Role of Potassium in Carbon Dioxide Assimilation in *Medicago sativa* L.  

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ABSTRACT

Alfalfa was grown hydroponically in 0, 0.6, and 4.8 millimolar K in order to determine the influence of tissue level of K on photosynthesis, dark respiration, photosynthesis, stomatal and mesophyll resistance to CO₂, photosystem I and II activity, and synthesis and activity of ribulose-1,5-bisphosphate carboxylase (RuBPc).

A severe (0.0 millimolar) and mild (0.6 millimolar) K deficiency, compared to plants grown at 4.8 millimolar K, produced a significant decrease in photosynthesis and photosynthesis, but an increase in dark respiration. Both deficient K levels increased hydrophylic resistance to CO₂, but only the severe deficiency increased stomatal resistance.

Photosystem I and II activity of isolated chloroplasts was not affected by K deficiency. The apparent activity of a crude RuBPc preparation was significantly reduced in severely deficient plants. Activity of the enzyme could not be restored to normal rates by the addition of K to the reaction medium.

The specific activity of RuBPc isolated from severely K-deficient and K-sufficient leaflets was not significantly different, suggesting that K does not function in RuBPc activity. Incorporation of ¹⁴C-leucine into RuBPc, as a measure of synthesis, by K-deficient leaflets was reduced to 15% of K-sufficient leaflets. The addition of K to the reaction medium stimulated [¹⁴C]leucine incorporation into RuBPc and 10 millimolar KNO₃ increased incorporation to 90% of K-sufficient leaflets. Actinomycin D and cycloheximide suppressed the K-stimulated incorporation of [¹⁴C]leucine into RuBPc, suggesting that the K-stimulated synthesis of RuBPc most likely represents de novo synthesis.

K deficiency has been shown to reduce the rate of photosynthesis in numerous higher plants (2, 10, 12, 13, 18, 19). Cooper et al. (3) attributed the decrease in photosynthesis of K-deficient alfalfa to a decrease in stomatal number and aperture size. Reduction in photosynthesis of K-deficient corn was attributed to increased stomatal resistance (4, 9). A combination of increased stomatal and mesophyll resistance to CO₂ was thought to reduce photosynthesis in K-deficient sugarbeets (20, 21). Since K is the major solute in turgid guard cells (6), it is reasonable to suggest that K deficiency will result in stomatal closure. In the presence of Na, low K increased stomatal resistance less, but mesophyll resistance remained high (21). These results suggest that the role of K in regulating photosynthesis may be within mesophyll cells.

The nature of increased mesophyll resistance in K-deficient tissue is not clear. Chloroplast electron transport was decreased in K-deficient spinach and sunflower (16) and tomato (18), but not in corn (1). Phosphoenolpyruvate-stimulated CO₂ fixation in enzyme preparations from K-deficient sweet potato leaves was significantly decreased and could not be restored to normal rates by the addition of K to the reaction buffer (22).

The purpose of this investigation was to determine the effect of tissue level of K on CO₂ assimilation, chloroplast electron transport, and diffusive resistances in alfalfa.

MATERIALS AND METHODS

**Plant Material.** Clonal propagules derived from a single plant of alfalfa (*Medicago sativa* L.) cv. 'Iroquois' were established in hydroponic sand culture. The composition of the nutrient solution in mm was: NH₄H₂PO₄, 1.0; Mg(NO₃)₂, 1.0; and MgSO₄, 2.0. Micronutrients were supplied in the following concentrations, expressed as μm: H₂BO₃, 38; CuSO₄, 10.0; MnCl₂, 9.2; H₂MoO₄, 0.5; monosodium ferric EDTA (Sequestrene NaFe), 172; and Sequestrene Na₂Zn, 1.0. K was supplied as KNO₃ at 0.0, 0.6, and 4.8 mm K. Concentration of Ca(NO₃)₂ was 6.5, 6.2, and 4.6 and that of CaCl₂ was 0.0, 0.3, and 2.4 mm for the 0, 0.6, and 4.8 mm K solutions, respectively. One plant per crock was established and the crows were arranged in a completely randomized block design with eight replications. Crocks were subirrigated every 2 h during the day and every 4 h at night. Nutrient solutions were changed every 2 weeks. The temperature in the greenhouse was 25/20 ± 5 C day/night, and the average daily solar radiation was approximately 310 cal cm⁻² day⁻¹. Plants were cut back to a 5-cm stubble when they reached the ½ bloom stage and had attained approximately 20 days of regrowth (early bud stage) at the time of measurement.

