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RESEARCH PAPER

Thermoperiodic growth control by gibberellin does not involve changes in photosynthetic or respiratory capacities in pea

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Abstract

Active gibberellin (GA_1) is an important mediator of thermoperiodic growth in pea. Plants grown under lower day than night temperature (negative DIF) elongate less and have reduced levels of GA_1 compared with plants grown at higher day than night temperature (positive DIF). By comparing the wild type (WT) and the elongated DELLA mutant *la cry^s*, this study has examined the effect of impaired GA signalling on thermoperiodic growth, photosynthesis, and respiration in pea. In the WT a negative DIF treatment reduced stem mass ratio and increased both root mass ratio and leaf mass ratio (dry weight of specific tissue related to total plant dry weight). Leaf, root and stem mass ratios of *la cry^s* were not affected by DIF. Under negative DIF, specific leaf area (projected leaf area per unit leaf dry mass), biomass, and chlorophyll content of WT and *la cry^s* plants were reduced. Young, expanding leaves of plants grown under negative DIF had reduced leaf area-based photosynthetic capacity. However, the highest photosynthetic electron transport rate was found in fully expanded leaves of WT plants grown under negative DIF. Negative DIF increased night respiration and was similar for both genotypes. It is concluded that GA signalling is not a major determinant of leaf area-based photosynthesis or respiration and that reduced dry weight of plants grown under negative DIF is caused by a GA-mediated reduction of photosynthetic stem and leaf tissue, reduced photosynthesis of young, expanding leaves, and reduced growth caused by low temperature in the photoperiod.

Key words: DIF, gibberellins, photosynthesis, *Pisum sativum*, plant morphology, respiration, stem elongation, thermoperiodism.

Introduction

Thermoperiodism was defined by Went (1944) as 'all effects of a temperature differential between light and dark periods on responses of the plants, whether they be flowering, fruiting or growth'. In general, when plants are grown at the same average diurnal temperature, plants grown under a negative temperature difference [negative DIF; day temperature (DT) < night temperature (NT)] elongate less than those grown under positive DIF (DT > NT). The DIF concept was introduced by Erwin *et al.* (1989) and has since become widely used for growth control in greenhouse production of ornamental plants. A negative DIF treatment often results in reduced total plant dry weight as compared with a positive DIF (Heuvelink, 1989; Myster *et al.*, 1997; Xiong *et al.*, 2002).

Gibberellins (GAs) are hormones contributing to the control of growth and development of plants throughout their life cycle and have been known for decades for their strong growth-promoting effect on stems. In pea (*Pisum sativum*), *Campanula isophylla*, and *Dendranthema grandiflorum*, regulation of GA metabolism has been hypothesized

to mediate the effects of diurnal temperature alternations on growth and morphology (Jensen *et al.*, 1996; Nishijima *et al.*, 1997; Grindal *et al.*, 1998; Stavang *et al.*, 2005). Thus, reduced stem elongation and leaf area in plants grown under negative DIF is caused by reduced amounts of active GA. However, it has also been shown that in *Lilium longiflorum* and cucumber, a negative DIF treatment reduces net photosynthetic rate and chlorophyll content compared with a positive DIF treatment (Berghage *et al.*, 1990; Agrawal *et al.*, 1993). Is GA also regulating photosynthesis? Recently, it has been shown that increased or decreased GA levels in transgenic *Populus* and tobacco plants improved or reduced growth and biomass production, respectively (Eriksson *et al.*, 2000; Biemelt *et al.*, 2004). Also, transgenic citrus with elevated levels of GAs are reported to have increased growth and photosynthesis (Huerta *et al.*, 2008).

In an early GA application experiment, spraying red clover with GA_3 increased photosynthesis and Rubisco activity, and a regulatory effect of the hormone on photosynthetic activity was suggested (Treharne and Stoddart, 1968). A positive effect of GA application on photosynthesis has also been found in other similar studies (Khan, 1996; Hayat *et al.*, 2001; Yuan and Xu, 2001). However, there are also published reports where applied GA stimulated growth, but decreased the rate of photosynthesis (Dijkstra *et al.*, 1990). Thetford *et al.* (1995) found that application of inhibitors of GA biosynthesis stimulated photosynthesis, while reducing elongation growth. There are also reports showing that application of GA biosynthesis inhibitors reduced both growth and photosynthetic rate (Bode and Wild, 1984; Heide *et al.*, 1985). Furthermore, Cramer *et al.* (1995) found no difference in photosynthetic rate between the wild type (WT) and a low GA mutant of tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*). Thus, the studies published so far are contradictory and do not show a clear relationship between GA levels and rates of photosynthesis, respiration, and growth (Nagel and Lambers, 2002).

