

Differential Pleiotropy in a *psbA* Gene Mutant of *Brassica napus* Implies Altered Temporal Photosynthesis and Thermal Tolerance

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Studies were conducted to test the hypothesis that the mutation to the *psbA* plastid gene that confers s-triazine resistance (R) also results in an altered diurnal pattern of photosynthetic carbon reduction (CER) relative to that of the susceptible (S) wild-type. In all experiments CER approximated the increasing and decreasing light levels during the diurnal. S CER exceeded that of R during the midday period, but R CER was greater early and late in the diurnal at 25 °C. R CER exceeded that of S for most of the diurnal at 35 °C and in older, crowded, nitrogen-starved plants. These studies support the stated hypothesis and indicate a more complex model of photosynthetic productivity than previously observed. An assessment of the photosynthetic competence of either biotype may be a function of the time of day or the environmental conditions the plants are exposed to, especially temperature.

Introduction

S-triazine resistance (R) in higher plants was discovered in 1970 [1]. Subsequent studies revealed this resistance was due to a single base pair mutation to the *psbA* chloroplast gene [2]. The *psbA* mutation caused a change at codon 264 in its product, the D-1 protein, a key functional element in photosystem II (PS II) electron transport [3]. This change in R leads to a profound reorganization of functional units in the chloroplast. This pleiotropic cascade includes both structural [4, 5] and functional changes [6]. These and other studies have led many to conclude that R mutants are inherently photosynthetically inferior to the susceptible (S) wild type [7]. Previous work in our laboratory indicated a constant, differential, pattern of variable chlorophyll fluorescence (F_T) between S and R over the course of a diel, *i.e.* R is a chronomutant [8]. What remains unknown is the relationship of this differential pattern in terminal fluorescence and changes in carbon fixation during the diurnal. Therefore, we hypothesized that the mutation to the *psbA* plastid gene that confers s-triazine resistance also results in an altered diurnal pattern of photosynthetic carbon reduction relative to that of

the susceptible biotype. We present data that supports this hypothesis. We also present evidence of other novel, temperature-dependent, functional differences.

Materials and Methods

Growth environments

Plants were established in a sunlit glasshouse with supplemental 1000 w metal halide lights. The photoperiod in that environment was 16 h light/8 h dark; photosynthetic photon flux densities (PPFD) reached 550–600 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at midday; relative air humidity was ca. 60% and the temperature 20–25 °C. Four–five days prior to each experiment the plants were moved to a Conviron model E-15 controlled environment growth chamber. The photoperiod length and relative air humidity were identical to that in the glasshouse. PPFD under controlled environmental conditions was 120 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at dawn and dusk with incremental increases and decreases either side of midday maxima of 1000 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. Several studies were conducted and then repeated. Of those reported here, two were performed at constant 25 °C temperatures, a third at 35 °C.

Plant material

Two nearly isonuclear biotypes of oilseed rape (*B. napus*) were evaluated in these experiments:

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triazine-susceptible *B. napus*, cv. ‘Tower’; and its triazine-resistant derivative, cv. ‘OAC Triton’ [9]. Plants evaluated were in the 3–4, or 8½–9½, leaf stages of growth. In the later stage of growth the plants were restricted in growth and the roots were noticeable pot-bound and nitrogen-starved.

Gas exchange measurements

Photosynthetic CO₂ gas exchange measurements (CER) were made with an ADC infrared gas analyzer. The measurements were made on the third leaf of each plant. Estimates of CER at each half-hourly of hourly measurement time for each biotype are the means of 9 separate plants at each individual time. Carbon reduction rates for early (0600–0950 or 1000), midday (1000 or 1010–1700 or 1800 or 1900), late (1800 or 1900 or 1910–2150) and all day (0600–2150) were calculated by integrating the CER area over that time period for the 9 plants of each biotype. The R and S mean carbon fixed for each period were compared using an F-test.

