for photosynthesis throughout the stress cycles (data not shown). However, recent studies by Kaiser (12) using a leaf disc O_2 electrode have suggested that dehydrated leaf tissue may require extremely high external [CO_2] to saturate photosynthesis. Therefore, 330 and 930 ppm external [CO_2] will be referred to in this report as 'low' and 'high' CO_2, respectively. All data obtained from gas exchange analyses are presented as the means of three replications ± the standard error.

Protoplast Studies

Protoplasts were isolated from leaves of plants grown at various K^+ levels as described previously (17), except that Cellulysin (Calbiochem, San Diego, CA) was used as a cellulase source in the enzymic digestion media. After purification and recovery from a 0/2%/5% dextran T-500 (Pharmacia, Piscataway, NJ) gradient, protoplasts were washed in resuspension medium containing 0.488 M betaine, 1 mM $CaCl_2$, and 5 mM Mes-NaOH (pH 6.0) which had a measured Ψ_s of −1.3 MPa. Aliquots of protoplasts were then added to resuspension medium which was brought to −1.3, −1.8, −2.3, or −2.8 MPa with betaine. After 5 min incubation in media of varying osmotic strength, protoplast volume, protoplast K^+, and chloroplast K^+ were measured using silicone oil microcentrifugation techniques (17). For determination of chloroplast K^+, protoplasts were passed through a 10 μm nylon net attached to the end of a 1 mL syringe six times prior to microcentrifugation for 30 s in a Beckman B microcentrifuge. Control experiments were performed to ensure the following: (a) virtually all protoplasts were broken at all treatments and at all Ψ_s after six passes through a 10 μm net; (b) replacement of the standard osmoticum (sorbitol) with betaine (this was done in order to allow the optimal silicone oil mixture [17, 22] to support media with an osmotic strength up to −2.8 MPa) did not alter protoplast or chloroplast K^+ at a given Ψ_s; and (c) after microcentrifugation of intact protoplasts, no cytoplasm (*i.e.* phosphoenol pyruvate carboxylase activity) was recovered in the supernatant above the silicone oil layer.

RESULTS AND DISCUSSION

Previous studies (17) have indicated that with well watered, growth chamber-grown wheat plants, 1 to 2 mM K^+ in nutrient solution applied 3 times per week was necessary to maintain maximal photosynthetic capacity. Increasing fertilizer K^+ to 6 mM has no beneficial or adverse effect under well-watered conditions, and decreasing K^+ to 0.2 mM results in a nonstomatally mediated inhibition in photosynthesis (17). This K^+ effect on photosynthesis was again demonstrated in the ex-

periment shown in Figure 1 (*i.e.* compare rates for 6, 2, and 0.2 mM K^+-grown plants at leaf water potentials approaching −0.5 MPa). In previous studies (17), leaf K^+ concentrations concomitant with these fertilizer regimes were exhaustively monitored. Typically, leaf K^+ was approximately 150 to 200 mM with 2 mM K^+ fertilization, was decreased by 100 to 150 mM at 0.2 mM K^+, and increased by 100 to 150 mM at 6 mM K^+. Plants kept well-watered during the course of the experiment shown in Figure 1 were found to have leaf K^+ of 68.5 ± 8.8 mM (0.2 mM K^+-fed plants), 234.0 ± 14.8 mM (2 mM K^+ plants), and 315.5 ± 30.5 mM (6 mM K^+ plants) prior to the experiment, and leaf K^+ concentrations of 46.0 ± 6.3 mM, 193.7 ± 14.8 mM and 310.9 ± 14.8 mM, respectively, after the completion of the experiment.

Altering leaf K^+ had a dramatic effect on photosynthesis at low CO_2 as leaf Ψ_w declined in water stressed wheat plants (Fig. 1B). With respect to the control (2 mM K^+-grown plants), photosynthesis at low Ψ_w was maintained to a greater degree at 6 mM K^+, and was more sensitive at 0.2 mM K^+. For example, at −1.75 MPa, photosynthesis of 2 mM K^+ plants was 15.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$. At this same Ψ_w, photosynthesis was 60% greater in 6 mM K^+ plants. At −1.75 MPa Ψ_w, photosynthesis in 0.2 mM K^+ plants was completely inhibited. These data differ with the results of similar experiments undertaken by Rao *et al.* (18) with sunflower.

