Net photosynthetic and transpiration rates in a chlorophyll-deficient isoline of soybean under well-watered and drought conditions

V.M. LUQUEZ*, J.J. GUIAME* and E.R. MONTALDI*

Instituto de Fitología Vegetal, Universidad Nacional de La Plata, c.c. 327, 1900-La Plata, Argentina*
Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048, U.S.A.**

Abstract

The gas exchange traits of wild type soybeans (cv. Clark) and a near-isogenic, chlorophyll-deficient line homozygous for the recessive allele y9 (y9y9) were compared under either well-watered or water-stress conditions. Mature leaves of y9 had a 65% lower chlorophyll content than wild type. However, the net photosynthetic rate ($P_N$) of y9 leaves was only 20% lower than in the wild type, irrespective of water availability. Transpiration rates ($E$) were significantly higher in leaves of y9, compared to the wild type, either under well-watered or stress conditions. The higher $E$ of y9 correlated with increased stomatal conductance, particularly in the abaxial epidermis, where more than 70% of the stomata were located. The combination of lower $P_N$ and increased $E$ resulted in a significant decrease of water use efficiency in y9, at both water availability levels. The relative water content decreased in stressed leaves, much more in y9 than in wild type leaves, probably because of the higher $E$ of the mutant line.

Additional key words: leaf sides; stomata density; stomatal conductance; water content; water use efficiency.

Introduction

Photosynthetic rate is remarkably affected by leaf temperature, mostly through the effects of temperature on the rate of the biochemical reactions of the photosynthetic carbon reduction cycle. Leaf temperature also influences the vapor pressure in the sub-stomatal cavity and intercellular air spaces, thereby affecting the rate of water

Received 8 July 1996, accepted 13 November 1996.

Abbreviations. Chl - chlorophyll, $E$ - transpiration rate, $g_s$ - stomatal conductance; PAR - photosynthetically active radiation; $P_N$ - net photosynthetic rate; PS - photosystem; RWC - relative water content; y9 - y9y9; WUE - water use efficiency.

Acknowledgements: This work was partially financed with funds from CONICET (Argentina). J.J. Guiamet is a researcher with C.I.C., Pcia. de Buenos Aires, Argentina.
loss. The temperature of a leaf reflects the balance between absorption of energy and energy dissipation in the form of convective heat, latent heat of evaporation, etc. (Campbell 1982). The absorption of radiant energy constitutes a large input of energy into leaves, and it depends to a large extent on their chlorophyll (Chl) content. Therefore, pale-green leaves may be cooler than their dark-green counterparts (Aase 1971, Ferguson et al. 1973, Xu et al. 1993) particularly under sunny skies and still air conditions.

There are several mutations that interfere with Chl accumulation and result in Chl-deficient phenotypes in various species (King 1990). While in some cases a partial deficiency of Chl correlates with a decrease in leaf $P_N$ (e.g., Estill et al. 1991, Xu et al. 1993), other mutant lines have $P_N$ similar to their corresponding wild types (Hesketh et al. 1981, Di Marco et al. 1989). Whole canopy carbon exchange rates may even be higher in lines with a reduced Chl content because of increased PAR penetration to lower leaf $strata$ (Pettigrew et al. 1989).

With lower leaf temperatures, Chl-deficient lines might have lower $E$ and higher water use efficiency (WUE) than dark-green lines. For example, in alfalfa, pale green plants had lower $E$ and higher WUE efficiency than their normal, green counterparts, particularly under water stress (Estill et al. 1991). Thus, lower Chl content might be a useful agronomic trait in environments with frequent and/or long periods of water stress. In this study, we report the gas exchange characteristics and WUE of a Chl deficient isole of soybean homozygous for the recessive, non-lethal, allele $y9$, both under well-watered or stress conditions.

