Modulation of Water Stress Effects on Photosynthesis by Altered Leaf K⁺

PAUL A. PIER AND GERALD A. BERKOWITZ*
Department of Horticulture and Forestry, Cook College, Rutgers University,
New Brunswick, New Jersey 08903

ABSTRACT

Wheat irrigated with nutrient solutions containing 0, 0.2, 0.5, 1, 2, or 6 millimolar K⁺ had maximum photosynthetic rates at 1 to 2 millimolar K⁺ concentrations. Rates in the 6 millimolar K⁺-grown plants were not higher than the 2 millimolar K⁺-grown wheat, and rates were inhibited below 0.5 millimolar K⁺. Photosynthesis was measured by both attached whole leaf CO₂ uptake and by ¹⁴C O₂ fixation of leaf slices in solution. Exposure of leaf slices from 0.2, 2, and 6 millimolar K⁺-grown wheat to various assay media water potentials showed that photosynthesis of the 0.2 millimolar K⁺-grown wheat decreased from control (high water potential) rates by 35%, that of the 2 millimolar K⁺-grown wheat by 20.4%, and that of the 6 millimolar K⁺-grown wheat by only 8.3% at -3.11 megapascals. Also, photosynthesis of the 6 millimolar K⁺-grown wheat was enhanced by 28% over that of the 2 millimolar K⁺ wheat at the most severe water stress (-3.11 megapascals), indicating that the excess leaf K⁺ in the 6 millimolar K⁺-grown wheat partially reversed dehydration effects on photosynthesis. Oligomycin eliminated the protective effects of high K⁺ on photosynthesis in dehydrated leaf slices. These results suggest that the protective effect of high K⁺ under water stress may involve the exchange of K⁺ in the cytoplasm for stroma H⁺, thus altering stromal pH and restoring photosynthesis. The protective effect of high K⁺ was also observed in attached whole leaf photosynthesis of in situ water-stressed wheat grown on 0.2, 2, and 6 millimolar K⁺. Under water stress, rates of the 6 millimolar K⁺-grown wheat were enhanced by 66.2% and 113.9% over that of the 2 millimolar K⁺-grown wheat in two separate experiments. Internal CO₂ concentration of the 6 millimolar K⁺-grown wheat was lower than that of the 0.2 and 2 millimolar K⁺-grown wheat. These results suggest that the high K⁺ effects on chloroplast photosynthesis seen in leaf slices also occur at the whole plant level.

Inhibition of photosynthesis in isolated chloroplasts, protoplasts, and leaf slices by dehydration in hypertonic solutions (4, 16) and determination of stomatal and mesophyll (i.e., biochemical) limitation to CO₂ uptake in leaves of water-stressed plants (15) indicate that nonstomatal factors can contribute substantially to decreased photosynthetic rates during water stress. Several groups of investigators have attempted to characterize the mechanism(s) mediating inhibition of chloroplast metabolism by water stress in subcellular studies (2, 6, 16). The results of these in vitro studies indicate that perturbations in the ionic milieu of the stroma may play an important role in mediating water stress inhibition of the photosynthetic process.

One line of evidence suggests that the stromal pH is too low to support optimal photosynthetic rates in dehydrated plastids, and that cellular processes which facilitate stromal neutralization may modulate stress effects on chloroplasts. Inhibition of photosynthesis of isolated chloroplasts in hypertonic medium was partially reversed by addition of K⁺ and NH₄Cl (2, 3), which raise stromal pH (13, 20). Berkowitz and Whalen (5) found that photosynthesis of leaf slices prepared from K⁺-deficient spinach was more sensitive to in vitro dehydration than leaf slices of control plants. The response of K⁺ appeared to be mediated by extrachloroplastic factors, since dehydration inhibited photosynthesis of chloroplasts isolated from control and K⁺-deficient plants to the same degree. These results suggest that raising the extrachloroplastic K⁺ concentration in plant cells by supplying the plant with excess K⁺ could possibly reduce water stress inhibition of photosynthesis. Many crop plants, when supplied with excess K⁺ fertilization below levels which would induce 'salt' or osmotic stress effects on water uptake, can accumulate superoptimal leaf K⁺ without deleterious effects on cell metabolism. In this study, wheat plants were supplied with a range of nutrient solution K⁺ concentrations, and photosynthesis was measured on whole leaves in water-stressed plants and on leaf slices dehydrated in hypertonic medium to determine the effect of altered cellular K⁺ levels on photosynthesis. The leaf slice procedure allows study of water stress effects with stomatal limitation to photosynthesis obviated, but with plastids still exposed to the in vivo regulation which occurs in the intact cell. Photosynthetic measurements of attached leaf slices were used to determine whether the leaf slice results were applicable at the whole plant level. The mechanism by which altered cellular K⁺ modulation of water stress effects on photosynthesis was studied using oligomycin. Oligomycin has been shown to inhibit the chloroplast envelope ATPase which facilitates and/or interacts with (either directly or indirectly) K⁺/H⁺ exchange across the limiting membrane of the plastid (20). Potassium concentrations in protoplasts, chloroplasts, and the extrachloroplastic cellular space were measured to further clarify the role of extrachloroplastic K⁺.