Results and Discussion

In all experiments CER approximated the increasing and decreasing light fluence levels during the diurnal. In 3–4 leaf plants grown at 25 °C, S carbon reduction rates exceeded those of R during the midday period (Fig. 1, Table I). R CER exceeded that of S during the early and late periods of the light period. At 25 °C, 3–4 leaf S plants reduced more carbon than did R plants for the day taken as a whole.

In 3–4 leaf plants grown at 35 °C, R CER exceeded that of S for the early and midday periods of the day (Fig. 2, Table II). R and S CER were similar late in the day. At 35 °C R fixed more car-

bon for the day period than did S. S CER were similar at 25 and 35 °C (Tables I, II). R CER was greater at 35 °C than at 25 °C.

In older (8½–9½ leaf) plants grown at 25 °C in pots with restricted space for root growth and expansion, R CER was greater than that of S in the early and midday periods of the day (Fig. 3). S CER exceeded R late in the day. For the day as a whole, older potbound R plants at 25 °C reduced more carbon than did comparable S plants. Neither leaf stomatal conductances nor intercellular CO₂ pressures differed between R and S for any time period in any experiment (data not reported).

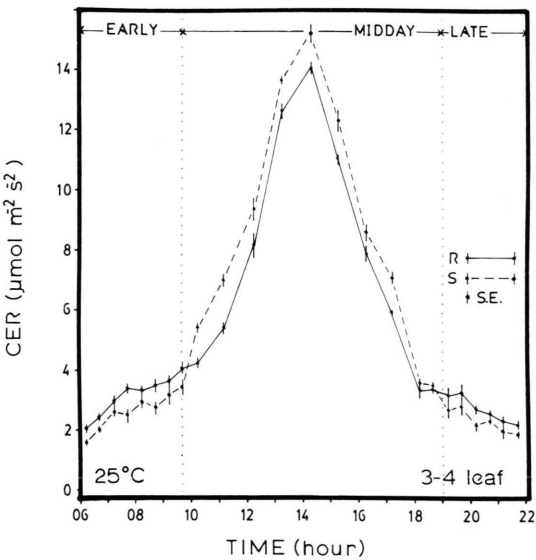


Fig. 1. Carbon exchange rate (CER) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at 25 °C in 3–4 leaf triazine-resistant (R) and -susceptible (S) *Brassica napus* plants through time (hour of the day); S.E. = \pm standard error of the mean, $n = 9$.

Table I. Cumulative carbon fixed ($\mu\text{mol CO}_2$ per period) at 25 °C in 3–4 leaf triazine resistant (R) and susceptible (S) *Brassica napus* plants during four periods (hour of the day) of the diurnal: early, midday, late and all day.

Biotype	Early (0600–0950)	Midday (1000–1900)	Late (1910–2150)	Day (0600–2200)
Resistant	13.6	73.8	5.3	92.6
Susceptible	11.7	82.3	4.6	98.6
P = *	0.0001	0.0001	0.0048	0.0001

* R and S period means within a time period compared with an F-Test, associated significance probability level (P = probability).

Table II. Cumulative carbon fixed ($\mu\text{mol CO}_2$ per period) at 35 °C in 3–4 leaf triazine resistant (R) and susceptible (S) *Brassica napus* plants during four periods (hour of the day) of the diurnal: early, midday, late and all day.

Biotype	Early (0600–0950)	Midday (1010–1700)	Late (1800–2200)	Day (0600–2150)
Resistant	17.8	80.8	9.2	104.8
Susceptible	13.1	71.4	8.9	93.2
P = *	0.0001	0.0001	NS	0.0001

* R and S period means within a time period compared with an F-Test, associated significance probability level (P = probability).