Although application of GAs and GA biosynthetic inhibitors often has been used as a fruitful approach in studies of GA physiology, differences in uptake and access to target tissue of the applied GA in different species, tissues, and experiments cannot be excluded. Thus, to study the effect of GAs on photosynthetic and respiratory capacity, using plants with different endogenous GA content or different response to GAs is preferable to application experiments. In WT pea, detailed analyses of GA content in different tissues have shown that the levels of active GA_1 are down-regulated under negative DIF as compared with positive DIF (Grindal *et al.*, 1998; Stavang *et al.*, 2005). Furthermore, the *la cry*^{s} mutant responds poorly to DIF treatments (Grindal *et al.*, 1998). This elongated pea mutant does not respond to applied GAs or GA biosynthesis inhibitors, but shows a saturated GA response independently of growth conditions (Potts *et al.*, 1985; Ingram and Reid, 1987). This mutant has now been identified as a double DELLA mutant (Weston *et al.*, 2008). Mutations in *LA* and *CRY*^{s} in pea result in non-functional DELLA proteins, and thus the growth inhibitory effect of these proteins is not present in the *la cry*^{s} mutant. Since stem elongation in the *la cry*^{s} mutant is similar to that of the WT given a saturating dose of active GA, it is suggested by Weston *et al.* (2008) that *LA* and *CRY* are the only DELLA-encoding genes involved in shoot growth in pea. Therefore, any regulation of GA levels occurring in *la cry*^{s} should have no effect in downstream signalling in the GA response pathway mediated by these DELLAs.

By exposing the WT and the DELLA mutant *la cry*^{s} to positive and negative DIF treatments, this study aimed to separate thermoperiodic growth mediated by GA regulation from thermoperiodic growth independent of GA signalling through these DELLAs in pea.

Materials and methods

Plant material and experimental conditions

Seed of *P. sativum* L. WT line 107 (cv. Torsdag) or the slender saturated GA response mutant *la cry*^{s}, which is mutated in the *LA* and *CRY*^{S} genes encoding DELLA proteins (Potts *et al.*, 1985; Ingram and Reid, 1987; Weston *et al.*, 2008), were sown in an 11 cm pot containing fertilized peat (Floralux, Nittedal Torvindustrier, Nittedal, Norway) and grown under controlled environmental conditions (Conviron growth chambers, Controlled Environments Ltd, Winnipeg, Canada). The humidity was adjusted to give 0.47±0.03 kPa water vapour deficit. The daily light period was 12 h (light period, 09:00–21:00; dark period, 21:00–09:00) with an irradiance of 160±10 μmol m $^{-2}$ s^{-1} at 400–700 nm [F96T12/CW/1500 fluorescent tubes (General Electric, Fairfield, CT, USA) enriched with light from incandescent lamps (OSRAM, Munich, Germany)]. The red/far red ratio was 1.7±0.1. The seedlings were watered daily with a complete nutrient solution of EC=1.5 mS cm^{-1}. The temperature was kept at 17 °C until the hypocotyls had straightened (6 d), then the plants were transferred to two separate growth chambers with different combinations of day (DT) and night temperature (NT), both at a daily average temperature of 17 °C. The effect of a DT/NT of 13 °C/21 °C treatment (negative DIF) was compared with a 21 °C/13 °C treatment (positive DIF). The DIF treatments started on day 6 when the light was turned on. Plant height was recorded every third or fourth day. Light level at the top of the plants was maintained at 160 μmol m $^{-2}$ s^{-1}during the experimental period. For chlorophyll measurements, some plants were held at constant temperatures of 21 °C and 13 °C.