MATERIALS AND METHODS

Plant Material. Wheat (Triticum aestivum L. cv Tyler) was germinated in flats of 1:1 peat:vermiculite and transplanted (one/pot) after 10 d to pots containing 1:1 washed sand/perlite. Pots were irrigated 3 times a week with nutrient solution containing 2 mM Ca(NO₃)₂, 2 mM NH₄NO₃, 0.5 mM NaH₂PO₄, 3 mM CaCl₂, 2 mM MgSO₄, 50 μM chelated Fe, 28 μM MnSO₄, 10 μM H₂BO₃, 1.4 μM CuSO₄, 1.4 μM ZnSO₄, and 0.3 μM (NH₄)₆Mo₇O₄₄. The potassium concentration of the nutrient solution (adjusted with K₂SO₄) was 0, 0.2, 0.5, 1.0, 2.0, and 6.0 mM. Plants were grown in a growth chamber at 21°C and 50% RH with an 11 h light
(250 \mu mol/m^2-s at plant height) period. The youngest (and occasionally the second youngest) fully exposed (i.e. auricles exposed on the culm) leaf of a tiller was used for all measurements, which began 40 d after planting.

**Water Relations.** Leaf \( \Psi \), was measured using a pressure chamber (Soil Moisture Equipment Corp.). Leaf \( \Psi \), was determined by measuring water potentials of frozen and thawed leaf discs with a Wescor C-52 leaf sample chamber and a HR 33T thermocouple psychrometer operating in the hygrometric mode. Turgor pressure was calculated as the difference between \( \Psi \), and \( \Psi \). Stomatal conductance measurements of the abaxial and adaxial surfaces of leaf blades with a Li-Cor 1600 porometer were used to calculate leaf conductance. All water relations measurements were made 7 to 8 h into the light period.

Water Stress. Plants were exposed to in situ water stress by withholding regular irrigations over an 8 d period, at which time net CO\(_2\) exchange and water status measurements were taken. Leaf \( \Psi \), was monitored during the stress period, and small amounts of water (as indicated in Fig. 4) were added to the pots to allow for a gradual decline in leaf \( \Psi \). The experiment was repeated a second time, with water stress extending over a 6 d period.

**Elemental Analysis.** Samples were taken over the course of the experiment to monitor changes in leaf \( K^+ \) concentration. The middle portion of the leaf blade (0.1–0.4 g) was weighed, dried, and wet-ashed in 2.0 ml concentrated H\(_2\)SO\(_4\) by heating until the leaf tissue dissolved. H\(_2\)O\(_2\) (30%) was then added dropwise with intermittent heating until the solution became clear. An aliquot of the sample was diluted with water to a final volume which contained 0.2% (w/v) LaCl\(_3\). Potassium concentration was determined using a Perkin-Elmer 2280 atomic absorption spectrophotometer.

**Photosynthetic Measurements.** During gas exchange studies, leaf temperature was maintained at 20 to 25°C and light was provided by a sodium vapor lamp (800 \mu mol/m\(^2\)-s) with 10 cm of water as a heat filter. CO\(_2\) uptake by intact leaves, positioned in a water-cooled Plexiglas leaf chamber (with an internal fan), was measured with a Beckman 865 Infrared Gas Analyzer arranged in an open system. Relative humidity was maintained at about 50%. For later experiments, an ADC portable gas exchange system was used for simultaneous measurement of CO\(_2\) uptake (\( A \)), leaf resistance to water vapor diffusion (\( L_w \)), transpiration (\( E \)), and internal leaf CO\(_2\) concentration (\( C_{i} \)). Using the accepted value of 1.6 as the ratio of the diffusivities of water vapor and CO\(_2\) in air, and a boundary layer resistance (\( B_d \)) value suggested by the manufacturer, leaf conductance to CO\(_2\) (\( g_c \)) was calculated according to the equation:

\[
g_c = 1/((1.6 \times L_w) + [1.37 \times B_d])
\]

and \( C_{i} \) was calculated using the measured CO\(_2\) concentration over the leaf (\( C_{out} \)) according to the equation:

\[
C_{in} = (g_c - E/2) \times C_{out} - A)/(g_c + E/2)
\]

These calculations of gas exchange parameters follow the analysis presented by Von Caemmerer and Farquhar (31).

Photosynthesis of leaf slices was measured by incorporation of \(^{14}\)CO\(_2\) as previously described (5). Two to four leaf discs (7.0 mm diameter) were cut into slices (0.75 mm average width) and vacuum-infiltrated twice for 15 to 25 s in 3.5 ml of reaction medium which contained 20 mM Hepes-NaOH (pH 7.6), 1 mM MgCl\(_2\), 1 mM MnCl\(_2\), 2 mM Na\(_2\)EDTA, 2 mM NaH\(_2\)PO\(_4\), and sorbitol. Preliminary experiments indicated that the presence of Mg\(^{2+}\), Mn\(^{2+}\), EDTA, and PO\(_4\)\(^{3-}\) were required for optimal photosynthetic activity.