**Gas Exchange.** Photosynthesis, transpiration, dark respiration, and stomatal and mesophyll resistance to CO₂ diffusion were measured on 20 individual attached leaves (fourth fully expanded leaf from the top) per treatment using an air-sealed leaf chamber (26). Beckman model 865 IR gas analyzer, and Cambridge model 880 dew point hygrometer. Each parameter was calculated according to previously derived equations (14). The apparatus and technique used for gas exchange measurements were described previously (14). Dark respiration was determined on the same leaf immediately following the light measurement. Photosynthesis and stomatal resistance measurements were repeated at the time of measurement according to the method of Thomas et al. (23).

**PSII Analysis.** Reduction of NADP⁺ and ferricyanide was determined by the spectrophotometric methods of Trebst (24) as a measure of PSII activity. Chloroplasts were isolated from 10 g depetiolated leaves from each plant in 25 ml extraction buffer (15). RuBPc.² The apparent activity of RuBPc (EC 4.1.1.39) was

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² Abbreviations: RuBPc: ribulose-1,5-bisphosphate carboxylase; RuBP: ribulose 1,5-bisphosphate.
determined on a crude enzyme extract from leaves of each plant (8). The crude enzyme extract was prepared by thoroughly grinding 3 to 4 g leaf tissue in a chilled mortar containing 10 ml buffer (0.2 M Tricine [pH 8.3], 10 mM MgCl₂, 20 mM 2-mercaptoethanol, 0.2 mM EDTA, PVP 16 g/l) and a small amount of acid-washed sand. The resulting homogenate was filtered through Whatman paper, centrifuged for 20 min at 30,000 g at 4 C, and the supernatant used for RuBPc assay.

The assay reaction mixture contained (in a volume of 200 μl): 30 μmol Tricine (pH 8.0), 10 μmol MgCl₂, 8 μmol 2-mercaptoethanol, 0.4 μmol RuBP, 2.0 μmol NaH¹⁴CO₃. Assays were initiated by the addition of 100 μl crude enzyme extract and run at 27 C for 5 min. Assays were terminated by the addition of 200 μl 25 % (v/v) acetic acid. Assay mixtures were counted by liquid scintillation spectroscopy. The protein content of the crude enzyme extract was determined by the Folin phenol method (11) and enzyme activity expressed as μmol CO₂ fixed/mg protein/h.

K was added to the reaction buffer to a final concentration of 0.0, 0.01, 0.05, 0.10, 0.50, 1.00, or 10.00 mM K as KNO₃. Enzyme from deficient leaves was assayed for activity at each level of KNO₃ and replicated 10 times.

A crude RuBPc extract was isolated from visually K-deficient and K-sufficient depetiolated leaf tissue (25). Ammonium sulfate was added to the crude extract to 35 % saturation (209 g/l) and allowed to stand for 30 min. The solution was then centrifuged at 9,000 g for 60 min and the pellet discarded. Ammonium sulfate (61 g/l) was then added to increase the concentration to 45 % saturation, allowed to stand for 30 min, and centrifuged for 60 min at 9,000 g. The supernatant was discarded and the protein pellet resuspended in 1.5 ml buffer (25 mM Tris-HCl [pH 7.4], 0.2 mM NaCl, 0.5 mM EDTA). The suspension was then applied to a Sephadex G-25 column (25 × 300 mm). The protein was eluted at a rate of 3 ml/20 min and collected in 3-ml fractions. The protein content of each fraction was determined spectrophotometrically. The concentration of protein in mg/ml equals the A at 280 nm minus the A at 260 nm × 0.76. Specific activity of the purified RuBPc was determined by incorporation of ¹⁴C into stable products.

Electrophoresis. Horizontal 7% (w/v) acrylamide gels in 0.005% (w/v) L-histidine (pH 7.0) were prepared for electrophoresis of the RuBPc-peak. Each gel was run for 5 h at 200 mamp at 4 C with 0.1 M Tris-citrate (pH 7.0). The gels were stained with 0.25% (w/v) Coomassie blue in 50% (v/v) methanol with 7% acetic acid for 24 h and destained in 50% methanol (v/v) with 10% acetic acid. ¹⁴C-leucine Incorporation. Twenty leaflets of similar age from K-deficient plants were floated on abaxial surface in contact with 10 ml of buffer solution (0.04 M Tris-HCl [pH 7.3], 0.005% [v/v] Triton X-100, 0.1 μCi [¹⁴C]leucine) in Petri plates (7). Specific activity of leucine was 55 mCi/mmol (New England Nuclear). K was added to the buffer to a final concentration of 0.0, 0.01, 0.05, 0.10, 0.50, 1.00, or 10.00 mM as KNO₃. Leaflets from plants grown at 4.8 mM substrate K were also used. The leaflets were incubated at 25 C for 20 h in light from two, 20-w cool-white fluorescent lamps which supplied a photon flux density of 80 nE cm⁻² s⁻¹ (400–700 nm). The Petri plates were gently swirled during incubation. Leaflets were also incubated in 10 mM K buffer with either cycloheximide (17 μg/ml) or actinomycin D (67 μg/ml) added to the buffer.