Photosynthesis was also monitored at high CO_2 during this water stress cycle (Fig. 1A). Photosynthesis at all treatments was higher when measured at high CO_2 than at near-ambient CO_2 throughout the stress cycle (*cf.* Fig. 1, A and B). Nonetheless, the modulation of low Ψ_w effects on photosynthesis by altered cellular K^+ was still evident at high assay CO_2. These results suggest that the K^+ effect on photosynthesis at low Ψ_w may not be mediated by treatment effects on stomatal resistance at low Ψ_w.

Previous research (2, 5, 14, 21) has indicated that acclimation of photosynthetic capacity to low Ψ_w due to physiological changes in leaf mesophyll cells can occur when plants are subjected to water deficits. Therefore, the interaction between cellular acclimation to low Ψ_w, and K^+ effects on photosynthesis at low Ψ_w was investigated. Photosynthesis at low (Fig. 2B) and high (Fig. 2A) CO_2 was monitored during a water stress cycle in plants which had been previously exposed to low Ψ_w. Photosynthesis at low Ψ_w was greater at any K^+ treatment in acclimated, as compared with nonacclimated plants. This occurred at either low assay CO_2 (*cf.* Figs. 1B and 2B) or at high CO_2 (*cf.* Figs. 1A and 2A). Even though plants grown at any K^+ level demonstrated an acclimation response with regard to the maintenance of photosynthesis at low Ψ_w, the K^+ effect of modulating low Ψ_w effects on photosynthesis was still evident in acclimated plants, both at low (Fig. 2B) and high (Fig. 2A) CO_2. The combination of acclimation and high K^+ (6 mM) fertilization resulted in substantial maintenance of photosynthesis at low Ψ_w compared to nonacclimated plants grown at a standard K^+ level (2 mM) which was sufficient to maintain optimal photosynthesis at high Ψ_w. For example, with 6 mM K^+ acclimated plants, photosynthetic rate under low CO_2 was 29.8 ± 1.7 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at −2.2 MPa (Fig. 2B). With 2 mM K^+ nonacclimated plants, photosynthetic rate was 6.1 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ under low CO_2 at −2.1 MPa (Fig. 1B).

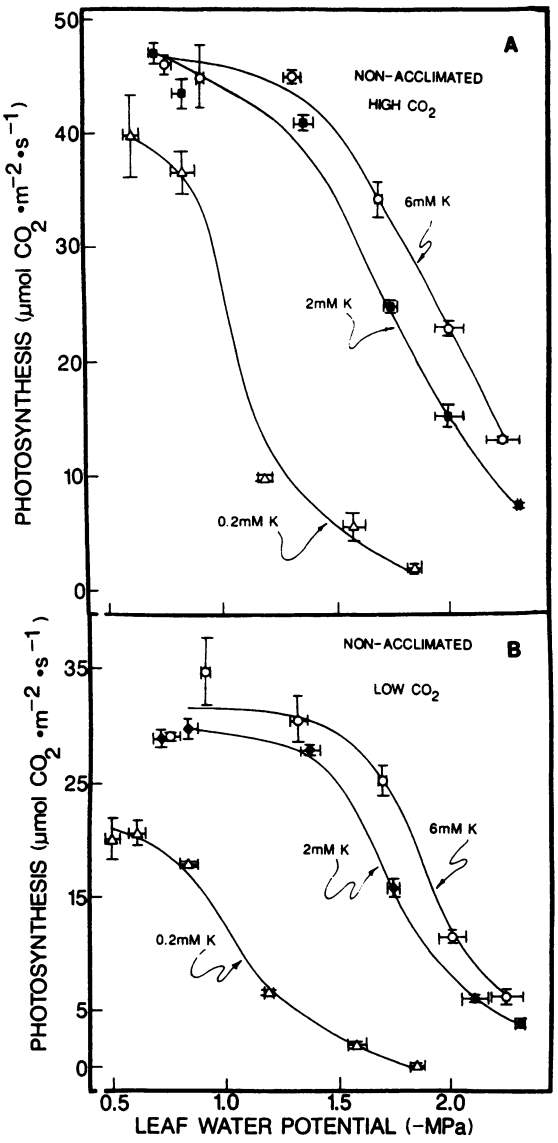

Figure 1. Light-saturated net photosynthesis at high (A) and low (B) external CO_2 of fully expanded, nonsenescing leaves of non acclimated wheat plants at declining Ψ_w during a stress cycle. Curves are presented for plants grown under different K^+ fertilization. Bars indicate ±SE (n = 3).