The sorbitol concentration was varied to alter the solution \( \Psi \).

\(^2\) Abbreviation: \( \Psi \), water potential; \( \Psi \), osmotic potential.
POTASSIUM AND WATER STRESS EFFECTS ON PHOTOSYNTHESIS

was 7.64 mm, and were 41.66 mm. K+-grown CHl was resuspended in 800 μl of chloroplast medium consisting of 0.5 mM sorbitol, 1 mM Na$_2$EDTA, and 5 mM Hepes-NaOH (pH 7.6). This preparation was sucked up and ejected six times through a 10 μm mesh nylon net fitted over the tip of a 1 ml plastic disposable syringe. The released chloroplasts were centrifuged at 250g for 90 s, and the pellet resuspended in 200 μl of chloroplast medium.

Chloroplast Isolation. A 200 μl aliquot from the protoplast preparation was added to 5 ml of wash medium and centrifuged at 250g for 5 min. The pellet was resuspended in 800 μl of chloroplast medium. This preparation was sucked up and ejected six times through a 10 μm mesh nylon net fitted over the tip of a 1 ml plastic disposable syringe. The released chloroplasts were centrifuged at 250g for 90 s, and the pellet resuspended in 200 μl of chloroplast medium.

Chloroplast and Protoplast Volume and K⁺ Determination. Chloroplast and protoplast volumes were determined following the double isotope measurement method of Heldt (14). Aliquots (100 μl) of the protoplast or chloroplasts in protoplast wash medium or chloroplast medium containing 10 μCi/ml $[^{14}\text{C}].$

![Image](https://example.com/image.png)

**Fig. 1.** Whole leaf photosynthesis, leaf conductance, and leaf K⁺ concentration of wheat grown on nutrient solution with varying K⁺ concentration. Values are means of 3 replications ± SE for photosynthesis and leaf conductance; for K⁺ concentrations, values are means of at least 4 replications.

![Image](https://example.com/image.png)

**Fig. 2.** $[^{14}\text{C}]\text{CO}_2$ fixation by leaf slices from wheat grown at varying K⁺. Values are means of three separate experiments. Leaf K⁺ concentrations were 41.66 ± 8.02 mm for 0 mm K⁺, 41.26 mm (no SE) for 0.2 mm K⁺, 102.92 ± 7.13 mm for 0.5 mm K⁺, 135.91 ± 5.25 mm for 1.0 mm K⁺, 198.95 ± 10.80 mm for 2.0 mm K⁺, and 331.48 ± 40.37 mm for 6 mm K⁺-grown plants. The maximum photosynthetic rate on an area basis was 7.64 μmol CO₂/m².s.

![Image](https://example.com/image.png)

**Fig. 3.** $[^{14}\text{C}]\text{CO}_2$ fixation by leaf slices of 0.2, 2, or 6 mm K⁺-grown wheat assayed in reaction media at varying $\Psi_s$. Values are means of two replications. Leaf K⁺ concentrations were 65.28 ± 6.02 mm for 0.2 mm K⁺, 234.12 ± 12.46 mm for 2 mm K⁺, and 432.38 ± 19.89 mm K⁺ for 6 mm K⁺-grown plants. The maximum photosynthetic rate on an area basis was 7.69 μmol CO₂/m².s.

Sorbitol and 7.5 μCi/ml $[^{3}\text{H}]\text{H}_2\text{O}$ were layered onto 100 μl of silicon oil in 0.4 ml microfuge tubes which had 20 μl of 14% HClO₄ below the oil layer. The tubes were centrifuged for 60 s in a Beckman Microfuge B with a horizontal rotor. Stromal and total protoplast volume was calculated by comparing the $[^{3}\text{H}]$ and $[^{14}\text{C}]$ below the oil layer with the supernatant. Subtraction of the $[^{14}\text{C}]$ sorbitol space from the $[^{3}\text{H}]\text{O}$ space gave the osmotic volume. The extrachloroplastic volume of the protoplasts was calculated as the difference between the stromal and protoplast osmotic volumes. $[^{3}\text{H}]$ and $[^{14}\text{C}]$ were determined using a TM Analytic liquid scintillation counter in the dual label mode with external standards ratio quench correction.

