Nutrient Influences on Leaf Photosynthesis

EFFECTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM FOR GOSSYPIUM HIRSUTUM L.\(^1\)

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ABSTRACT

The net rate of CO\(_2\) uptake for leaves of Gossypium hirsutum L. was reduced when the plants were grown at low concentrations of NO\(_3^-\), PO\(_4^{3-}\), or K\(^+\). The water vapor conductance was relatively constant for all nutrient levels, indicating little effect on stomatal response. Although leaves under nutrient stress tended to be lower in chlorophyll and thinner, the ratio of mesophyll surface area to leaf area did not change appreciably. Thus, the reduction in CO\(_2\) uptake rate at low nutrient levels was due to a decrease in the CO\(_2\) conductance expressed per unit mesophyll cell wall area (g\(_{\text{cell}}\)). The use of g\(_{\text{cell}}\) and nutrient levels expressed per unit of mesophyll cell wall provides a new means of assessing nutrient effects on CO\(_2\) uptake of leaves.

Plant mineral status can markedly affect photosynthesis. For instance, J\(_{\text{CO}_2}\) is reduced in leaves that are deficient in N (10, 16, 18, 25), P (16, 20, 22), or K (3, 16, 17, 21, 24). Describing J\(_{\text{CO}_2}\) as a diffusion process controlled by g\(_{\text{meso}}\) and g\(_{\text{cell}}\) (8) facilitates examination of nutrient effects on photosynthesis. With decreasing N content, g\(_{\text{meso}}\) appears to be the main control of J\(_{\text{CO}_2}\) in C\(_3\) plants (11, 18). For sugar beet and clover, g\(_{\text{meso}}\) is more responsive to decreasing P or K than is g\(_{\text{cell}}\) (17, 18, 21). Measurement of leaf anatomy allows division of g\(_{\text{meso}}\) into a geometrical component, \(A^{\text{meso}}/A\), and g\(_{\text{cell}}\). The cellular term includes the diffusion pathway into the mesophyll cells, as well as the initial biochemical step of CO\(_2\) fixation (14). Nutrient status could affect \(A^{\text{meso}}/A\), since packing of mesophyll cells (6), the amount of cell wall per unit leaf dry weight (19), specific leaf weight (16, 25), and leaf thickness (2) have all been shown to vary with N and/or P levels. Nutrient effects on g\(_{\text{cell}}\) are also probable, since reductions in total soluble protein and total Chl are correlated with low N levels (12). The objective of the present study was to examine the specific effects of N, P, and K on \(A^{\text{meso}}/A\) and g\(_{\text{cell}}\) for cotton.

MATERIALS AND METHODS

Seedlings of Gossypium hirsutum L. var. Acala SJ-2 were trans-ferred to hydroponic culture 12–14 days after germination. The concentrations of NO\(_3^-\), PO\(_4^{3-}\), and K\(^+\) were individually varied from one sixty-fourth to four times their concentration in full strength Hoagland No. 1 solution (with Hoagland minor solution and 0.08 meq 1\(^{-1}\) Fe\(^{3+}\) sequestered with EDTA; ref. 7). Two seedlings were grown per 8-liter container in growth chambers using a 12-h day at 26 °C with 375 ± 50 \(\mu\)E m\(^{-2}\)s\(^{-1}\) PAR (provided by cool-white fluorescent lamps supplemented 8% with incandescent lights) and a 12-h night at 21 °C.

Solution concentrations were expressed relative to Hoagland solution No. 1, which contains 16 meq 1\(^{-1}\) NO\(_3^-\), 2 meq 1\(^{-1}\) PO\(_4^{3-}\), and 4 meq 1\(^{-1}\) K\(^+\). For NO\(_3^-\) variation, the solution was modified by omitting KNO\(_3\), varying the concentration of Ca(NO\(_3\))\(_2\), adding 2.5 meq 1\(^{-1}\) K\(^+\) as K\(_2\)SO\(_4\), and adding CaCl\(_2\)-2 H\(_2\)O as needed to give a minimum Ca\(^{2+}\) concentration of 5 meq 1\(^{-1}\). For PO\(_4^{3-}\) variation, the KH\(_2\)PO\(_4\) concentration was changed. For K\(^+\) variation, K\(_2\)HPO\(_4\) was omitted, K\(_2\)SO\(_4\) was varied, and 2 meq 1\(^{-1}\) PO\(_4^{3-}\) as Ca(H\(_2\)PO\(_4\)) was added. The range in solution osmotic potential from low to high NO\(_3^-\), PO\(_4^{3-}\), and K\(^+\) averaged 0.17 MPa, which has little effect on morphology and photosynthetic response of cotton (9).