Results of work in this laboratory (5, 21, 22) and others (7, 14) have been consistent with the hypothesis first raised by Kaiser (11) that photosynthetic inhibition at low Ψ_w may be fundamentally linked to the degree of chloroplast dehydration (*i.e.* volume reduction) at low Ψ_w. Cellular-level acclimation to low Ψ_w has been shown to occur concomitantly with the maintenance of greater RWC (7, 10), protoplast volume (5, 11, 21), and chloroplast volume (2, 4, 13, 22) at low Ψ_w. RWC measurements have been used to estimate the contribution of protoplast volume maintenance at low Ψ_w to photosynthetic acclimation to low Ψ_w (7). Therefore, photosynthesis of plants subjected to the two water stress cycles shown in Figures 1 and 2 was also plotted as a function of declining RWC during

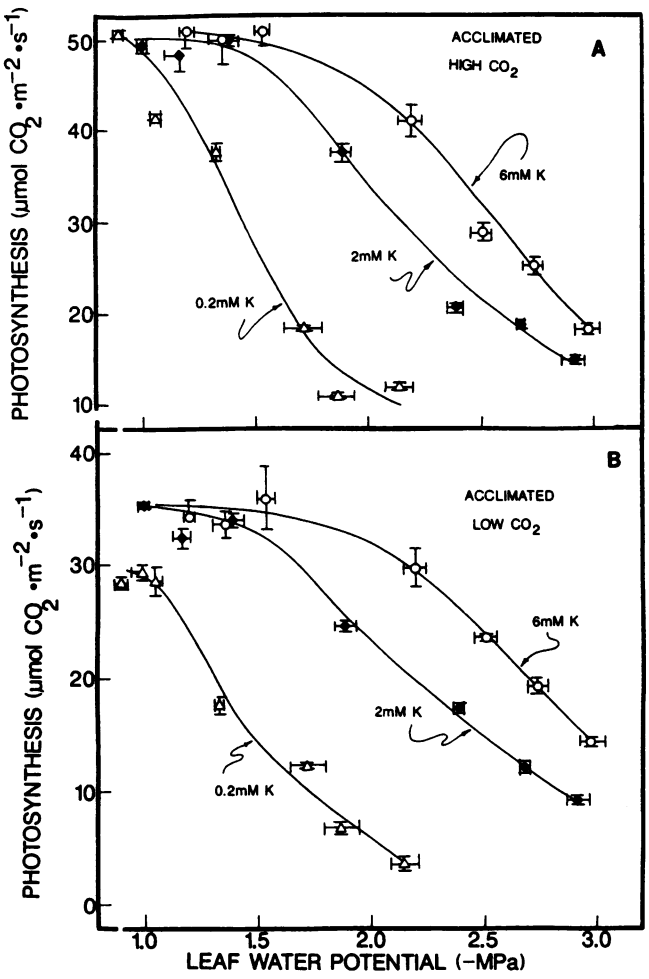

Figure 2. Light-saturated net photosynthesis at high (A) and low (B) external CO_2 of leaves of acclimated wheat plants at declining Ψ_w during a second stress cycle. Curves are presented for plants grown under different K^+ fertilization. Bars indicate ±SE (n = 3).