Following incubation, the leaflets were rinsed in distilled deionized H₂O and the RuBPc isolated and purified from the leaflets as described above. The RuBPc pellet was suspended in 1.0 ml 0.1 N KOH and the solution counted in a liquid scintillation spectrometer. Counting efficiency was determined by internal standardization with [¹⁴C]toluene. Synthesis was calculated as dpm mg RuBPc⁻¹ h⁻¹. Each treatment was replicated four times.

RESULTS AND DISCUSSION

Plants supplied with sufficient levels of K (4.8 mM) maintained a vigorous growth rate whereas deficient levels of K (0.6 and 0.0 mM) tended to suppress growth of both the roots and tops. Deficient levels of K also tended to hasten maturity of the plants with first bud occurring approximately 3 days earlier than sufficient plants. Photosynthesis, photorespiration, and leaf K were significantly reduced by decreased levels of substrate K (Table I). The ratio of photorespiration to photosynthesis was similar in the sufficient (4.8 mM) and mildly deficient (0.6 mM) plants, 0.26 and 0.27, respectively. Severe K deficiency resulted in an increased ratio (0.34), indicating that the decrease in photorespiration was less than the decrease in photosynthesis. K deficiency increased dark respiration, but the increase was statistically significant only at the more severe K deficiency. A mild K deficiency increased mesophyll resistance, but did not significantly increase stomatal resistance (Table II). Stomatal resistance increased significantly only with more severe K deficiency.

The effect of K level on various components of mesophyll resistance are presented in Table III. PSI and PSII activities were not significantly affected by K deficiency, but the activity of the crude RuBPc preparation was reduced by a severe K deficiency. Addition of K to the reaction medium in quantities failed to restore the crude extract to normal activity (data not shown). The specific activity of purified RuBPc from K-deficient leaves was not significantly reduced compared to the K-sufficient enzyme (data not shown). Since the specific activity of RuBPc was not affected by K deficiency it was presumed that K was not involved in RuBPc activity (5).

Table I. The effect of three levels of substrate potassium on carbon dioxide exchange in alfalfa leaves. Each figure represents the mean of 20 measurements.

<table>
<thead>
<tr>
<th>Substrate potassium (mM)</th>
<th>Photosynthesis mg dm⁻² h⁻¹</th>
<th>Photorespiration dpm/dpm²</th>
<th>Dark respiration mg dm⁻² h⁻¹ %</th>
<th>Leaf K</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>11.88 a</td>
<td>4.00 a</td>
<td>7.56 a</td>
<td>1.28 a</td>
</tr>
<tr>
<td>0.6</td>
<td>21.72 b</td>
<td>5.87 b</td>
<td>5.34 b</td>
<td>1.98 b</td>
</tr>
<tr>
<td>4.8</td>
<td>33.97 c</td>
<td>8.96 c</td>
<td>3.06 b</td>
<td>3.84 c</td>
</tr>
</tbody>
</table>

1 Means within a column followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.

2 ¹⁴CO₂ evolved per ¹⁴CO₂ absorbed by the leaflets.
Acrylamide gel electrophoresis of the purified RuBPc peak indicated that only RuBPc was present in the sample and that the enzyme preparation was purified RuBPc. The R2 of the enzyme was 0.11 in the 7.0% gel, which corresponds to an R2 value of 0.11 using 7.5% acrylamide gels (7).

The incorporation of [14C]leucine into RuBPc isolated from K-deficient leaflets was reduced to 15% that of the K-sufficient leaflets (Table IV). Increasing levels of K in the incubation medium led to an increase in [14C]leucine incorporation. When Ca(NO3)2 was added in place of KNO3, [14C]leucine incorporation was not increased in K-deficient leaflets. These data indicate that K is involved in the synthesis of RuBPc in alfalfa leaves and the observed responses were not due to increased NO3. The concentration of RuBPc in K-deficient leaves was significantly reduced compared to K-sufficient leaves (1.29 and 2.88 mg g-1 fresh weight, respectively), further indicating that K deficiency results in decreased RuBPc available for CO2 fixation.

In the presence of 67 µg/ml actinomycin D and 17 µg/ml cycloheximide, RuBPc synthesis was inhibited (32.3 and 51.4 dpm mg protein-1 24 h-1, respectively, compared with 525.9 dpm mg protein-1 24 h-1 for the control). Since the K-stimulated formation of RuBPc was suppressed by actinomycin D and cycloheximide, it appears that the mechanism by which K is involved in RuBPc synthesis involves DNA-dependent RNA synthesis and subsequent protein synthesis. A similar finding was made for the zinc-stimulated formation of RuBPc in bean (7). A suppression of RuBPc synthesis in K-deficient leaves led to a reduction in RuBPc levels in leaves.