Gas exchange and anatomical measurements were made on the third or fourth leaf above the cotyledonary leaves. These were mature leaves that had developed under a given nutrient treatment for 21–28 days. Rates of water vapor loss and J\(_{\text{CO}_2}\) were determined at 2,000 ± 100 \(\mu\)E m\(^{-2}\)s\(^{-1}\) PAR (light saturation) on attached leaves of at least two plants from each nutrient using a null-point closed-circuit flow system with circulating air containing approximately 1% O\(_2\) (15). The low O\(_2\) minimized effects of respiration and photosynthesis. Leaf temperature was maintained at 30 ± 1 °C, and the water vapor pressure difference between leaf and air was 1.6 ± 0.2 kPa.

Gas exchange rates were analyzed using the appropriate conductances (8, 14). The transpiration rate divided by the water vapor concentration drop from leaf to air gave g\(_{\text{meso}}\). J\(_{\text{CO}_2}\) was plotted versus the CO\(_2\) concentration in the intercellular air spaces next to the stomates, which was the CO\(_2\) concentration outside the leaf minus 1.56 J\(_{\text{CO}_2}/g_{\text{meso}}\); the slope of the line connecting the CO\(_2\) compensation point and the J\(_{\text{CO}_2}\) value at ambient CO\(_2\) concentration (340 µl 1\(^{-1}\)) was designated g\(_{\text{cell}}\) (9, 14). Individual J\(_{\text{CO}_2}\) values varied less than 10% from the reported means. To determine \(A^{\text{meso}}/A\), fresh sections cut from the side of the leaf midvein were infiltrated with distilled H\(_2\)O and examined using a Zeiss microscope with a camera lucida. Cell surface areas were calculated assuming that palisade cells were cylindrical with hemispherical ends and spongy cells were spheres (13). Using \(A^{\text{meso}}/A\) and g\(_{\text{meso}}\), g\(_{\text{cell}}\) was calculated:

\[
g_{\text{cell}} = g_{\text{meso}}/(A^{\text{meso}}/A)
\]

Tissue NO\(_3^-\) concentration was measured with a nitrate ion electrode (Orion 92-07) on samples ground in 25 mM Al\(_2\)(SO\(_4\))\(_3\)

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Abbreviations: J\(_{\text{CO}_2}\): net CO\(_2\) exchange rate per unit leaf area; g\(_{\text{meso}}\): water vapor conductance (primarily stomatal); g\(_{\text{cell}}\): CO\(_2\) mesophyll conductance; \(A^{\text{meso}}/A\): surface area of mesophyll cells per unit leaf surface area; g\(_{\text{cell}}\): cellular CO\(_2\) conductance expressed on a mesophyll surface area basis.
RESULTS

The hydroponic treatments produced a wide range of leaf nutrient levels. Leaf NO$_3^-$-N ranged from 0.02 to 0.55% (dry weight/dry weight) from the low to high NO$_3^-$ treatment, leaf P ranged from 0.05 to 1.34% for the PO$_4^{2-}$ treatments, and K$^+$ ranged from 0.16 to 2.97% for the K$^+$ treatments. Total Chl was reduced 50% in the low NO$_3^-$ and K$^+$ treatments, but only about 8% in low PO$_4^{2-}$ (Table 1). Specific leaf weights for all leaves used in JCO$_2$ measurements averaged 4.4 mg cm$^{-2}$, 10–20% higher values occurring at the lower nutrient levels. Soluble protein/cm$^2$ leaf area (assayed for the NO$_3^-$ treatment only) increased 300% from one sixty-fourth to one-fourth strength NO$_3^-$ and remained constant at higher concentrations.

Nutrient treatment did not affect epidermal thickness (upper surfaces averaged 15 μm and lower surfaces, 16 μm), and so differences in leaf thickness mirrored differences in mesophyll thickness. For the lowest nutrient concentrations the mesophyll region was 8–12% thinner than the average value (Table 1). However, A$^{\text{mean}}$/A was relatively constant for all treatments (Fig. 1).

At the lowest nutrient concentrations, JCO$_2$ was approximately 50% of the value found at one-fourth strength Hoagland solution (Fig. 2A). Stomatal conductance (indicated by g$_{st}$) changed little with increasing concentration of each nutrient (Fig. 2B). The increase in JCO$_2$ with increasing nutrient levels reflected a greater g$_{st}$ (Fig. 2C), which in turn was due to changes in g$_{cc2}$. In fact, g$_{cc2}$ more than doubled from the low to one-fourth strength NO$_3^-$, PO$_4^{2-}$, and K$^+$ (Fig. 3). For each nutrient, g$_{cc2}$ rose rapidly at low nutrient levels (expressed as amount per unit of mesophyll cell surface) and then remained constant at about 0.15 mm s$^{-1}$ over a large range (Fig. 4).

