High Photosynthetic Rate of a Chlorophyll Mutant of Cotton

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ABSTRACT

In a chlorophyll mutant (virescent) and wild-type cotton (Gossypium hirsutum L.), a number of photosynthetic parameters have been measured and compared with those published for other chlorophyll mutants. (a) The photosynthetic rates at 230 w/m² (400–700 nm) from a tungsten lamp were 36.8 mg CO₂ fixed/dm²·hr (virescent) and 39.5 mg CO₂ fixed/dm²·hr (wild-type). On a chlorophyll basis, the photosynthetic rates were 36.8 and 12.1 mg CO₂ fixed/mg chl·hr, respectively. (b) The photosynthetic rates at 13 w/m² (400–700 nm) from a tungsten source were 7.1 mg CO₂ fixed/dm²·hr (virescent) and 7.4 mg CO₂ fixed/dm²·hr (wild-type). On a chlorophyll basis, the photosynthetic rates were 6.0 and 1.4 mg CO₂ fixed/mg chl·hr, respectively. (c) The chlorophyll a/b ratios of the virecent and wild-type leaves were 3.3 and 4.1. (d) The chlorophyll/carotenoid ratios for the virecent and wild-type leaves were 3.2 and 7.3, respectively. (e) The photosynthetic carbon metabolism of the chlorophyll mutant was through the reductive pentose phosphate cycle. (f) The CO₂ compensation points for the virecent and wild-type plants were similar. (g) The mutant and wild-type leaves have the same quantum yield in the red part of the visible spectrum, but the virecent leaves have a lower quantum yield in the blue part of the spectrum. (h) Virecent and wild-type leaves contain similar levels on a protein basis of several reductive pentose phosphate cycle enzymes.

MATERIALS AND METHODS

Materials. 3-Phosphoglyceric phosphokinase, glyceraldehyde-3-P dehydrogenase, d(3)-phosphoglyceric acid tricyclohexylammonium salt, d-fructose-1, 6-dip-tetracyclohexylammonium salt, d-glucose-6-P disodium, ribose-1, 5-dip tetrasodium salt, NADH, NADPH, ATP, and GSH were obtained from Sigma Chemical Company. Phosphoenolpyruvic acid monocychohexylammonium salt was obtained from Nutritional Biochemical Co.

Plants. Mutant and normal green plants of Gossypium hirsutum L. were grown in the greenhouse. Killough and Horlacher (15) have described the inheritance of the mutant virecent cotton, and it differs from normal cultivated cotton by recessive alleles at a single locus. The long term inbred TM-1 was used as the source of normal green plants. Virecent plants were derived by five generations of backcrossing to TM-1 and maintained by self-pollination. All of the cotton plants sampled in this study had 9 to 15 leaves. The 3rd and 4th leaves were used for the enzyme and photosynthetic studies.

Preparation of Soluble Leaf Protein. Four to six mutant and normal green leaves were harvested, rinsed with distilled H₂O, blotted, and weighed. The leaves were ground in a chilled...
mortar in 0.1 M tris buffer, pH 7.5, containing 0.1 mM GSH and sand. The brei was squeezed through two layers of cheese-
cloth and centrifuged 30 min at 27,000g in a Sorvall refrigerat-
centrifuge. The soluble supernatant fraction was removed
and used as the source of enzymes.

**Enzyme Assays.** Ribulose-1,5-diphosphate carboxylase was assayed
by the procedure described by Fuller and Gibbs (5). The reaction
mixture contained in μmoles: 100, tris buffer, pH 7.5; 10,
MgCl₂; 2.5, GSH; 2.0, ribulose-1,5-diphosphate; 50, KHCO₃ containing
10 μC of radioactivity; 0.05 to 0.10 ml of protein extract and
H₂O₂ to a final volume of 1.0 ml. The reaction was incubated
15 min at 37°C. The reaction was stopped by adding 1.0 ml of
concentrated HCl to each tube. The tube contents were evapo-
rated to dryness. The residue was dissolved in H₂O and assayed
for radioactivity.