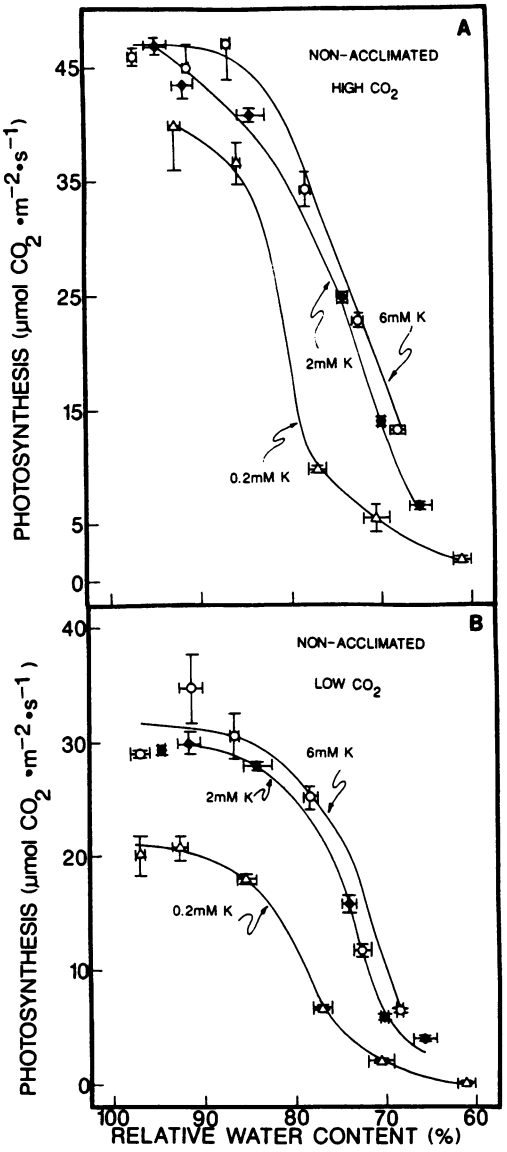

Figure 3. Light-saturated net photosynthesis at high (A) and low (B) external CO_2 of leaves of non acclimated wheat plants at declining RWC during a stress cycle. Curves are presented for plants grown under different K^+ fertilization. Bars indicate ±SE (n = 3).

the stress periods (Figs. 3 and 4). In all cases, the effect of varying K^+ fertilization on the maintenance of photosynthesis during the stress cycles was much less pronounced. This comparison of K^+ treatment effects in terms of photosynthesis at declining RWC, *versus* declining Ψ_w, supports Kaiser's recent contention (12) that a significant portion of the K^+ effect of imparting water stress resistance to plants (in terms of enhancement of photosynthetic capacity at low Ψ_w) may be due to K^+ effects on the relationship between RWC and Ψ_w. This point is supported by the analyses presented in Figures 5 and 6. With both nonacclimated (Fig. 5) and acclimated plants (Fig. 6), 6 mM K^+ plants tended to maintain greater RWC, and 0.2 mM K^+ plants tended to display lower RWC than 2 mM K^+ plants as leaf Ψ_w declined during the water stress cycles.

An altered RWC/Ψ_w relationship can be facilitated by osmotic adjustment during drought periods (7, 10). Therefore, K^+ effects on capacity for osmotic adjustment during the two water stress cycles were determined by evaluating the relationship between RWC and Ψ_s during the stress cycles. The solute-concentrating effects of dehydration should cause a linear decline in Ψ_s when the data are plotted in a double logarithmic fashion (21) as is shown in Figures 7 and 8. In the case of nonacclimated plants, the plot of leaf Ψ_s and RWC during the stress cycle indicated that at all three K^+ treatments, plants osmotically adjusted (Fig. 7). Surprisingly, 0.2 mM K^+ plants displayed the greatest, and 6 mM K^+ plants displayed the least degree of osmotic adjustment during the water stress cycle. With acclimated plants exposed to a second stress cycle (Fig. 8), no osmotic adjustment occurred in plants with any K^+ treatment. As shown in Figure 8, Ψ_s decline in 2 and 0.2 mM K^+ plants during the initial phase of the stress cycle (*i.e.* between RWC of 100 and 75%) could be accounted for entirely by the solute-concentrating effect of dehydration. Below 75% RWC, 2 and 0.2 mM K^+ plants lost solutes. During the entire stress cycle, 6 mM K^+, acclimated plants lost solutes

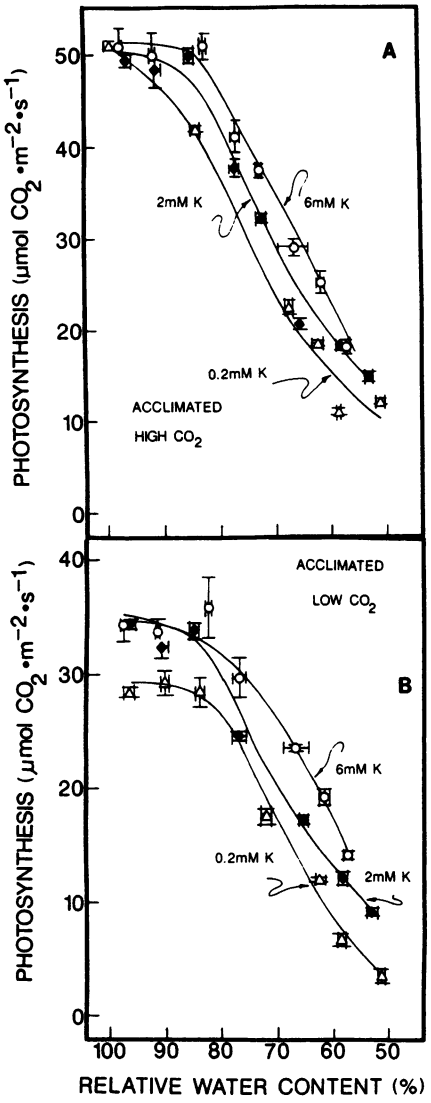

Figure 4. Light-saturated net photosynthesis at high (A) and low (B) external CO_2 of leaves of acclimated wheat plants at declining RWC during a second stress cycle. Curves are presented for plants grown under different K^+ fertilization. Bars indicate ±SE (n = 3).