DISCUSSION

Reduction in the net rate of CO$_2$ uptake occurred at low concentrations of NO$_3^-$, PO$_4^{2-}$, and K$^+$ for cotton (Fig. 2). The reduction was primarily due to a decrease in g$_{cc2}$, similar to findings for nutrient effects on other species (11, 18, 20, 21). Cell dimensions decreased slightly at the lowest nutrient levels, but in such a way that A$^{\text{mean}}$/A varied little (Fig. 1). Thus, nutrient effects

Table 1. Total Chl and Leaf Thickness for Plants Grown at Various Concentrations of NO$_3^-$, PO$_4^{2-}$, or K$^+$

<table>
<thead>
<tr>
<th>Solution Concentration (Hoagland units)</th>
<th>1/4</th>
<th>1/8</th>
<th>1/4</th>
<th>1</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Chl (μg cm$^{-2}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>21</td>
<td>39</td>
<td>48</td>
<td>46</td>
<td>49</td>
</tr>
<tr>
<td>PO$_4^{2-}$</td>
<td>42</td>
<td>50</td>
<td>46</td>
<td>47</td>
<td>51</td>
</tr>
<tr>
<td>K$^+$</td>
<td>26</td>
<td>48</td>
<td>46</td>
<td>50</td>
<td>46</td>
</tr>
<tr>
<td>Mesophyll thickness (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>198</td>
<td>214</td>
<td>211</td>
<td>223</td>
<td>232</td>
</tr>
<tr>
<td>PO$_4^{2-}$</td>
<td>208</td>
<td>214</td>
<td>220</td>
<td>242</td>
<td>226</td>
</tr>
<tr>
<td>K$^+$</td>
<td>200</td>
<td>228</td>
<td>240</td>
<td>240</td>
<td>224</td>
</tr>
</tbody>
</table>

on JCO$_2$ reflected changes at the cellular level as represented by g$_{cc2}$ (Fig. 3), similar to previous results on Plectranthus parviflorus (13). Such responses are in contrast to those for differences in illumination during leaf development, which affects primarily A$^{\text{mean}}$/A (13, 14), and salinity, which can affect both A$^{\text{mean}}$/A and g$_{cc2}$ (9).

Changes in stomatal conductance (deduced by measuring g$_{ss}$) did not appreciably affect JCO$_2$ at any nutrient concentration (Fig. 2). Although previous work with C$_3$ plants indicated that variations in NO$_3^-$ mainly influenced g$_{cc2}$ (11, 18), appreciable changes

FIG. 1. A$^{\text{mean}}$/A from leaves of cotton grown hydroponically under various concentrations of NO$_3^-$ (C), PO$_4^{2-}$ (△), or K$^+$ (□).

FIG. 2. JCO$_2$ and related conductances, g$_{st}$, g$_{cc2}$, for plants grown under various concentrations of NO$_3^-$ (C), PO$_4^{2-}$ (△), or K$^+$ (□).

FIG. 3. g$_{cc2}$, for plants grown under various concentrations of NO$_3^-$ (C), PO$_4^{2-}$ (△), or K$^+$ (□).
in $g_{av}$ can occur. Terry and Ulrich (20, 21) showed that $g_{av}$ declined under PO$_4$$^2-$ and K$^+$ stress, but this occurred after declines in $g_{c10}$ had substantially lowered $J_{CO2}$. Likewise, as K$^+$ deficiency increased in Medicago sativa, $g_{c10}$ responded before $g_{av}$ (17). Therefore, changes in $g_{av}$ may be a secondary response to nutrient stress.

Although $A_{max}/A$ varied little (Fig. 1), low nutrient levels had an effect on several leaf properties. Cotton leaves from plants developing under nutrient stress were thinner than those developing under normal concentrations (Table I), unlike two Australian tree species, in which leaf thickness increased with N and P stress (2). Similar to wheat (16), but contrary to rice (25), specific leaf weight of cotton was highest at the lowest NO$_3$- concentration (as well as lowest PO$_4$$^2-$ and K$^+$ for cotton). As in previous reports (11, 12), total Chl was reduced in low NO$_3$-, low K$^+$, and to a lesser extent low PO$_4$$^2-$; soluble protein was reduced in low NO$_3$-.

Our results suggest that some chemical component related to the photochemistry or biochemistry of photosynthesis was leading to the lower $g_{c10}$ under low nutrient treatments.

Relating $g_{c10}$, which varied more with nutrient treatment than the other factors controlling $J_{CO2}$, to nutrient level per unit cell surface area (Fig. 4) provides a direct assessment of the nutrient level necessary for maximum $g_{c10}$. The lower the level necessary to produce a maximum $g_{c10}$, the better potential there is for adaptation of the plant to low nutrient values. This type of comparison could be used to quantify adaptation to nutrient stress of different species or to screen genotypes of crop species for use in nutrient-poor soil.

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FIG. 4. $g_{c10}$ at different nutrient levels per unit area of mesophyll cell wall.