

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

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

*Instituto de Fisiología 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 isoline 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 cm³ pots filled with soil. The seedlings were grown in a greenhouse [irradiance 200–800 $\mu\text{mol}(\text{PAR}) \text{ m}^{-2} \text{ s}^{-1}$, 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 $\mu\text{mol m}^{-2} \text{ s}^{-1}$, 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 $\mu\text{mol}(\text{PAR}) \text{ m}^{-2} \text{ s}^{-1}$, 30-70 % relative humidity, and 330-370 $\mu\text{mol}(\text{CO}_2) \text{ mol}^{-1}$. Stomatal conductance (g_s) and E were measured under the same conditions with a *Licor LI-1600 Steady State Porometer*.

Relative water content: Leaf disks (15 mm diameter) were excised, weighed (w_i) and floated on distilled water at 12 $\mu\text{mol m}^{-2} \text{ s}^{-1}$ PAR for 4 h. Then, disks were weighed again (w_f), and dried at 80 °C for 48 h for the determination of dry mass (w_d). Relative water content (RWC) was calculated as:

$$\text{RWC} = (w_i - w_d) / (w_f - w_d) \times 100$$

Stomatal density: Microscope observations of the leaf epidermis were carried out as described by Rumi and Carpinetti (1981) with only minor modifications. Small (4 mm²) 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 Inskeep 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 samples were taken for Chl analysis at the start of the treatments (day 1), 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.

		Day 1			Day 10		
		Chl <i>a</i>	Chl <i>b</i>	Chl (<i>a+b</i>)	Chl <i>a</i>	Chl <i>b</i>	Chl (<i>a+b</i>)
Clark	Well watered	0.416 a	0.142 a	0.558 a	0.242 a	0.090 a	0.344 a
	Stressed				0.292 b	0.120 b	0.412 b
$y9$	Well watered	0.157 b	0.043 b	0.199 b	0.069 c	0.025 c	0.094 c
	Stressed				0.070 c	0.016 c	0.086 c

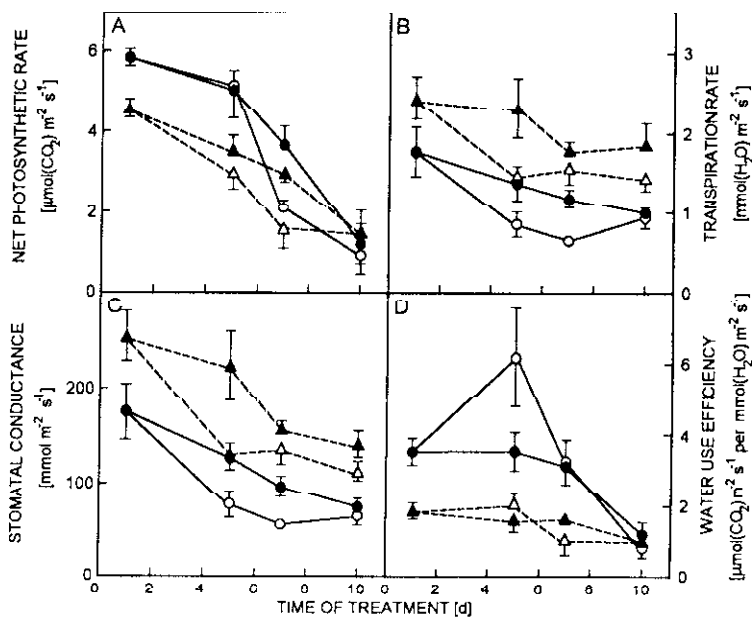


Fig. 1. Net photosynthetic rate (A), transpiration rate (B), stomatal conductance (C), and water use efficiency (D) of the first trifoliate leaf of wild type cv. Clark (solid lines) and Chl-deficient $y9$ (broken lines) soybeans under well watered (soil water potential around -0.03 MPa) and stress (soil 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_s . Compared to the wild type, leaves of $y9$ had higher g_s 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 Chl-deficient ($y9$) soybeans. Means followed by the same letter do not differ significantly at 5 % level according to Tukey's test.