Leaf chlorophyll fluorescence recording, chlorophyll estimation, leaf area, and dry weight measurements

On day 12 after the start of the DIF treatments, relative chlorophyll content was estimated with a Hansatech CL-01-chlorophyll content meter (Hansatech Instruments, King's Lynn, Norfolk, UK) in leaf 1–5 or 6 counted from the bottom of WT and *la cry*^{s} plants. Ten plants per genotype were harvested 14 d after the start of the DIF treatments. Plants were divided into three fractions: leaves, roots, and stem tissue (stem tissue included stem, tendrils, and petioles and is henceforth referred to as stem). Leaf area was determined with a Li-Cor LI-3100 area meter (Li-Cor Biosciences, Lincoln, NE, USA). The root was rinsed with water before drying, and dry masses were determined after drying in an oven at 70 °C for 5 d.

Leaf chlorophyll fluorescence was recorded with a portable, modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany). Chlorophyll fluorescence parameters were measured on seven plants per treatment, and on each plant measurements were performed both on a young expanding (leaf 5) and on a fully expanded leaf (leaf 3). The measurements were performed on dark-adapted leaves in the morning before the lights were turned on on day 13. The following protocol was used for the quenching analysis: first F_0 and F_m were determined for dark-adapted leaves using a saturating pulse of 0.6 s duration. Then, after 10 s, actinic irradiance (210 μmol m^{-2} s^{-1} from internal red diodes in the fluorometer) was turned on. Steady-state chlorophyll fluorescence (F_t) was reached after 5 min, and F_m' was determined with a new saturating pulse. Finally, the actinic light was turned off and F_0' was determined after far-red irradiation for 3 s. The following parameters were determined: (i) maximal photochemical quantum yield of photosystem II [PSII; $F_v/F_m=(F_m-F_0)/F_m$]; (ii) irradiance-adapted quantum yield of PSII [$\Phi PSII=F_v'/F_m'=(F_m'-F_t)/F_m'$] (Genty *et al.*, 1989); (iii) non-photochemical quenching, $NPQ=(F_m-F_m')/F_m'$ (Bilger and Björkman, 1990); (iv) electron transport rate, $ETR=\Phi PSII\times PDF\times 0.5$ (Genty *et al.*, 1989); and (v) proportion of open PSII, $Qp=(F_m'-F_t)/(F_m'-F_0')$ (e.g. Maxwell and Johnson, 2000).

Stem elongation rate recordings

After 10 d of growth of the WT and the *la cry*^{s} mutant at 17 °C, negative or positive DIF treatments were introduced. For comparison, a subset of plants was also kept at 17 °C (zero DIF) or moved to constant 13 °C and constant 21 °C. The stem elongation rate was then continuously measured every tenth second for 3 d according to Torre and Moe (1998) by an angular Displacement Transducer, series 604 (Trans-Tec. Ellington, CT, USA) connected to a data logger, type CR10-AM416 (Campbell Scientific Inc., Shepshed, UK). The water vapour deficit could not be precisely controlled in these chambers and relative humidity varied from 45% to 65%.

GA measurements

After 12 d of DIF treatment, leaf 3 and 5 counted from the bottom of WT and *la cry*^{s} mutant plants were harvested in liquid nitrogen. For each DIF treatment, genotype, and leaf number, four samples consisting of leaves from 20 plants were extracted and analysed for their GA contents according to Stavang *et al.* (2007) and Olsen and Junttila (2002). This included extraction in cold methanol, addition of [17, 17-^{2}H]GA_{19}, [17, 17-^{2}H]GA_{20}, [17, 17-^{2}H]GA_{29}, [17, 17-^{2}H]GA_1, and [17, 17-^{2}H]GA_8 (LN Mander, Australian National University, Canberra, Australia), partition against ethyl acetate, use of QAE Sephadex A25 (Pharmacia, Uppsala, Sweden) anion-exchange columns combined with 0.5 g Sep-Pak Vac C18 cartridges (Varian, Harbor City, CA, USA), methylation, and purification on 0.1 g bond elute aminopropyl cartridges (Varian), followed by reverse-phase HPLC. Combined HPLC fractions were trimethylsilylated and subjected to combined gas chromatography–mass spectrometry in the selected ion monitoring mode. For each GA, two characteristic ions and their deuterated analogues were recorded.