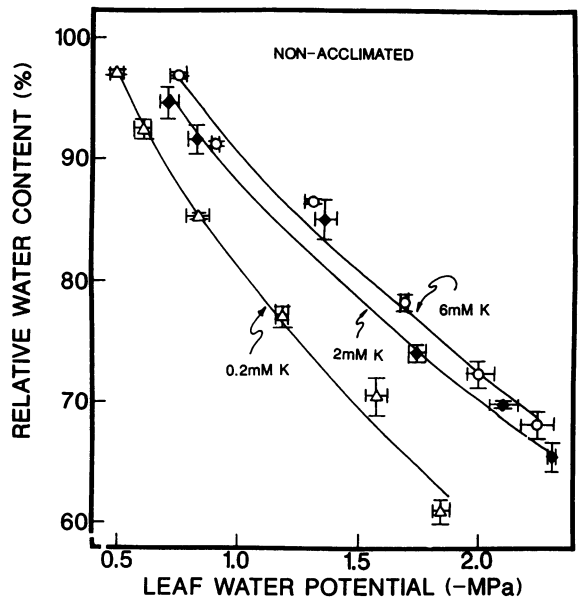

Figure 5. Relationship between declining RWC and Ψ_w in mature, nonsenescing leaves of nonacclimated wheat plants exposed to a stress cycle. Curves are presented for plants grown on 0.2 mM (△), 2 mM (◆), and 6 mM (○) K^+ fertilization. Horizontal and vertical bars represent ±SE of Ψ_w and RWC, respectively.

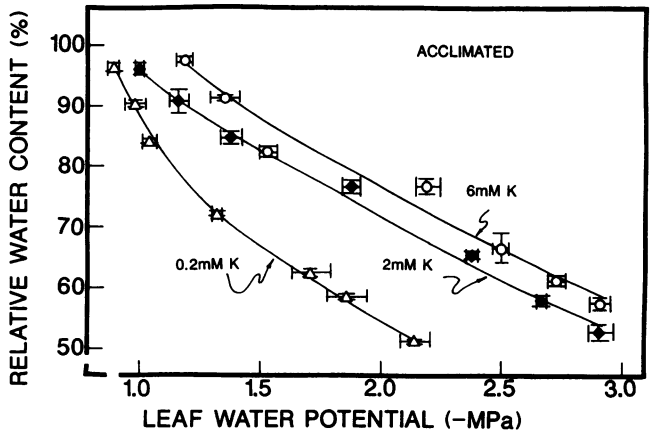

Figure 6. Relationship between declining RWC and Ψ_w in mature, nonsenescing leaves of acclimated wheat plants exposed to a second stress cycle. Curves are presented for plants grown on 0.2 mM (△), 2 mM (◆), and 6 mM (○) K^+ fertilization. Horizontal and vertical bars represent ±SE of Ψ_w and RWC, respectively.

(Fig. 8). K^+ effects on the relationship between RWC and Ψ_w (Figs. 5 and 6) and photosynthesis at low Ψ_w (Figs. 1 and 2) cannot be explained by altered capacity for osmotic adjustment during a water deficit episode, with either nonacclimated or acclimated plants. However, the data presented in Figures 7 and 8 do indicate that K^+ effects on the osmotic relations of leaf cells can explain the altered RWC/Ψ_w relationship evidenced by plants grown with different K^+ during water stress episodes as shown in Figures 5 and 6. With nonacclimated plants, even though the degree of osmotic adjustment was greatest in 0.2 mM, and least in 6 mM K^+ plants, the leaf Ψ_s was lowest in 6 mM, and greatest in 0.2 mM K^+ plants over the entire range of RWC decline during the water stress cycle (Fig. 7). At high RWC, leaf solute concentration was substantially higher in 6 mM K^+ plants than 2 mM K^+ plants, and 2 mM K^+ well watered plants had a greater solute concentration than 0.2 mM K^+ plants (Fig. 7). This difference in solute content between well watered plants grown at varying K^+ persisted throughout the stress cycle. This situation also occurred with acclimated plants. Leaf Ψ_s at any K^+ level was lower in plants which were rehydrated after a stress cycle as compared to well watered, nonacclimated plants (*cf.* Ψ_s values at RWC near 100% in Figs. 7 and 8). However, there was still a substantial difference in Ψ_s due to K^+ treatment in the acclimated plants at the onset of the second stress cycle (*cf.* Ψ_s at RWC near 100% of 6, 2, and 0.2 mM K^+ plants in Fig. 8). This differential in Ψ_s between 6, 2, and 0.2 mM K^+ plants again persisted throughout a second water stress cycle. The data presented in Figures 5 to 8 suggest that increased solute