	Number of stomata [mm ⁻²]	
	Abaxial side	Adaxial side
Clark	39 a	15 b
$y9$	39 a	13 b

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 Chl-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.

	Relative water content [%]	
	Well-watered	Stressed
Clark	83.3 a	73.8 b
$y9$	88.8 c	68.8 d

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 (PS) 2, with a lesser effect on the photosynthetic reaction centers of PS1 and PS2 (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 Guamet, 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 $y11$ 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

- Aase, J.K.: Growth, water use, and energy balance comparisons between isogenic lines of barley. - *Agron. J.* **63**: 425-428. 1971.

- Campbell, G.S.: Fundamentals of radiation and temperature relations. - In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): *Physiological Plant Ecology I*. Pp. 11-40. Springer-Verlag, Berlin - Heidelberg - New York 1981.
- Di Marco, G., D'Ambrosio, N., Giardi, M.T., Massacci, A., Tricoli, D.: Photosynthetic properties of leaves of a yellow green mutant of wheat compared to its wild type. - *Photosynth. Res.* **21**: 117-122, 1989.
- Droppa, M., Ghirardi, M.L., Horvath, G., Melis, A.: Chlorophyll *b* deficiency in soybean mutants. II. Thylakoid membrane development and differentiation. - *Biochim. biophys. Acta* **932**: 138-145, 1988.
- Eskins, K., Delmastro, D., Harris, L.: A comparison of pigment-protein complexes among normal, chlorophyll-deficient and senescent soybean genotypes. - *Plant Physiol.* **73**: 51-55, 1983.
- Eskins, K., Jiang, C.Z., Shibles, R.: Light-quality and irradiance effects on pigments, light-harvesting proteins and Rubisco activity in a chlorophyll- and light-harvesting-deficient soybean mutant. - *Physiol. Plant.* **83**: 47-53, 1991.
- Estill, K., Delaney, R.H., Smith, W.K., Ditterline, R.L.: Water relations and productivity of alfalfa leaf chlorophyll variants. - *Crop Sci.* **31**: 1229-1233, 1991.
- Ferguson, H., Eslick, R.F., Aase, J.K.: Canopy temperatures of barley as influenced by morphological characteristics. - *Agron. J.* **65**: 425-428, 1973.
- Ferguson, H.: Use of variety isogenes in plant water-use efficiency studies. - *Agr. Meteorol.* **14**: 25-29, 1974.
- Hesketh, J.D., Ogren, W.L., Hageman, M.E., Peters, D.B.: Correlations among leaf CO₂-exchange rates, areas and enzyme activities among soybean cultivars. - *Photosynth. Res.* **2**: 21-30, 1981.
- Inskeep, W.P., Bloom, P.R.: Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. - *Plant Physiol.* **77**: 483-485, 1985.
- King, J.: *The Genetic Basis of Plant Physiological Processes*. - Oxford University Press, Oxford 1990.
- Leopold, A.C.: Senescence in plant development. - *Science* **134**: 1727-1732, 1961.
- Pettigrew, W.T., Hesketh, J.D., Peters, D.B., Woolley, J.T.: Characterization of canopy photosynthesis of chlorophyll-deficient soybean isolines. - *Crop Sci.* **29**: 1025-1029, 1989.
- Rumi, C.P., Carpinetti, R.M.: Effect of sunlight on the development of *Tropaeolum majus* L. II. Leaf development. - *Fyton* **41**: 129-137, 1981.
- Xu, D.Q., Chen, X.M., Zhang, L.X., Wang, R.F., Hesketh, J.D.: Leaf photosynthesis and chlorophyll fluorescence in a chlorophyll-deficient soybean mutant. - *Photosynthetica* **29**: 103-112, 1993.