Gas exchange measurements

All gas exchange measurements were performed with a portable leaf cuvette system [CIRAS-2 portable photosynthesis system attached to a Parkinson leaf cuvette (PLC6), PP systems, Hitchin, Hertfordshire, UK]. The CIRAS-2 Remote Control Software (PP systems) was used to program the parameters of the analyses and to collect and store data. In addition to showing recordings of CO_2 assimilation (P_{net}), cuvette temperature, and light, this software automatically calculates internal CO_2 concentrations (*Ci*) and stomatal conductance (g_S). All gas exchange measurements were performed 11–13 d after the start of the DIF treatments. Night respiration (R_{night}) measurements were made on leaf pair 3, 4, and 5 (counted from the soil). Leaf 3 was fully expanded, while leaf 4 and leaf 5 (the youngest) were still expanding. R_{night} measurements were performed from 23:00 to 07:30, and results were averaged. Net photosynthesis (P_{net}) and day respiration (R_{day}) were measured from 10:00 to 20:00 on day 12, and averages of the measurements are presented. P_{net} and R_{night} measurements were done at ambient leaf temperature and for 6–7 plants in each case (in the light period leaf temperatures were ~2 °C above air temperature). P_{net} was measured at a photon flux density of 160 μmol m^{-2} s^{-1}.

In preliminary studies, light response curves were made to establish which photon flux densities were saturating. It was then found that a photon flux density of 1000 μmol m^{-2} s^{-1} was saturating under both positive and negative DIF. This photon flux density gave only slightly (but not statistically significantly, *t*-test; $P \geqslant 0.05$) higher CO_2 assimilation than both 600 μmol m^{-2} s^{-1} and 800 μmol m^{-2} s^{-1} under negative DIF. CO_2 response curves were recorded at saturating light conditions (1000 μmol m^{-2} s^{-1}) at day 12 (leaf 5) and day 13 (leaf 3), following the recommendations by Long and Bernacchi (2003). Intercellular CO_2 response curves (*A*/*Ci* curves) were analysed as follows (n=7). First, estimates of maximum carboxylation velocity of Rubisco, V_{cmax}, and day respiration rate, R_{day}, were obtained by fitting the following equation to the rates of CO_2 assimilation, *A*, at low intercellular CO_2 partial pressures (von Caemmerer and Farquhar, 1981):

$$A=V_{cmax}\left(Ci-\Gamma^*\right)/\left[Ci+K_c\left(1+O/K_o\right)\right]-R_{day} \quad (1)$$

Ci is the intercellular partial pressure of CO_2 (here assumed to be equal to that at the sites of carboxylation), Γ* is the CO_2 photocompensation point in the absence of non-photorespiratory CO_2 evolution, R_{day}, K_c and K_o are the Michaelis–Menten constants of Rubisco for CO_2 and O_2, respectively, and *O* is the oxygen concentration. The kinetic constants for Rubisco were assumed to be equal to those determined for tobacco (von Caemmerer *et al.*, 1994), namely 36.9 μbar for Γ* and 730 μbar for *K*′ at 25 °C. When calculating V_{cmax} and J_{max}, parameter values were adjusted using the Arrhenius equation and activation energies given by de Pury and Farquhar (1997). Having derived the best fit for the lower range of *Ci*, the estimate of maximum electron transport rate contributing to RuBP regeneration, J_{max}, was then obtained by fitting the following equation to the rates of CO_2 assimilation at high intercellular CO_2 partial pressures (von Caemmerer and Farquhar, 1981):

$$A=tJ_{max}\left(Ci-\Gamma^*\right)/\left(4Ci+8\Gamma^*\right)-R_{day} \quad (2)$$

The relative effect of stomatal limitation on photosynthesis (S%) was estimated by the following equation (Farquhar and Sharkey, 1982):

$$S\%=(A_{Ci}-A_{Ce})/A_{Ci})\times 100 \quad (3)$$

where A_{Ce} represents the net photosynthesis at an ambient external CO_2 concentration of 350 ppm and A_{Ci} represents the photosynthesis rate if there were no stomatal limitation to *A*, e.g. *Ci*=*Ce* (Farquhar and Sharkey, 1982).