K⁺ is known to leak out of isolated chloroplasts at high rates (8, 26), the wire-net silicone oil centrifugation technique (29) was used to instantaneously isolate and separate chloroplasts from protoplasts for determination of chloroplast stromal K⁺. Aliquots (100 μl) of protoplasts were layered onto silicone oil/wire net tubes. These tubes were constructed as follows. Wash medium (30 μl) was layered onto 100 μl of silicone oil in 0.4 ml microfuge tubes which had their caps removed. The tips of 1 ml disposable syringes were cut off and used as protoplast reservoirs. Glue was placed around the end of the cutoff tip, a piece of nylon net (10 μm mesh) was placed over the end of the tip, and the nylon net was inserted into the microfuge tube. During centrifugation, chloroplasts were released as the protoplasts pass through the net. The plastids pass through the silicone oil layer, while the extrachloroplastic contents of the protoplasts stay in the wash medium layer above the oil. Total protoplast K⁺ was determined by microcentrifuging protoplasts (100 μl) through 100 μl silicone oil. Compartmentalized and total protoplast K⁺ was ascertained by measuring the K⁺ in fractions above and below the silicone layer. The silicone oil used for all experiments was a mixture with a ratio (by weight) of 0.14167:0.6917:0.1667 of 200 (2 centistoke):550:710 Dow Corning oils (William F. Nye, Inc., New Bedford, MA). Stromal K⁺ was corrected for 5% cytoplasmic contamination (29).

**RESULTS AND DISCUSSION**

To supply sufficient K⁺ for growth, wheat plants grown hydroponically are normally supplied with 0.75 to 4.5 mm K⁺ in the
Table I. Effect of Oligomycin on Leaf Slice Photosynthesis of K*-grown Wheat under Dehydration Stress

This experiment was repeated with the 6 mm K* tissue, and similar trends were found. The maximum photosynthetic rate on an area basis was 10.2 μmol CO2/m²-s.

<table>
<thead>
<tr>
<th>Oligomycin</th>
<th>2 mm K*-grown Wheat Photosynthesis</th>
<th>6 mm K*-grown Wheat Photosynthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg/ml</td>
<td>change*</td>
<td>μg/ml</td>
</tr>
<tr>
<td></td>
<td>-0.95 MPa</td>
<td>-3.11 MPa</td>
</tr>
<tr>
<td></td>
<td>-0.95 MPa</td>
<td>-3.11 MPa</td>
</tr>
<tr>
<td>0</td>
<td>134.5</td>
<td>60.9</td>
</tr>
<tr>
<td>6</td>
<td>111.9</td>
<td>58.8</td>
</tr>
<tr>
<td>20</td>
<td>113.6</td>
<td>52.7</td>
</tr>
</tbody>
</table>

*The photosynthetic rate at -3.11 MPa reaction medium water potential is compared to the rate at -0.95 MPa for a given level of oligomycin.

Table II. K* Concentration of Chloroplasts, Protoplasts, and Extrachloroplastic Space of K*-Grown Wheat

Values are means of 4 replications ± se. Chloroplast K* for the 0.2 mm treatment is not reported because too little K* was pelleted in the wire-net silicone oil experiment for analysis. Leaf K* was assayed on the leaves used for protoplast isolation 1 week prior to the experiment.

<table>
<thead>
<tr>
<th>K* in Nutrient Solution</th>
<th>Chloroplast K* Concentration</th>
<th>Extrachloroplastic K* Concentration</th>
<th>Protoplast (Chloroplast + Extrachloroplastic) K* Concentration</th>
<th>Protoplast K* Concentration</th>
<th>Leaf K*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>73.34 ± 7.84</td>
<td>98.66 ± 6.31</td>
<td>44.99 ± 3.96</td>
<td>54.51 ± 5.47</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>177.52 ± 5.88</td>
<td>195.16 ± 4.12</td>
<td>137.41 ± 1.29</td>
<td>194.56 ± 11.00</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>286.44 ± 4.32</td>
<td>293.89 ± 5.77</td>
<td>215.06 ± 1.34</td>
<td>314.73 ± 22.57</td>
<td></td>
</tr>
</tbody>
</table>

nutrient solution (11, 22). Osman et al. (23) found that 1.25 mm K* was the optimum concentration for wheat grown in a 1:1 mixture of sand and grit. In our study, maximum photosynthetic rates in well-watered wheat plants occurred with 1 mm K* in the nutrient solution (Fig. 1). Rates were inhibited at K* concentrations below 0.5 mm K*, and 6 mm K* was in excess of that needed for maximum photosynthesis. Leaf conductance measurements shown in Figure 1B were taken immediately after leaves had reached a steady state of CO2 exchange (under 800 μmol/m²-s light) and had been removed from the leaf chamber after photosynthetic measurements. Leaf conductances were the same for all K* treatments except at 0 mm K* (Fib. 1B), and plants showed this pattern of response when measurements were taken several times during the growth period (data not shown). The results of these experiments indicate that differences in net CO2 uptake were not due to variations in stomatal resistance at 0.2 mm nutrient solution K* and above. These data also suggest that with decreasing nutrient solution K* concentrations, chloroplast photosynthetic capacity in well-watered plants can be inhibited prior to decreased stomatal conductance caused by low K*. Leaf K* concentrations were dramatically altered by the K* treatments in the nutrient solution (Fig. 1C). The leaf K* concentration at 6 mm K* was almost double that at 2 mm K*, so the similar rates of photosynthesis (Fig. 1A) at 2 and 6 mm K* were not due to similar K* concentrations in the leaf.