Materials and methods

Plants: Seeds of soybean [Glycine max (L.) Merr.] cv. Clark and its near-isogenic line homozygous for the recessive $y9$ allele ($y9y9$) were obtained from Dr. R.L. Bernard, Department of Agronomy, University of Illinois, Urbana, IL 61801, U.S.A. Seeds were germinated for 3 d in Petri dishes with moistened filter paper at 25 °C, and then transplanted into 500 cm$^3$ pots filled with soil. The seedlings were grown in a greenhouse (irradiance 200–800 μmol(PAR) m$^{-2}$ s$^{-1}$, day/night temperatures 23/18 °C, 10 h natural photoperiod) for 24 d, and then placed in an environmental control chamber at an irradiance of 700 μmol m$^{-2}$ s$^{-1}$, 10 h photoperiod, and day/night temperature of 22/18 °C. Irradiance in the chamber was provided by 1000 W tungsten halogen lamps filtered through a 10 cm running water bath.

Water stress treatment: Starting at 27 d after planting, water stress was imposed on a group of plants by withholding watering until soil water potential reached -0.5 MPa. Thereafter, the amount of water lost by transpiration was calculated every day from measurements of changes in pot mass, and added to each pot to maintain soil water potential constant around -0.5 MPa. Control pots were watered every day to field capacity (-0.03 MPa). To avoid excessive loss of water through evaporation from the soil surface, the top of the soil was covered with a 1 cm layer of polystyrene beads.
which effectively reduced soil evaporation, as judged from measurements of water loss in pots without plants.

Gas exchange measurements were started 27 d after planting, simultaneously with the water stress treatment. All measurements were carried out on the first trifoliate leaf between 11:00 and 15:30 h. The first trifoliate leaf, which was fully expanded at the start of the experiment, was used for all determinations. The \( P_N \) was measured with a Licor LI-6200 Portable Photosynthesis System at an irradiance of 700 \( \mu \text{mol(PAR)} \text{m}^{-2} \text{s}^{-1} \), 30-70 % relative humidity, and 330-370 \( \mu \text{mol(CO}_2 \text{)} \text{mol}^{-1} \). Stomatal conductance \( (g_s) \) and \( E \) were measured under the same conditions with a Licor LI-6000 Steady State Porometer.

Relative water content: Leaf disks (12 mm diameter) were excised, weighed (\( \text{wi} \)) and floated on distilled water at 12 \( \mu \text{mol m}^{-2} \text{s}^{-1} \text{PAR} \) for 4 h. Then, disks were weighed again (\( \text{wf} \)), and dried at 80 °C for 48 h for the determination of dry mass (\( \text{wd} \)). Relative water content (\( \text{RWC} \)) was calculated as:

\[
\text{RWC} = \frac{(\text{wi} - \text{wd})}{(\text{wf} - \text{wd})} \times 100
\]

Stomatal density: Microscopic observations of the leaf epidermis were carried out as described by Rumi and Carpinteri (1981) with only minor modifications. Small (4 mm\(^2\)) pieces of leaves were fixed in ethanol:formaldehyde (37 %):glacial acetic acid:water (48:10:5:37, v/v) for two weeks, then transferred to 30 % ethanol for 10 min, and washed with distilled water (10 min). Specimens were stained with ruthenium red (0.02 %, m/v) for 24 h, washed with distilled water, and mounted in gelatine-glycerine for observation with a Zeiss Photomicroscope II fitted with a green interference filter.

Chl content: Leaf disks (15 mm diameter) were extracted with dimethyl formamide at room temperature, and Chl content was calculated using the equations developed by Inskop and Bloom (1985).

Results

Chl content of the mature first trifoliate leaf of \( y9 \) was 65 % lower than in the wild type, with a slightly larger decrease of Chl \( b \) (70 %) than Chl \( a \) (62 %) (Table 1). During the 10-d experimental period, wild type leaves lost 20 % of their Chl, whereas more than 40 % of the initial Chl was degraded in \( y9 \) leaves. The decrease in Chl content was attenuated in water-stressed plants of the wild type, possibly due to a reduction in apex growth (results not shown) that halted the symptoms of progressive senescence (Leopold 1961).