Glyceraldehyde-3-P dehydrogenase was assayed in the reverse
direction by the procedure of Gibbs (6). The reaction
mixture contained in μmoles: 100, tris buffer, pH 7.5; 10,
MgCl₂; 10, ATP; 5, GSH; 0.25, NADH or NADPH; 0.5 mg of
crystalline 3-phosphoglycerate phosphokinase in 2.7 m
(NH₄)₂SO₄ suspension, 10, glyceraldehyde-3-P tricyclohexylam-
monium salt; 0.1 ml of protein extract and H₂O₂ to a final
volume of 3.0 ml. The decrease in absorbancy at 340 nm was
recorded for 5 min in a Beckman DK-2 recording spectro-
photometer.

Glycerate-3-P kinase was assayed in the reverse direction.
The reaction mixture was the same as the glyceraldehyde-3-P
dehydrogenase activity assay mixture except the 3-phos-
phoglycerate phosphokinase was replaced with 0.5 mg of glyceralde-
hyde-3-P dehydrogenase in a 0.25 m (NH₄)₂SO₄ suspension.

Fructose-1,6-diphosphate aldolase was assayed by coupling the en-
zyme to glyceraldehyde-3-P dehydrogenase. The reaction
mixture contained in μmoles: 100, tris buffer, pH 7.5: 0.25,
NADH; 50, Na,H₄AsO₄; 0.5 mg of crystalline 3-glyceralde-
hyde-3-P dehydrogenase in a 2.7 m (NH₄)₂SO₄ suspension, 10,
fructose-1,6-diphosphate tricyclohexylammonium salt, 0.1 ml of
protein extract and H₂O₂ to a final volume of 3.0 ml. The in-
crease in absorbancy at 340 nm was linear for 1 min.

**Pigment Analysis.** The chlorophylls and carotenoids were
extracted from the leaves and examined spectrophotometri-
cally by the following procedure. The leaf tissue was placed in
absolute methyl alcohol. The residue was reextracted with
methyl alcohol until it was colorless. The methyl alcohol was
brought to a specified volume and visible absorption spectrum of
this extract was determined with a Beckman DK-2 record-
ing spectrophotometer. The amount of chlorophyll a + b was
determined by the method of MacKinney (16). To extract the
carotenoids the methanol extract was saponified by treatment
with 30% methanolic KOH at room temperature for 8 hr in
the dark. The carotenoids were transferred to hexane by parti-
tion and the visible absorption spectrum of the hexane fraction
determined. The carotenoid concentration in the extracts were
calculated using a ε₅₂₅ of 2500 for carotene (1).

**Photosynthetic Rate.** The method for measuring the rate of
photosynthetic fixation in leaves was similar to the technique
described by Zelitch (24). Leaf punches of 1 cm² were equi-
ibrated for 20 min in the light at 0.06 to 0.08% CO₂ in air at
30°C and 230 w/m² (400–700 nm) of incandescent light, fol-
lowed by a 5-min CO₂ fixation period. The CO₂ remaining in
the atmosphere was determined by a Beckman CO₂ analyzer or
Beckman GC 4 gas chromatograph equipped with a thermal
conductivity detector and a 6 foot × ⅛ inch stainless steel gel
column.

**Spectral Absorbance and Quantum Yield Determinations.**
Spectral quantum yields of photosynthesis were determined by
the method of McCree (17).

**Measurement of Radioactivity.** The amount of radioactivity
in the aqueous samples was assayed in a Beckman liquid scintillation
system. Each radioactive sample was added to 15.0
ml of scintillation fluid containing 5 g of PPO, 100 g of
naphthalene, 10 ml of H₂O and dioxyne to 1 liter. The scintilla-
tion vials were dark adapted for several hours, and the counts
were assayed with ±0.2% error.

**Determination of the ¹³C/¹²C Ratio of the Virescent and
Wild-Type Fractions.** The mutant and wild-type plants were
grown in a greenhouse in College Station, Texas, atmosphere.
The plant material was harvested and washed with deionized
H₂O. The lipid, protein, carotenoid, amino acid, organic acid,
and sugars fractions were extracted from the leaves as previ-
ously described (23).

Isotope ratios of the CO₂ gas was measured on a modified
60° sector, Nier-type mass spectrometer especially designed for
isotope ratio determinations.

The results are expressed in ¹³C units as shown below:

\[ \delta^{13}C(^{100}) = \left( \frac{^{13}C/^{12}C \text{ sample}}{^{13}C/^{12}C \text{ standard}} - 1 \right) \times 10^3 \]

The reference standard is Chicago PDB limestone.