Statistical analyses

The effects of the two experimental factors, DIF treatment and genotype, on measured growth parameters were analysed using a general linear model (GLM) approach in the Minitab statistical software (Minitab 15.1, Minitab Inc., PA, USA). The model used was: mean=DIF treatment+genotype+replicate+DIF treatment×genotype+replicate×DIF treatment+DIF treatment×genotype+replicate×DIF treatment×genotype. For analysis of leaf chlorophyll fluorescence, chlorophyll level, A_{sat}, V_{cmax}, J_{cmax}, and S%, the model was: mean=DIF treatment+genotype+leaf

number+DIF treatment×leaf number+DIF treatment×leaf number+genotype×leaf number+DIF treatment×genotype×leaf number. For these analyses, values from two replicate experiments were pooled. In the analysis of GA content, each leaf was analysed separately and the model was: mean=DIF treatment+genotype+DIF treatment×genotype. Tukey's test was used for testing for differences between means.

Results

Temperature, light, darkness, and gibberellin interact to determine stem elongation rate

It has been shown earlier that the level of active GA_1 in stem and leaf tissue of pea is down-regulated under negative DIF as compared with positive DIF (Grindal *et al.*, 1998; Stavang *et al.*, 2005). To investigate in more detail the influence of GAs in thermoperiodic control of stem elongation, WT pea and the saturated GA response DELLA mutant *la cry*^{s} (Weston *et al.*, 2008) were both grown under negative and positive DIF for subsequent analysis of stem elongation. Negative DIF reduced the height of WT plants by >30% compared with positive DIF, whereas height of the *la cry*^{s} mutant was not affected by the DIF treatments within the experimental period (Fig. 1). After 13 d the *la cry*^{s} mutant was on average 65% and 100% higher than the WT at positive and negative DIF, respectively.

By using triangular transducer equipment it was possible to examine the influence of growth temperature on the stem elongation rate in more detail. At constant temperatures, (13 °C, 17 °C, and 21 °C), the rate of stem elongation was lower in the light period than in the dark for both genotypes (Fig. 2A), and the diurnal stem elongation rate was affected similarly by night and day cycling in the WT and *la cry*^{s}. From the results presented in Fig. 2A, the average stem elongation rate during the day and night for each temperature was calculated separately (Fig. 2B). For both WT and *la cry*^{s} plants, a linear correlation between stem elongation rate and DT and NT was observed (Fig. 2B). To study the effects of DIF on stem elongation rate, an experiment with negative, zero, and positive DIF (with all plants having the same average daily temperature) was conducted. In contrast to the stem elongation rate under constant temperature (zero DIF), the stem elongation rate under positive DIF was higher during the light period than during the night for both genotypes (Fig. 2C). Under negative DIF, the stem elongation rate of the WT was strongly inhibited during the daytime, and, importantly, the WT did not compensate for the reduced stem elongation rate during the day the following night. However, the *la cry*^{s} plants grown under negative DIF compensated for the reduced growth in the daytime under negative DIF by having a very high stem elongation rate the following night(s) (Figs 1, 2C). When averages of stem elongation rate in the day or night were plotted against temperature, a linear correlation was observed between stem elongation rate and DT, but not for NT in the WT. In *la cry*^{s} plants a linear correlation was found for both DT and NT (Fig. 2D). Since negative DIF reduces GA_1 in WT plants (Grindal *et al.*, 1998; Stavang *et al.*, 2005), and *la cry*^{s} plants show a GA-saturated response, this response of the WT to negative DIF indicates that GA regulation is important for mediating a DIF response and that the stem elongation rate is determined by ambient growth temperature, light/darkness, and GA levels/GA response in an interacting manner. In conclusion, DELLAs, light, and low temperatures inhibit stem elongation, and an intact GA signalling pathway is needed to mediate the DIF effect on stem elongation.