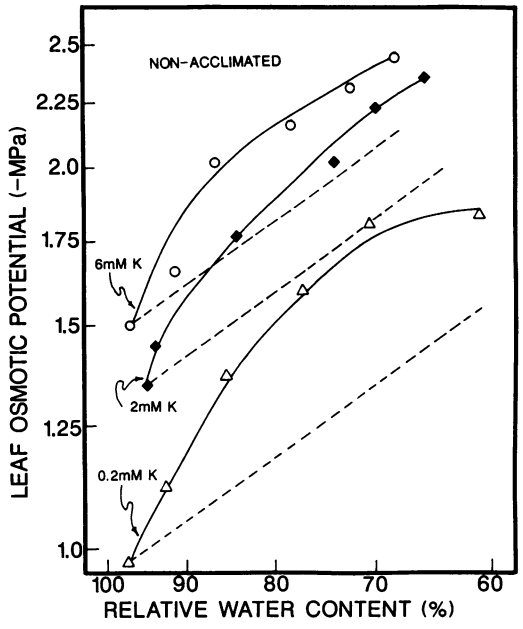

Figure 7. Double logarithmic plot of declining Ψ_s and RWC during a stress cycle in nonacclimated plants. The solute-concentrating effects of dehydration (RWC decline) on Ψ_s of 6 mM (○), 2 mM (♦), and 0.2 mM (△) K^+ plants are represented by the broken lines drawn through the data recorded on well watered plants (data points closest to 100% RWC). Degree of osmotic adjustment during stress can be evaluated by comparing actual Ψ_s decline under each treatment with the respective broken line. If the data points fall above the broken line for a treatment, osmotic adjustment occurred during the stress cycle. For clarity, bars representing the standard error of the means for RWC and Ψ_s are not represented in this figure. The standard errors of the RWC means are shown in Figure 3. The average standard error for the Ψ_s means was 0.041 MPa, and the maximum standard error for Ψ_s in this figure was 0.067 MPa.

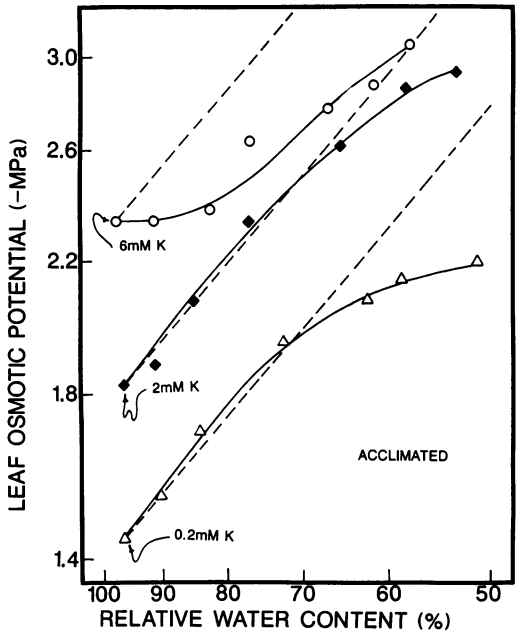

Figure 8. Double logarithmic plot of declining Ψ_s and RWC during a stress cycle in acclimated plants exposed to a second stress cycle. The solute-concentrating effects of dehydration (RWC decline) on Ψ_s of 6 mM (○), 2 mM (♦), and 0.2 mM (△) K^+ plants are represented by the broken lines drawn through the data recorded on well watered plants (data points closest to 100% RWC). For clarity, bars representing the standard error of the means of RWC and Ψ_s are not represented in this figure. The standard errors of the RWC means are shown in Figure 4. The average standard error for the Ψ_s means was 0.036 MPa, and the maximum standard error for Ψ_s in this figure was 0.104 MPa.

concentration at varying K^+ treatment in well watered, or rehydrated plants resulted in an altered Ψ_w/RWC relationship which persisted throughout the water deficit episodes. These K^+ treatment-induced alterations in leaf cell osmotic relations likely were responsible for the bulk of the difference in photosynthetic capacity at low Ψ_w between plants grown on varying K^+ as shown in Figures 1 and 2.