**Determination of Virescent and Wild-Type CO₂ Compensation
Points.** The determination of CO₂ compensation points
was carried out as described by Goldsworthy and Day (7). The
virescent or wild-type leaf was allowed to photosynthesize in a
Mylar bag in a Petri dish containing 10 ml of H₂O for 1.5 hr
at 1000 ft-c at 50°C. The gas in the bag was squeezed out into
an infrared gas analyzer, and the CO₂ concentration was meas-
ured.

**RESULTS**

**Photosynthetic Characteristics.** Some of the photosynthetic
characteristics of the mutant and wild-type cotton leaves are
described in Table I. At high light levels the photosynthetic rate
per dm² of leaf area of the mutant leaves is equal to that of
the wild-type leaves. The photosynthetic rate per mg of chlorophyll
of the mutant is higher than that of the wild-type leaves. The
same is true at low light levels. The rates of photosynthetic
CO₂ uptake/dm².hr for the mutant and wild-type leaves are
about equal to those found in cotton leaves at 600 μl/l CO₂, 42
to 45 mg CO₂ fixed/dm².hr (9). As shown in Table I, the green
cotton leaves contain about 5 times as much chlorophyll a + b
chlorophyll content and the activity of a number of enzymes of the reductive pentose phosphate cycle in *Chlamydomonas reinhardtii* and *Medicago sativa* (8, 12). The activity of a number of the enzymes of the reductive pentose phosphate cycle in the mutant and wild-type cotton leaves is shown in Table II. The levels of the enzymatic activity of RuDP-carboxylase, NADP-linked glyceraldehyde-3-P dehydrogenase, phosphoglyceric acid kinase and fructose-1,6-diP aldolase are essentially the same in the mutant and wild-type leaves.

**CO₂ Compensation Point.** The CO₂ compensation point for the mutant and wild-type leaves is shown in Table I. Both types of leaves have similar values at 30°C. The CO₂ compensation point is not altered in the mutant leaves.

**Quantum Yields.** The absorbance and the relative quantum yields of the mutant and wild-type leaves are shown in Figure 2. The absorbance of the yellow leaves is below that of the control leaves throughout the visible spectrum. The photosynthetic yield per quantum absorbed (relative quantum yield) in the mutant leaves is close to that of the wild-type leaves in the red and far red portions of the spectrum but less in the blue portion of the spectrum. As the virescent leaves matured and became green, both the absorbance and relative quantum yield in the blue and red portions of the spectrum became in-

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**Fig. 1.** δ³¹C values of the metabolic intermediates of virescent and wild-type cotton. The plants were grown in an atmosphere of −7 %. The δ³¹C values are shown in the table below.

### Table II. Activity of Some Enzymes of the Reductive Pentose Phosphate Cycle in Virescent and Wild-Type Cotton Leaves

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Wild-type</th>
<th>Virescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribulose-1,5-diP carboxylase</td>
<td>27.0</td>
<td>23.0</td>
</tr>
<tr>
<td>Glyceraldehyde-3-P dehydrogenase (NADP)</td>
<td>4.8</td>
<td>5.1</td>
</tr>
<tr>
<td>3-Phosphoglyceric acid kinase</td>
<td>195.0</td>
<td>318.0</td>
</tr>
<tr>
<td>Fructose-1,6-diP aldolase</td>
<td>5.1</td>
<td>6.7</td>
</tr>
</tbody>
</table>

as the mutant leaves. The chlorophyll *a/b* ratio is nearly the same for both types of leaves, but the chlorophyll/carotenoid ratio is much higher in the wild-type leaves. It is clear that the chlorophyll mutant of cotton has a high photosynthetic rate per mg of chlorophyll.

**C₄ Type of Plant.** The type of carbon metabolism in most chlorophyll mutants has not been determined. The rate of photosynthesis of the mutant leaves on a chlorophyll basis is 2 to 5 times the rate of wild-type leaves (3, 11, 20) and in this respect approaches the rate of CO₂ fixation of C₄ plants. The light level necessary to saturate photosynthesis in some chlorophyll-deficient mutants is similar to the level necessary to saturate photosynthesis in C₄ plants.