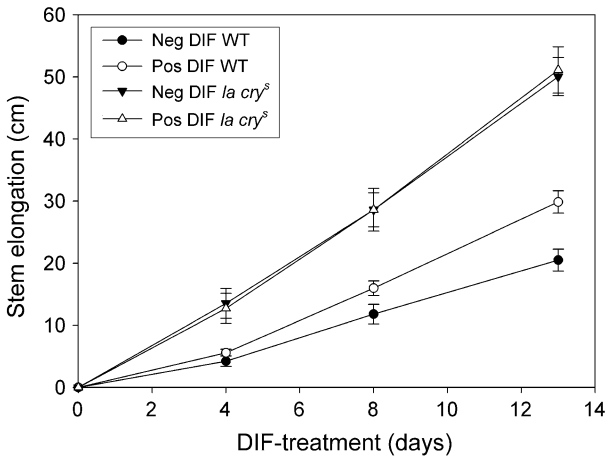

Fig. 1. Effect of 12 d of different (DIF) day and night temperatures (DT/NT) on stem elongation of the *la cry*^{S} mutant and WT of pea. Seedlings were grown for 6 d at a constant temperature of 17 °C prior to the start of the DIF treatments. The temperature regimes were negative DIF (13 °C/21 °C) and positive DIF (21 °C/13 °C). Results are the mean ±SD of 10 plants.

Thermoperiodic regulation of GA levels in leaves of the WT and the DELLA mutant la cry^S

To study effects of GAs on the photosynthetic and respiratory capacity of pea, it was relevant to measure GA_1 levels in the leaves in parallel, and therefore GA measurements were also included in this study. It turned out that both genotypes responded to a negative DIF treatment by down-regulating the level of GA_1. The GA_1 content of young leaves (leaf 5) of WT and *la cry*^{s} plants grown under negative DIF was reduced by 51% and 80%, respectively (Fig. 3). There was no statistically significant effect of genotype, but DIF treatment (P <0.01) and the interaction term DIF×genotype (P <0.01) were both highly significant. Thus, the data suggest that the mutant is more sensitive to the temperature regimes with respect to regulation of the GA_1 level. However, due to lack of functional DELLA proteins resulting in a GA-saturated response, there was no effect of DIF on stem elongation in *la cry*^{s} within the time frame of the experiment (Figs 1, 2). Irrespective of this, the *la cry*^{s} mutant generally followed the pattern of the WT regarding the effect of DIF on the levels of other GAs, even though the levels of GA_{44}, GA_{29}, and GA_{19} were higher in the mutant. However, the level of GA_{20} was almost a 3-fold