The results of leaf slice photosynthesis with K*-treated wheat showed trends similar to that of whole leaf photosynthesis (Fig. 2). Rates of CO2 fixation by leaf slices were inhibited below 0.5 mm K*, and rates at 2 and 6 mm K* were the same.

The minimum K* required for optimum photosynthesis in a number of crops was similar to that found with wheat. For photosynthesis, threshold levels of K* concentration in the nutrient solution were between 0.3 to 1.2 mm for Phaseolus vulgaris L. (24), 0.8 mm K* for Gossypium hirsutum L. (19), and 0.5 mm K* for Zea mays L. (10). Photosynthesis remained constant or changed only slightly above these threshold K* concentrations. Net carbon exchange of Beta vulgaris L. leaves was the same at fertilization levels of 2 and 10 mm K*, further indicating that K* concentrations above an optimal level do not alter photosynthetic rates (12). Threshold levels of leaf tissue K* for optimal photosynthesis occurred at 1.5 to 2.0% K* in alfalfa K*-grown wheat (11, 12), and 1% in P. vulgaris L. (24), and 1.5 to 2.0% in corn (10, 28), similar to the results in this study with wheat, in which the threshold leaf K* concentration for photosynthesis occurred between 35 and 80 mm K* (Fig. 1, A and C). These data indicate that plants supplied with extra K* can drastically alter leaf K* concentration without affecting photosynthesis under well-watered conditions. For subsequent studies, 0.2, 2, and 6 mm K* were used to give suboptimal, optimal, and suprathermal leaf K* concentrations for photosynthesis, respectively.

Photosynthesis of Dehydrated Leaf Slices. The results of ^14CO2 fixation by leaf slices from K*-treated plants and dehydrated by exposure to assay media of various water potentials are shown in Figure 3. The 0.2 mm K*-grown wheat was most sensitive to dehydration. Photosynthetic rates decreased with the first decrease in water potential, and at -3.11 MPa rates had decreased by 35% from the highest rate at 0 MPa. Berkowitz and Whalen (5) found that photosynthesis of leaf slices from spinach depleted of K* was more susceptible to dehydration than control plants, similar to the results with wheat found here. The 2 mm K*-grown wheat was also sensitive to dehydration, although less so than the 0.2 mm K* plants. Photosynthesis was inhibited by 20.4% at -3.11 MPa when compared to the highest rates at -0.95 MPa. The shift to 0.95 MPa for maximum CO2 fixation indicated that possibly a change in water potential of the assay medium was needed to maintain isotonicity and support maximum photosynthesis. This may have been due to higher K* concentrations (and ambient cell solute level in the 2 mm K*-grown plant compared to the 0.2 mm K*-grown plant. Maximum rates for the 6 mm K*-grown wheat were at -1.57 MPa ψw, with only an 8% decrease in photosynthesis at -3.11 MPa. When compared to the rate of the 2 mm K* tissue at -3.11 MPa, the photosynthetic rate of the 6 mm K* leaf tissue was 28% greater.
Although the leaf slice photosynthetic rates of 6 mM K⁺ and 2 mM K⁺ wheat were similar at high \( \Psi_w \) (Figs. 2 and 3), the inhibitory effect of low \( \Psi_w \) on photosynthesis was nearly eliminated in the presence of high leaf K⁺, as shown in Figure 3. It should be noted that the inhibition of photosynthesis at low \( \Psi_w \) in leaf slice experiments was not as great as that found in whole leaf, gas exchange studies (e.g., Table III). As noted in the legend of Figure 4, the \( \Psi_w \) of wheat leaves was about -1.7 to -1.9 MPa. Therefore, exposure to solution \( \Psi_w \) of <3 MPa represented only a 1.1 to 1.3 MPa stress. Also, leaf photosynthesis was presumably monitored under saturating internal [CO₂], while this was not the case in the gas exchange studies (e.g., Table III). These factors may have contributed to the apparent differences in whole leaf and leaf slice response to low \( \Psi_w \).

The K⁺ protective effect was studied using oligomycin (Table I), which has been shown to inhibit a chloroplast membrane ATPase which interacts with K⁺/H⁺ exchange across the envelope membrane (20). The 2 mM K⁺ plant showed no change of inhibition under water stress in the presence of oligomycin. For the 6 mM K⁺ plant, however, the protective effect of the high K⁺ at low \( \Psi_w \) was lost in the presence of oligomycin, and photosynthesis was inhibited to almost the same degree as in the 2 mM K⁺ plant at low \( \Psi_w \). Since oligomycin is known to inhibit mitochondrial ATPase activity (25), oligomycin effects on respiration of leaf slices were investigated using an \( \text{O}_2 \) electrode. The results indicated that respiration was less than 10% of control photosynthesis (on a leaf area basis) and that oligomycin effects on respiration of 2 and 6 mM K⁺ leaf slices under high and low reaction media \( \Psi_w \) could not account for changes in photosynthesis in the presence of oligomycin (data not shown). Vanadate, a plasma membrane ATPase inhibitor (30), and the ATPase inhibitor ouabain (20) did not affect leaf slice photosynthesis of the 2 and 6 mM K⁺-grown wheat under control and water stress conditions (data not shown). It can be hypothesized that oligomycin was effective in reducing K⁺/H⁺ exchange across the chloroplast envelope, and this eliminated the protective effect of high K⁺ on chloroplast photosynthesis.