Gas exchange. Mature, non-stressed leaves of \( y9 \) had \( P_N \) 20 % lower than the wild type (Fig. 1A). The \( P_N \) declined in the following 10-d experimental period, more rapidly in water-stressed plants. Overall, the trend for slightly higher \( P_N \) in wild type was maintained over time in both conditions, although at day 10 leaves of both treatments and genotypes had similar and very low \( P_N \). In spite of leaf temperatures
Table 1. Chlorophyll (Chl) content [g m\(^{-2}\)] in leaves of a wild type soybean (cv. Clark) and its near-isogenic line homozygous for the y9 allele. Plants were maintained at two soil water potentials (-0.03 or -0.5 MPa), and sampled were taken for Chl analysis at the start of the treatments (day 0), and 10 d thereafter. For each parameter and sampling date, means followed by the same letter do not differ significantly at 5% level according to Tukey’s test.

<table>
<thead>
<tr>
<th></th>
<th>Day 1 Chl a</th>
<th>0.416 a</th>
<th>0.142 a</th>
<th>0.558 a</th>
<th>0.242 a</th>
<th>0.090 a</th>
<th>0.344 a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chl b</td>
<td>0.292 b</td>
<td>0.120 b</td>
<td>0.412 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chl (a+b)</td>
<td>0.292 b</td>
<td>0.120 b</td>
<td>0.412 b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clark</td>
<td>Well watered</td>
<td>0.416 a</td>
<td>0.142 a</td>
<td>0.558 a</td>
<td>0.242 a</td>
<td>0.090 a</td>
<td>0.344 a</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>y9</td>
<td>Well watered</td>
<td>0.157 b</td>
<td>0.043 b</td>
<td>0.199 b</td>
<td>0.069 c</td>
<td>0.025 c</td>
<td>0.094 c</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Net photosynthetic rate (A), transpiration rate (B), stomatal conductance (C), and water use efficiency (D) of the first trifoliate leaf of wild type cv. Clark (solid lines) and Chl-deficient y9 (broken lines) soybeans under well watered (soil water potential around -0.03 MPa) and stress (soil water potential around -0.50 MPa) conditions. ●: Clark, well-watered; ○: Clark, stressed; ▲: y9, well watered; △: y9, stressed. Vertical bars represent the standard error of the mean.

about 1 °C lower (values not shown), non-stressed, mature leaves of the y9 mutant exhibited a higher E than the wild type (Fig. 1B). Water stress significantly reduced E of leaves of both wild type and y9, but nonetheless, water-stressed leaves of y9 still exhibited significantly higher E than water-stressed wild type plants. In fact, E in water-stressed leaves of y9 was similar or even higher than those of well-watered leaves of the wild type cultivar. The differences in E correlated with similar changes
in g. Compared to the wild type, leaves of y9 had higher g. at all sampling dates in both water availability treatments (Fig. 1C). This was not due to increased stomatal density, as the number of stomata per unit area was the same in both lines (Table 2).

Table 2. Number of stomata per unit area in fully expanded leaves of wild type (cv. Clark) and Chlorite-deficient (y9) soybeans. Means followed by the same letter do not differ significantly at 5 % level according to Tukey’s test.

<table>
<thead>
<tr>
<th></th>
<th>Abaxial side</th>
<th>Adaxial side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clark</td>
<td>39 a</td>
<td>15 b</td>
</tr>
<tr>
<td>y9</td>
<td>39 a</td>
<td>13 b</td>
</tr>
</tbody>
</table>

Thus, differences in the extent of stomatal aperture were responsible for the increased E in y9. Also E of whole plants, computed on the basis of daily measurements of the changes in soil water content, was higher in y9 than in the wild type, both for well-watered and stressed plants (results not shown).

The combination of lower P_N and increased E reduced the instantaneous WUE of y9 leaves to about 50 % that in the wild type (Fig. 1D). Except for an increase in WUE at day 5, water stress did not have an appreciable effect on WUE in either line.

Relative water content: At the end of the 10-d water stress period, the relative water content (RWC) of stressed leaves of Clark and y9 was significantly lower than that of their respective controls (Table 3). Remarkably, water-stressed leaves of y9 had a lower RWC than stressed leaves of cv. Clark, i.e., y9 maintained their stomata open and sustained high E in spite of very severe leaf water stress.