One objective of the work presented in this report was to evaluate the contribution of nonstomatal factors to the K^+ effect on photosynthesis at low Ψ_w. Therefore, an attempt was made to partition any possible effects of the K^+ treatments on stomatal resistance in stressed plants from the overall K^+ effect on photosynthesis under stress (as shown in Figs. 1–4). This was done by evaluating stomatal resistance as a function of declining RWC during the stress cycles (Fig. 9). It can be seen that in nonacclimated plants, K^+ had no effect on the increase in stomatal resistance with declining RWC during the stress cycle (data for 0.2, 2, and 6 mM K^+ plants all fell on the same line). Although there was some scatter between K^+ treatments with acclimated plants below 65% RWC, this was also the case with declining RWC in acclimated plants (Fig. 9). These data, then, indicate that K^+ effects on photosynthesis during the water stress cycles (Figs. 1–4) are likely not mediated by treatment effects on stomatal resistance (Fig. 9).

The data presented in this report are consistent with the contention raised by Kaiser (12) that the bulk of the difference in photosynthetic rates at low Ψ_w between plants grown on varying K^+ is due to altered leaf solute content. However, differences between photosynthesis at the three K^+ treatments were still evident when photosynthesis was plotted as a function of declining RWC during the stress cycles (Figs. 3B and 4B). These differences persisted, in both nonacclimated and acclimated plants, at high assay [CO_2] (Figs. 3A and 4A) which was 'apparently' saturating or near-saturating (data not shown). Therefore, these results do not preclude the possibility raised in earlier work (6, 17) that high cytoplasmic K^+ may modulate, to some degree, water stress effects on chloroplast metabolism by reducing net K^+ efflux in dehydrated chloroplasts. Although subsequent work by Robinson (19) clearly illuminated the limitations of the isolated chloroplast as a model system to study water stress effects on photosynthesis, Berkowitz and Gibbs. (3) have shown that increased extrachloroplastic K^+ substantially increases photosynthesis in osmotically stressed chloroplasts. This effect was later evidenced in osmotically stressed plant cells (6). Pertinent to this line of reasoning is a different study by Robinson (20) which found that K^+ fluxes out of chloroplasts result in loss of photosynthetic capacity. Demmig and Gimmler (8) and Maury *et al.*

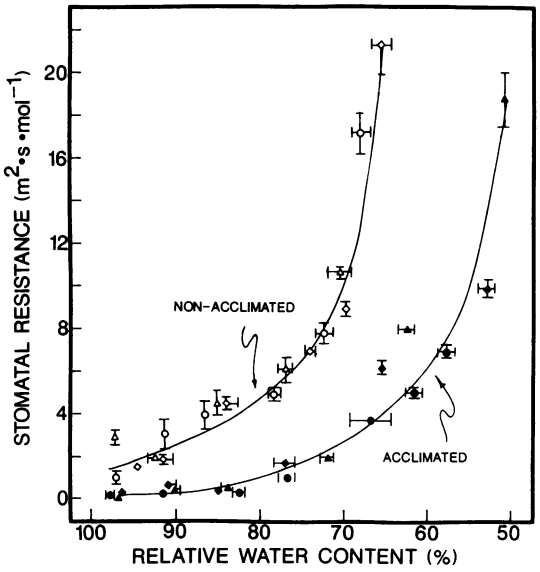

Figure 9. Stomatal resistance of nonacclimated (open symbols) and acclimated (closed symbols) plants at declining RWC during a stress cycle. Data are shown for 6 mM K^+ (○, ●), 2 mM K^+ (◇, ◆), and 0.2 mM K^+ (△, ▲) plants. Horizontal and vertical bars indiate ±SE (n = 3) of RWC and stomatal resistence, respectively. For clarity, standard error bars were omitted when the standard error was extremely small.

(15) have associated chloroplast K^+ loss and photosynthetic inhibition with reduced stromal alkalinization in the light. Oja *et al.* (16) have recently shown that light-induced stromal alkalinization occurs in intact leaves, and may have significant effects on cell metabolism *in vivo*.