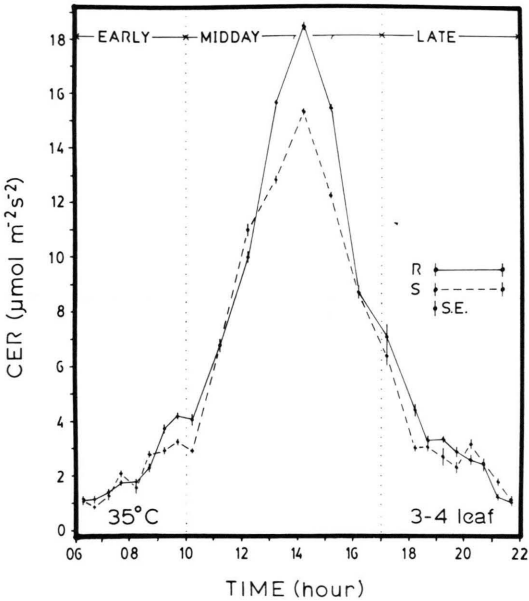


Fig. 2. Carbon exchange rate (CER) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at 35 °C in 3–4 leaf triazine-resistant (R) and -susceptible (S) *Brassica napus* plants through time (hour of the day); S.E. = \pm standard error of the mean, $n = 9$.

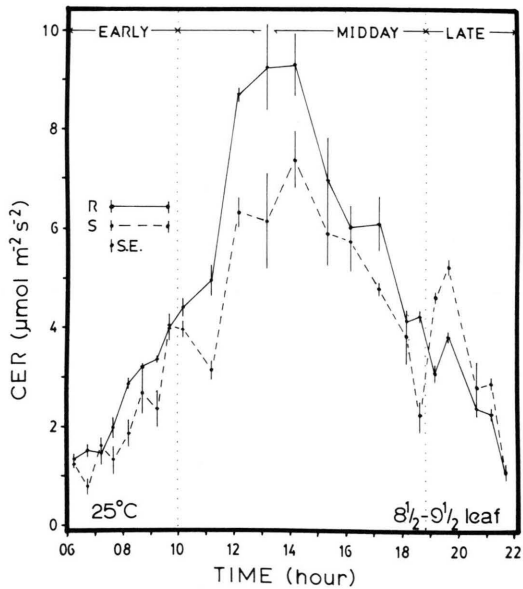


Fig. 3. Carbon exchange rate (CER) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at 25 °C in 8½–9½ leaf triazine-resistant (R) and -susceptible (S) *Brassica napus* plants through time (hour of the day); S.E. = \pm standard error of the mean, $n = 9$.

Table III. Cumulative carbon fixed ($\mu\text{mol CO}_2$ per period) at 25 °C in 8½–9½ leaf triazine resistant (R) and susceptible (S) *Brassica napus* plants during four periods (hour of the day) of the diurnal: early, midday, late and all day.

Biotype	Early (0600–0950)	Midday (1010–1850)	Late (1900–2200)	Day (0600–2150)
Resistant	10.4	57.9	9.3	77.7
Susceptible	8.5	44.9	11.1	64.5
P = *	0.0001	0.0004	0.0004	0.0001

* R and S period means within a time period compared with an F-Test, associated significance probability level (P = probability).

These studies indicate a more complex model of photosynthetic productivity than previously observed. The photosynthetic superiority of one biotype relative to the other is a function of the time of day. Consistent with previous reports [7], S had greater CER than R during the midday period under optimal environmental and ontogenetic conditions. Unlike previous studies, there appears to be times in the diurnal when R is photosynthetically superior to S: early and late in the day. These results indicate that R may be better adapted to a range of unfavorable environmental conditions than S: early and late in the day with lower available light levels and under hyper-optimal temperatures, under crowded conditions and with soil nitrogen deficits. These results support the hypothesis tested: *psbA* plastid gene mutation conferring R also confers a different diurnal pattern of photosynthetic function than that in S. This work is also

consistent with the pattern of differential chlorophyll fluorescence (F_T) early and late in the diurnal previously reported [8].

Conclusions

It can be envisioned that there were ecological conditions before the advent of s-triazine herbicides in which R had an adaptive advantage over the more numerous S individuals in a population of a species. Under certain conditions R might have exploited a photosynthetic niche under-utilized by S. Under these conditions R survival and continuity could have been ensured at a higher frequency of occurrence than that due to the mutation rate of the *psbA* plastid gene alone. These conditions could have been the lower light level occurring early and late in the day or the lower light levels in the understory of a plant canopy, and under hot and crowded growth conditions.

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