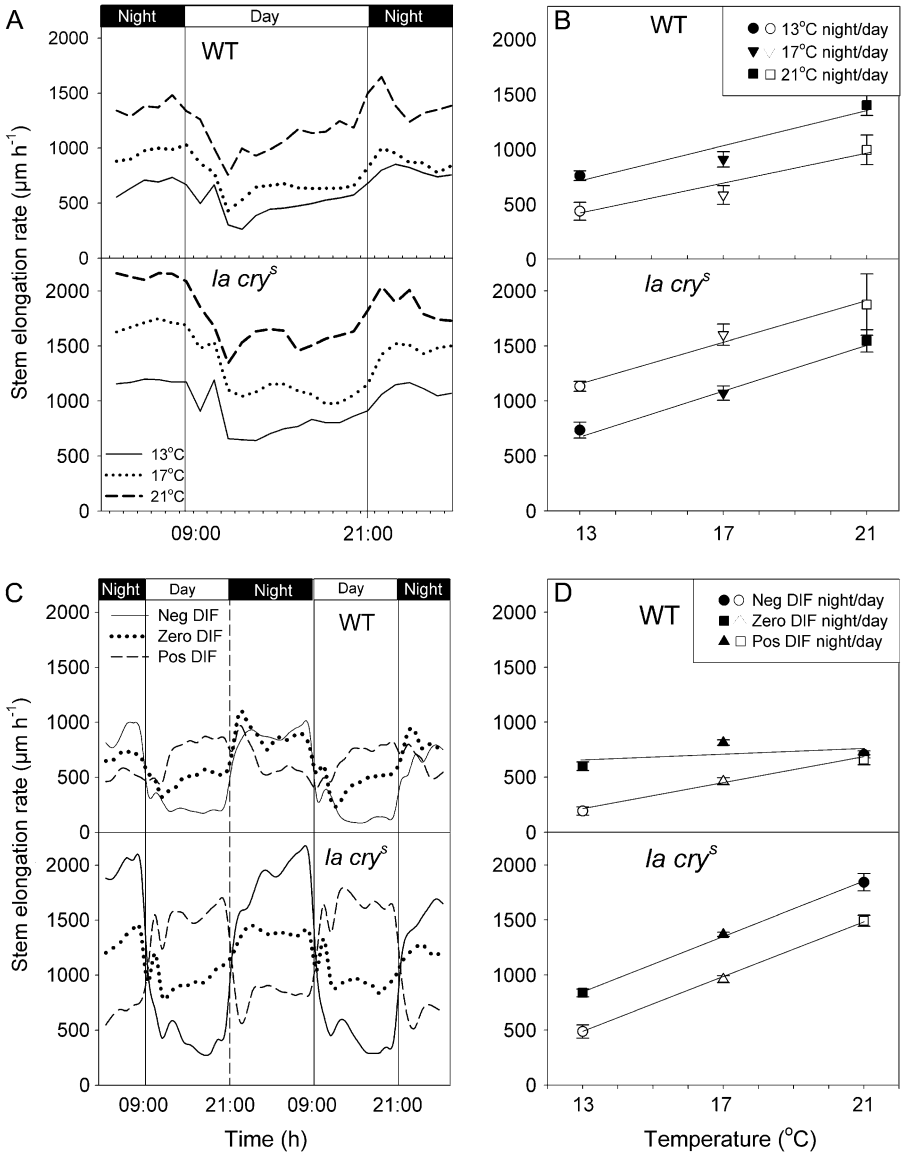

Fig. 2. Stem elongation rate recordings of plants grown under different temperature regimes. (A) Diurnal growth rhythms of the *la cry*^{s} mutant and WT pea as affected by different constant growth temperatures (13 °C/13 °C, 17 °C/17 °C, and 21 °C/21 °C DT/NT). (B) Average stem elongation rate ±SE in the light and dark of WT and *la cry*^{s} plants grown under constant temperatures (calculated from A) plotted against growth temperature. A regression line is fitted to the dark and light mean recordings. (C) Diurnal growth rhythms of the *la cry*^{s} mutant and WT pea as affected by different day and night temperature (DT/NT) combinations. The temperature regimes were negative DIF (13 °C/21 °C DT/NT), zero DIF (17 °C/17 °C), and positive DIF (21 °C/13 °C). (D) Average stem elongation rate ±SE in the light and dark of WT and *la cry*^{s} plants grown under negative, zero, and positive DIF (calculated from C) plotted against growth temperature. A regression line is fitted to the dark and light mean recordings. In all experiments seedlings were grown for 10 d at a constant temperature of 17 °C prior to the start of treatments. Stem elongation rate recordings show days 11–13. Results are the average of eight individual plants.

higher in expanding leaves of *la cry*^{s} plants grown at positive DIF than under negative DIF, whereas in the WT there was no significant difference between the DIF treatments (Fig. 3). In fully expanded leaves (leaf 3) GA_1 levels were low in both genotypes. This might at least partly be due to high inactivation rates, since GA_1/GA_8 and GA_{20}/GA_{29} ratios were increased as compared with those in younger leaves (see also Ross *et al.*, 2003). Also, there were no significant differences between WT and *la cry*^{s} plants or between DIF treatments in the expanded leaves.

Non-functional DELLA proteins restrain changes in dry matter allocation in response to alternating diurnal temperatures

To study the effects of the DIF treatments on growth and biomass allocation in WT and *la cry*^{s} plants, the dry weights of several tissues were measured at the end of the experimental period. The *la cry*^{s} seeds were heavier than WT seeds [*la cry*^{s}, 0.331±0.015 (mean±SE) g seed^{-1}, n=10; and WT, 0.247±0.007 g seed^{-1}, n=10]. Total dry weight

accumulation (dry weight of shoot and root tissue–seed weight) of WT plants grown under negative DIF was reduced from 0.317 g plant^{-1} to 0.215 g plant^{-1} as compared with plants grown at positive DIF (P <0.01). Stem was the tissue most affected by negative DIF in the WT, with a reduction in dry weight of 34% (P <0.01). Negative DIF reduced leaf dry weight by 16% (P <0.01) (Table 1). Leaf area was also significantly reduced under negative DIF (9%; P=0.05), but less than dry weight. Accordingly specific leaf area (SLA; projected leaf area per unit leaf dry mass) increased under negative DIF, from 615 cm^2 g^{-1} to 670 cm^2 g^{-1} (P <0.01), indicating that the leaves got thinner or became less dense under negative DIF. Root dry weight was reduced by 5% in the WT (not significant). In *la cry*^{s}, dry weight accumulation was reduced from 0.203 g plant^{-1} to 0.121 g plant^{-1} (P <0.01). In contrast to the WT where stem was the tissue most affected by negative DIF, the reduction of dry weight in *la cry*^{s} was quite similar for all tissue types, ~14–17%. The roots of the *la cry*^{s} mutant developed poorly as compared with the WT, and even with the remnants of the seeds included, the dry weight of the roots of *la cry*^{s} was significantly lower than that of the WT