The mechanism of the K⁺ protective effect under water stress is indicated by several lines of evidence. Maury et al. (20) described the K⁺/H⁺ antiport on the chloroplast envelope membrane discussed above. In the presence of Mg²⁺, which activated the envelope ATPase, and at low extrachloroplastic K⁺, K⁺ moved out of the stroma of isolated chloroplasts in exchange for H⁺, causing stromal acidification which in turn inhibited photosynthesis. The reverse occurred at high extrachloroplastic K⁺ with K⁺ moving in and H⁺ out, preventing stromal acidification and restoring photosynthesis. Berkowitz and Gibbs (3) found that using the stromal alkalization agent NH₄Cl to raise the stromal pH of isolated chloroplasts exposed to low \( \Psi_w \), partially reversed the dehydration-induced inhibition of photosynthesis. These data suggest that suboptimal pH of the stroma during water stress was inhibiting photosynthesis. Last, the K⁺ protective effect appears to be extrachloroplastic, since chloroplasts isolated from control and K⁺-deficient plants were inhibited to the same extent by decreased \( \Psi_w \) of the assay solution (5).

These earlier studies and the results presented here suggest that the protective K⁺ effect in the 6 mM K⁺ plant may be due to increased exchange of extrachloroplastic K⁺ for stromal H⁺ during water stress, thus reversing the suboptimal stroma pH and restoring photosynthesis. The effect of oligomycin on leaf slice photosynthesis implies that the K⁺/H⁺ exchange and the subsequent stromal alkalization were reduced in the 6 mM K⁺ leaf slices during dehydration, allowing for dehydration-induced inhibition of photosynthesis similar to that in the 2 mM K⁺ tissue. The results of the oligomycin experiment (Table I) offer only indirect evidence to support the hypothesis that high leaf K⁺ reduced low \( \Psi_w \); inhibition of photosynthesis due to increased K⁺ import and H⁺ export during dehydration. K⁺ and H⁺ fluxes under the conditions used to induce cell dehydration were not measured in this study. Therefore, our analysis of the oligomycin effect is preliminary. However, considering the results of the control experiments involving oligomycin effects on respiration, and our experiments with other ATPase inhibitors, which Maury et al. (20) found to have no effect on K⁺/H⁺ exchange across the

![Figure 4](https://example.com/figure4.png)

**Figure 4.** Leaf water potentials of 0.2, 2, and 6 mM K⁺-grown wheat subjected to a drying cycle. Control (well watered) \( \Psi_w \) was -1.65 MPa for 0.2 mM K⁺, -1.71 MPa for 2 mM K⁺, and -1.90 MPa for 6 mM K⁺; \( \Psi_w \) at the end of drying cycle was -1.48 MPa for 0.2 mM K⁺, -2.22 MPa for 2 mM K⁺, and -2.59 MPa for 6 mM K⁺ grown plants. Control (well watered) turgor pressures were 0.64 MPa for 0.2 mM K⁺, 0.58 MPa for 2 mM K⁺, and 0.83 MPa for 6 mM K⁺; turgor pressures at the end of the drying cycle were zero turgor for 0.2 mM K⁺, 0.49 MPa for 2 mM K⁺, and 0.22 MPa for 6 mM K⁺-grown plants.

Table III. Effect of Water Stress on Whole Leaf CO₂ Uptake and Leaf Conductance in K⁺-Grown Wheat

<table>
<thead>
<tr>
<th>K⁺ in Nutrient Solution</th>
<th>Control Photosynthesis</th>
<th>Leaf Water Potential Decline</th>
<th>Leaf Conductance</th>
<th>Photosynthesis</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>21.96 ± 2.29</td>
<td>0.75</td>
<td>0.02676 ± 0.001161</td>
<td>6.68 ± 1.21</td>
<td>-36.5</td>
</tr>
<tr>
<td>2</td>
<td>24.78 ± 1.44</td>
<td>0.71</td>
<td>0.05074 ± 0.00724</td>
<td>10.52 ± 0.91</td>
<td>+66.2</td>
</tr>
<tr>
<td>6</td>
<td>26.07 ± 0.76</td>
<td>1.30</td>
<td>0.05856 ± 0.00193</td>
<td>17.48 ± 1.50</td>
<td>+66.2</td>
</tr>
</tbody>
</table>

* Differences between leaf water potentials at the beginning and end of the stress cycle shown in Figure 4.