Table 3. Relative water content (RWC) of the first trifoliate leaf of wild type (cv. Clark) and Chlorite-deficient (y9) soybeans subjected to water stress for 10 d. Mean values followed by different letters differ significantly at 5 % level according to Tukey’s test.

<table>
<thead>
<tr>
<th></th>
<th>Well-watered</th>
<th>Stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clark</td>
<td>83.3 a</td>
<td>73.8 b</td>
</tr>
<tr>
<td>y9</td>
<td>88.8 c</td>
<td>68.8 d</td>
</tr>
</tbody>
</table>

Discussion

The y9 mutation of soybean seemed particularly appropriate to study the impact of Chl content on leaf temperature and E because this mutation is non-lethal and affects mostly the accumulation of the light-harvesting complex associated with photosystem (P3) 2, with a lesser effect on the photosynthetic reaction centers of P61 and P62 (Eskins et al. 1983, 1991, Droppa et al. 1988). Since the light-harvesting complex of
PS2 is an accessory antenna with no photochemical activity, leaves of y9 may maintain relatively normal \( P_N \) under non-limiting irradiances. In fact, the pronounced decrease of Chl content in this genotype does not translate into a corresponding decline of photosynthetic activity (Hesketh et al. 1981). In our conditions, under moderate irradiance, with a 65 % lower Chl content, \( P_N \) of y9 leaves is only about 20 % less than in the wild type, and we speculate that this difference might be even smaller in the field, under higher irradiance. Several other reports have shown Chl-deficient lines to have similar \( P_N \) than their green counterparts (Ferguson 1974, Di Marco et al. 1989, Pettigrew et al. 1989, Xu et al. 1993). Thus, Chl-deficient lines might achieve relatively high \( P_N \) with less absorption of radiant energy and, therefore, lower leaf temperatures.

Although leaf temperature was about 1 °C lower in y9 than in the wild type and, therefore, we estimated that water vapor pressure in the intercellular air spaces of y9 leaves was 6 % lower, \( E \) was significantly higher in y9 than in cv. Clark, both in water-stress and well-watered treatments. This was mostly due to a significant increase in \( g_s \) of the abaxial epidermis that offset the effects of the lower leaf temperatures in y9. Leaves of y9 maintained a higher \( g_s \) than cv. Clark throughout the experimental period, even as \( P_N \) and \( E \) declined in both genotypes. Similarly, mature leaves of y9 plants grown in the field have higher \( g_s \) than the wild type cv. Clark (Barreiro, Beltrano and Guiamet, unpublished). As far as we know, this is the first report of increased \( g_s \) and \( E \) in Chl-deficient mutants.

In soybean, over 70 % of stomata are located in the abaxial epidermis (Table 2), and are shaded by the spongy and mesophyll palisade cells above them. Thus, the higher \( g_s \) of y9 leaves might be related to an increase in the flux of radiation transmitted through the mesophyll reaching the lower epidermis guard cells. However, Chl-deficient soybeans heterozygous for the y1/1 allele exhibit similar \( g_s \) and \( E \) as the wild type (Xu et al. 1993), whereas \( g_s \) and \( E \) are lower in a Chl-deficient line of alfalfa (Estill et al. 1991). Thus, the higher \( g_s \) of y9 was probably not due to increased transmission of PAR to the lower epidermis.

Leaves of y9 responded to water stress by reducing \( g_s \) by a similar proportion as the wild type, although their \( g_s \) nonetheless remained higher than in water-stressed leaves of the wild type. Interestingly, water-stressed leaves of y9 maintained higher \( g_s \) than the wild type with significantly lower RWC (i.e., more severe stress).

Our results indicate that the increased PAR reflectance and reduced PAR absorption of Chl-deficient plants, while reducing their leaf temperature, may not necessarily result in lower \( E \) and improved WUE. On the contrary, we show that in one of such mutants Chl-deficiency is actually linked to increased \( E \) and reduced WUE. The mechanism linking Chl-deficiency with \( g_s \) in y9 is presently unknown.

References

PHOTOSYNTHESIS AND TRANSPERSION IN SOYBEAN