This study agrees with the results with sugar beets, in which K deficiency decreased both photosynthesis and photorespiration and increased stomatal and mesophyll resistance to CO2 diffusion (20, 21). Stomatal resistance of K-deficient sugar beet leaves did not increase until leaf K decreased below approximately 200 meq/kg while mesophyll resistance increased when blade K decreased to approximately 400 meq/kg (20). The sensitivity of mesophyll resistance, compared to stomatal resistance, was also observed in the present study, where a mild K deficiency increased mesophyll resistance, but did not significantly affect stomatal resistance. The level at which K is deficient in alfalfa leaves, as shown in this study, was higher than in sugar beet leaves. An increase in mesophyll resistance was found at a K concentration of 1.98% (506 meq/kg). Stomatal resistance increased at a leaf level of 1.28% (327 meq/kg).

The role of mesophyll resistance in the reduction of photosynthesis in K-deficient leaves has been described (2, 20, 27); however, no consistent results have been presented to describe the component responsible for the increased resistance under K-deficient conditions. Chloroplasts isolated from K-deficient corn showed no difference in PSI and PSII activities compared to K-sufficient plants (1). In several other species, it was shown that a reduction in PSI and PSII activity would occur in K-deficient chloroplasts (16, 17). K deficiency did not reduce PSI and PSII activities in this study.

The apparent activity of a crude RuBPc preparation was reduced in K-deficient leaves. Since a crude preparation was used to measure RuBPc activity, it was not possible to ascertain if the specific activity or the quantity of RuBPc was responsible for the observed reductions in RuBPc fixation.

In this study, reduced photosynthetic rates in mildly K-deficient alfalfa leaves (levels at which visible symptoms were not apparent) was associated with decreased synthesis of RuBPc. Photosynthetic rates also declined with mild K deficiency. Stomatal resistance

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**Table IV.** The effect of K+ on incorporation of 14C-leucine into ribulose 1,5-bisphosphate carboxylase in leaves of potassium deficient alfalfa. Each value represents the mean of four measurements.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>14C-leucine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO3</td>
<td>dpm-mg RuBPc-1 24 h-1</td>
</tr>
<tr>
<td>Control</td>
<td>655.5a^1</td>
</tr>
<tr>
<td>0.0</td>
<td>525.9b</td>
</tr>
<tr>
<td>1.00</td>
<td>274.5c</td>
</tr>
<tr>
<td>0.10</td>
<td>219.8cd</td>
</tr>
<tr>
<td>0.01</td>
<td>166.6de</td>
</tr>
<tr>
<td>0.00</td>
<td>99.4e</td>
</tr>
</tbody>
</table>

^1 Means within a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.

^2 Normal leaflets were obtained from potassium sufficient plants.

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**Table II.** The effect of three levels of substrate potassium on carbon dioxide diffusive resistances in alfalfa leaves. Each value represents the mean of 20 measurements.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Stomatal resistance</th>
<th>Mesophyll resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>sec cm-1</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>9.32a^1</td>
<td>8.65a</td>
</tr>
<tr>
<td>0.6</td>
<td>6.80b</td>
<td>7.01a</td>
</tr>
<tr>
<td>4.8</td>
<td>5.95b</td>
<td>2.99b</td>
</tr>
</tbody>
</table>

^1 Means within a column followed by the same letter are not significantly different (P<0.05) according to Duncan's multiple range test.

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**Table III.** The effect of three levels of substrate potassium on photosystem I and II and ribulose 1,5-bisphosphate carboxylase activity of alfalfa. Each value represents the mean of 10 measurements.

<table>
<thead>
<tr>
<th>Substrate potassium</th>
<th>Photosystem I activity</th>
<th>Photosystem II activity</th>
<th>Ribulose bisphosphate carboxylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>umol acceptor reduced mg Chl-1 h-1</td>
<td>mg CO2 mg protein-1 h-1</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>238a^1</td>
<td>282a</td>
<td>1.79a</td>
</tr>
<tr>
<td>0.6</td>
<td>262a</td>
<td>294a</td>
<td>4.51b</td>
</tr>
<tr>
<td>4.8</td>
<td>241a</td>
<td>285a</td>
<td>6.06b</td>
</tr>
</tbody>
</table>

^1 Means within a column followed by the same letter are not significantly different (P< 0.05) according to Duncan's multiple range test.
did not increase until K deficiency progressed (visible symptoms evident).

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