* The photosynthetic rate of water stressed, 0.2 mm and 6 mm K⁺ plants are compared to the rates of water stressed, 2 mm K⁺ plants.
chloroplast envelope, the proposed hypothesis for the oligomycin effect on photosynthesis of 6 mM K⁺ tissue at high and low Ψₑ, appears to be the most likely explanation. Recent studies (e.g., Robinson [26]) have demonstrated only a loose association between stromal ATP and transenvelope pH in isolated chloroplasts. These data suggest that an envelope ATPase may not be involved in H⁺ movement across the plastid-limiting membrane in all cases. However, an important distinction between this study (26) and the earlier study demonstrating the involvement of an oligomycin-sensitive ATPase in K⁺/H⁺ antiport (20), is the requirement for free Mg²⁺ (or other divalent cations) outside the chloroplast in order for ATPase-mediated K⁺/H⁺ antiport to occur. The lack of ATPase involvement in H⁺ movement was demonstrated when virtually no free Mg²⁺ was present in the reaction media (26). Therefore, the recent studies of Robinson (26) do not necessarily suggest a reassessment of the hypothesis developed by Maury et al. (20). Presumably, in the photosynthesis experiments reported here, some free Mg²⁺ was present in the cytoplasm of the leaf tissue which could activate the ATPase-mediated K⁺/H⁺ antiport.

Cell Compartment K⁺ Analysis. Studies were undertaken with protoplasts isolated from wheat plants grown on varying K⁺ fertilization in order to determine changes in compartmentalized K⁺ concentrations in the leaf cells (Table II). Wire-net silicone oil centrifugation techniques were used in these experiments to instantaneously sequester chloroplasts for K⁺ analysis as soon as they were liberated from protoplasts. Total protoplast K⁺ was estimated two different ways in these experiments; both are presented. In Table II, the 'Protoplast (chloroplast + extrachloroplastic) K⁺' figures refer to the pooling of K⁺ found in the chloroplast pellet and supernatant fractions of wire-net silicone oil centrifugation experiments. The Protoplast K⁺ figures refer to K⁺ assayed in the protoplast pellet from silicone oil centrifugation experiments. A comparison of these data indicates that the pooling of the two fractions (i.e. the chloroplast pellet and the reaction medium remaining above the oil) generated in the wire-net experiment yielded more cellular K⁺ than was obtained by separating protoplasts from the reaction medium. This discrepancy could be explained by slow leakage of K⁺ from the isolated protoplasts. This was unexpected, as previous studies have shown that isolated protoplasts retain endogenous K⁺ against leakage to the suspending media (18). A comparison of the two measurements with the total leaf K⁺ determined for a particular treatment (Table II) indicates that the pooled totals for the 2 and 6 mM tissue are nearly identical to the leaf measurements. Therefore, this comparison supports the supposition of some degree of leakage from these protoplasts; about 28% in the case of 2 and 6 mM K⁺ protoplasts, and 54% with 0.2 mM K⁺ protoplasts. However, both sets of data show quite clearly the same effect; about a 60% increase in cellular K⁺ when K⁺ fertilization was increased from 2 to 6 mM. Protoplast (i.e. chloroplast and extrachloroplastic) K⁺ concentration was found to be reduced by 50% in tissue prepared from 0.2 mM grown plants as compared to 2 mM grown plants in this experiment (Table II). Analysis of leaf tissue (Table II) indicated that compared to 2 mM grown leaf tissue, 6 mM tissue K⁺ level was increased by 62% and 0.2 mM tissue K⁺ level decreased by 72%.

In contrast to whole protoplast K⁺ levels, chloroplast K⁺ concentration was not increased in well-watered leaves of 6 mM grown plants as compared to chloroplasts of 2 mM grown plants (Table II). This indicates that the increase in protoplast K⁺ in 6 mM tissue was associated with an increase in extrachloroplastic K⁺, and the decrease in 0.2 mM protoplast K⁺ was associated with a decrease in extrachloroplastic K⁺ (Table II). The data presented in Table II indicate that high K⁺ fertilization reverses the concentration gradient between chloroplast and extrachloroplastic K⁺. Movement of K⁺ according to this concentration gradient from the cytoplasm to the chloroplast during cell dehydoration could be responsible for high K⁺ protection of photosynthesis during cell dehydration (Fig. 2), and could explain the reversal of this effect by oligomycin (Table I). Studies of K⁺ deficiency indicate that cytoplasmic K⁺ concentration can be maintained with declining leaf K⁺, and alterations in leaf K⁺ due to low K⁺ feeding are often manifested in altered vacuole K⁺ (17). However, high vacuole K⁺ is freely available to the cytoplasm (17). Therefore, whether the extrachloroplastic cell K⁺ in 6 mM tissue (table II) was in the vacuole or cytoplasm, it would still be available for import into the chloroplast.