Smith and Epstein (22) have shown that the δ³¹C/³⁰C ratios for most C₄ and C₃ plants is −25 to −10, respectively. Whelan et al. (23) have examined the δ³¹C composition of intermediates of C₃ and C₄ plants. These isotope values support the conclusion that the carbon for C₃ and C₄ plants is exclusively fixed through the ribulose-1,5-diP carboxylase or P-enolpyruvate carboxylase step. The δ³¹C/³⁰C ratios for constituents of mutant and wild-type cotton are shown in Figure 1. The δ³¹C values for most of these constituents are about −22 to −35. There are no significant differences in δ³¹C values of constituents from either plant. These values are typical of plant constituents whose carbon is derived solely through the ribulose-1,5-diP carboxylase step.

**Enzymes of the Reductive Pentose Phosphate Cycle.** The mutation of a single nuclear gene has been shown to alter...
distinguishable from the wild-type leaves. We have previously shown that as the virescent leaves mature there is a formation of chloroplast grana (from agranal chloroplasts in the young yellow leaves) indistinguishable from the wild-type leaves (3).

**DISCUSSION**

The work in this paper and in previous publications (2, 3) lead to a general description of the photosynthetic characteristics of the chlorophyll mutant of cotton. The mutant is due to homozygous recessive alleles at a single locus that produce a virescent phenotype. Mutant seedlings are yellow and become greener, until at maturity they are indistinguishable from wild-type plants. The mutant (at the yellow stage) contains about 3 to 5 times less chlorophyll than the wild-type and the chlorophyll a/b ratio is not altered in the mutant leaves. On a chlorophyll basis, the mutant has a 3 to 5 times higher net photosynthetic rate than leaves of the wild-type, and this high rate is exhibited at high or low light levels. The photosynthetic rate on a leaf area basis is the same for mutant and wild-type leaves. The photosynthetic rate saturates at the same light level for the mutant and wild-type leaves. Examination of the chloroplasts in the mutant leaves shows a general lack of grana associated with the period of low chlorophyll content. As the mutant leaves mature, grana develop to the same extent as the grana in the chloroplasts of the wild-type leaves. The mutant leaves have a normal C3 type of carbon metabolism and the mutation does not lead to an alteration of the CO2 compensation point nor an alteration in the level of activity of some of the enzymes of the reductive pentose phosphate cycle. The relative quantum yield of the mutant is lower than the wild-type in the blue portion of the spectrum but equal to the wild-type in the red and far red portions of the spectrum. On maturing, the relative quantum yield in the blue part of the spectrum is the same in the mutant and wild-type leaves. In the blue part of the visible spectrum, the relative quantum yield of the virescent leaves is not equal to that of the green leaves until the mutant leaves have developed the same chlorophyll content as the green leaves. The low quantum yield of the mutant leaves at the shorter wavelengths is characteristic of the yellow stage of leaf development of the mutant leaves. The low relative quantum yield of the mutant cotton leaves in the blue wavelengths cannot be due to an altered chlorophyll a/b ratio, but might be due to an altered pigment arrangement in the chloroplast due to an altered chlorophyll/carotenoid content. This could result in a less efficient transfer of energy from the accessory pigments to chlorophyll at the shorter wavelengths.

The lower chlorophyll content of the mutant cotton leaves, unlike the low chlorophyll content of pea and tobacco (11, 19), does not lead to a lower photosynthetic rate at low light levels using tungsten lamps. The higher light levels required for the saturation of photosynthesis in pea and tobacco mutants are also different than photosynthesis in the cotton mutant. The same quantum efficiency of the mutant cotton and wild-type leaves in the red and far red part of the spectrum might predict that photosynthesis in the mutant leaves would saturate at the same light level as the wild-type leaves and that the rate of photosynthesis on a leaf area basis would be similar to the wild-type at low and high light levels.

The high net photosynthetic rate of the mutant cotton per mg of chlorophyll is not due to an altered carbon metabolism, a lower CO2 compensation point, an increased level of activity of some of the enzymes of the reductive pentose phosphate cycle, or an increased quantum efficiency. The high photosynthetic rate seems to be directly related to the lower chlorophyll content of the mutant leaves. It may be the result of smaller photosynthetic units in the yellow cotton plants. Thus the chlorophyll mutant of cotton seems to have this characteristic in common with the chlorophyll-deficient mutants of tobacco and pea plants (11, 19).

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