Attempts were made in this study to investigate the possibility that altered K^+ fertilization could result in reduced net K^+ efflux from chloroplasts in water stressed leaves. In this experiment, protoplasts were isolated from 6 and 2 mM K^+ plants. Protoplast and chloroplast K^+, and protoplast volume were measured in protoplasts incubated in medium at a range of Ψ_s. As can be seen in Table I, osmotic stress resulted in protoplast dehydration (*i.e.* volume loss). Little to no K^+ leakage occurred from the 6 or 2 mM K^+ protoplasts due to the osmotic dehydration. However, protoplast dehydration apparently caused significant loss of stromal K^+ in both 6 and 2 mM K^+ protoplasts. With 2 mM K^+ protoplasts subjected to −2.8 MPa solution Ψ_s, stromal K^+ was only 38% of that at −1.3 MPa. With 6 mM K^+ protoplasts at −2.8 MPa, the extent of efflux of stromal K^+ was reduced by nearly half. These results indicate that protoplast dehydration *in vitro* can be associated with substantial loss of stromal K^+, and that this K^+ efflux is not as severe in protoplasts prepared from 6 mM K^+ plants, as compared to protoplasts from 2 mM K^+ plants. This experiment was undertaken with a model system; isolated protoplasts subjected to instantaneous osmotic dehydration. Therefore, extension of these results to the situation *in situ* with regard to slowly developed leaf water deficits should be made with caution. However, if intracellular K^+ fluxes occur during cell dehydration *in situ*, and if K^+ flux effects on chloroplast photosynthetic capacity *in situ* are similar to the effects previously documented with the isolated chloroplast (3, 6, 8, 15), then K^+ status in the dehydrated leaf cell could, to some degree, modulate the severity of photosynthetic inhibition as shown in Figures 1 to 4 by altered intracellular K^+ movement.

Table I. *Effects of Dehydration on Volume, Total Protoplast K^+, and Chloroplast K^+ in Protoplasts Isolated from Wheat Plants Grown on 6 and 2 mM K^+*

The 6 and 2 mM K^+ plants used for this experiment had 286.7 ± 25.8 mM and 178.7 ± 20.3 mM leaf K^+, respectively, as determined by atomic absorption spectroscopy analysis of leaf tissue. To facilitate comparison between changes in the different measured parameters, results are presented as percentage change from the measurement at −1.3 MPa for each parameter. An analysis of variance was made on the measured values of each parameter. In each row, percentages followed by different letters represent means of measured values which were significantly different (Duncan's multiple range test). The means ± the standard error of values at −1.3 MPa were: 220.2 ± 7.6 μL/mg Chl, 204.6 ± 9.7 μL/mg Chl, 58.9 ± 5.3 μmol/mg Chl, 38.7 ± 2.5 μmol/mg Chl, 6.09 ± 0.45 μmol/mg Chl, and 6.77 ± 1.33 μmol/mg Chl, respectively, for 6 and 2 mM K^+ protoplast volume, 6 and 2 mM K^+ protoplast K^+, and 6 and 2 mM K^+ chloroplast K^+.

Tissue	% of Value at −1.3 MPa Solution Ψ_s			
	−1.3 MPa	−1.8 MPa	−2.3 MPa	−2.8 MPa
Protoplast Volume				
6 mM K^+ plants	100a	61.6b	53.2b	28.6c
2 mM K^+ plants	100a	56.0b	48.1b	40.0b
Protoplast K^+				
6 mM K^+ plants	100a	94.2a	92.1a	80.3a
2 mM K^+ plants	100a	93.6ab	86.6ab	85.7b
Chloroplast K^+				
6 mM K^+ plants	100a	78.0ab	64.5ab	70.8b
2 mM K^+ plants	100a	49.2b	43.6b	38.1b

In summary, evidence has been presented in this report to document that in wheat, increasing leaf K^+ above that required for optimal photosynthesis in well watered plants allows for the maintenance of higher photosynthetic rates at low Ψ_w during a period of water stress. Lowering leaf K^+ from the control level has the opposite effect. K^+ effects on photosynthetic capacity in stressed plants are likely not mediated by alterations in stomatal conductance under water deficits. We conclude that the bulk of this protective effect of high leaf K^+ is due to an altered cell volume/Ψ_w relationship due to changes in cell solute level which persist through a period of water stress. Other factors may contribute to the overall K^+-protective effect. These may include altered intracellular K^+ fluxes in dehydrating tissue.

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