Whole Leaf Photosynthesis of Water-Stressed K⁺-Treated Wheat. In order to determine whether the protective effect of high K⁺ could be observed in whole plants, wheat plants grown on 0.2, 2, and 6 mM K⁺ were water stressed over an 8 d period (Fig. 4). Leaf water potentials declined from −0.84, −1.02, and −1.07 MPa for 0.2, 2, and 6 mM K⁺ plants, respectively, to −1.59, −1.73, and −2.37 MPa during the 8 day stress cycle shown in Figure 4. CO₂ uptake by whole leaves under stress (Table III) showed responses similar to that of leaf slices at low Ψₑ (Fig. 3). Under in situ water stress conditions, photosynthesis of the 0.2 mM K⁺-grown plants was inhibited 36.5% compared to the 2 mM K⁺ plants. Rates for the 6 mM K⁺ wheat, however, were enhanced by 66.2% over that of the 2 mM K⁺ plants, whereas under well-watered conditions, photosynthetic rates (Table III; Fig. 1A) for the 2 and 6 mM K⁺ plants were similar. This photosynthetic rate enhancement occurred in 6 mM K⁺ grown plants under water stress despite similar levels of leaf conductance in 2 and 6 mM K⁺-grown plants (Table III). These data, then, indicate that high K⁺ enhancement of photosynthesis in water-stressed wheat plants is not mediated by altered stomatal response.

This experiment was repeated with a second set of plants

Table IV. Effect of Water Stress on Leaf Water Potential, Leaf Conductance, Internal CO₂ Concentration, and Whole Leaf CO₂ Uptake in K⁺-Grown Wheat

<table>
<thead>
<tr>
<th>K⁺ in Nutrient Solution</th>
<th>Leaf Water Potential</th>
<th>Leaf Conductance</th>
<th>Internal CO₂ Concentration</th>
<th>Photosynthesis</th>
<th>Change*</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm</td>
<td>MPa</td>
<td>mol H₂O/m²·s</td>
<td>μL/L</td>
<td>μmol CO₂/m²·s</td>
<td>%</td>
</tr>
<tr>
<td>0.2</td>
<td>0.560 ± 1.080</td>
<td>0.03824 ± 0.00551</td>
<td>266.7 ± 27.1</td>
<td>2.60 ± 0.48</td>
<td>−3.5</td>
</tr>
<tr>
<td>2</td>
<td>0.453 ± 1.425</td>
<td>0.03813 ± 0.00543</td>
<td>265.2 ± 14.3</td>
<td>2.70 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.543 ± 1.405</td>
<td>0.04383 ± 0.00382</td>
<td>197.3 ± 47.9</td>
<td>5.77 ± 1.03</td>
<td>+113.9</td>
</tr>
</tbody>
</table>

*The photosynthetic rates of water-stressed 0.2 and 6 mM K⁺ plants are compared to the rates of water-stressed 2 mM K⁺ plants.
Leaf $K^+$ of well-watered plants was similar under the different $K^+$ fertilization regimes (Table IV). During the stress cycle, small amounts of water were intermittently added to the pots of the stressed plants in a fashion similar to the protocol for the experiment shown in Table III and Figure 4. After 6 d of stress, leaf $K^+$ had dropped about 1 MPa in the 2 and 6 mm $K^+$ plants, and by 0.5 MPa in the 0.2 mm $K^+$ plants (Table IV). Photosynthetic rates of water-stressed 6 mm $K^+$ plants were 113.9% higher than the rates found with stressed 2 mm $K^+$ plants, and the leaf conductance was somewhat higher in the 6 mm $K^+$ plants as compared to the 2 mm $K^+$ plants (Table IV). However, the internal $CO_2$ concentration of the water-stressed 6 mm $K^+$ plants was lower than that of stressed 2 mm $K^+$ plants (Table IV), supporting the contention that the high $K^+$ effect on photosynthesis under stress is not due to stomatal effects. The data presented in Tables III and IV extend the hypotheses developed using the model system of leaf slices vacuum-infiltrated with saturating $CO_2$ and high concentrations of sorbitol as shown in Figures 2 and 3, and in Ref. 5.

In summary, the data presented here support the hypothesis that excess $K^+$ in the leaf partially protects photosynthesis against the deleterious effects of water stress. High levels of $K^+$ prevented inhibition of photosynthesis under dehydration stress in both leaf slices and attached whole leaves. This protective effect appears to be mediated by extrachloroplastic $K^+$ in the plant cells, possibly acting on chloroplast photosynthesis through the mechanism of a $K^+/H^+$ antiport system.

LITERATURE CITED

1. ARNON DI 1949 Copper enzyme in chloroplasts. Polyphenoloxidases in Beta vulgaris. Plant Physiol 24: 1–4
17. LEIGH RA, RG WYN JONES 1984 A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol 97: 1–13
27. ROBINSON SP 1986 Improved rates of $CO_2$ fixation by intact chloroplasts isolated in media with KCl as the osmoticum. Photosynth Res 10: 93–100