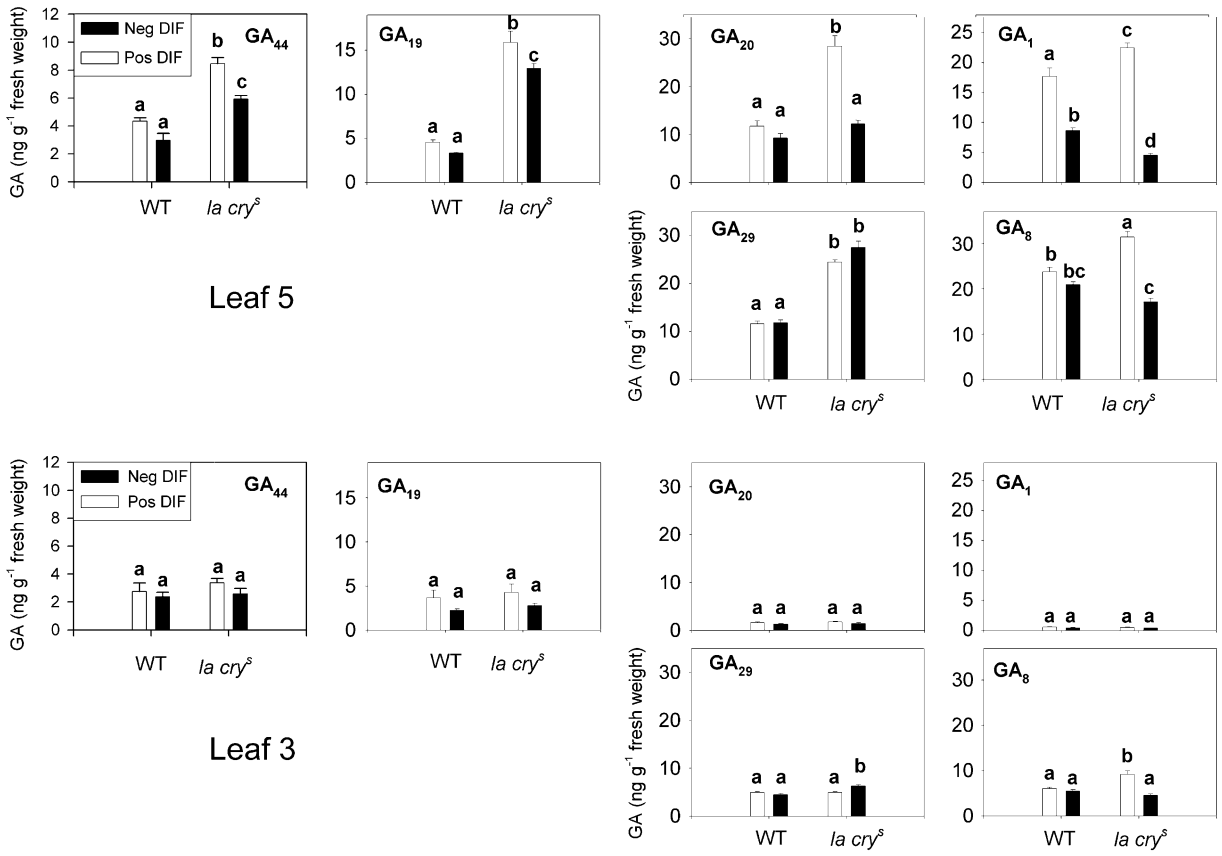

Fig. 3. GA levels in young expanding (leaf 5) and expanded (leaf 3) leaves of WT and the *la cry*^{s} mutant of pea seedlings after 12 d of growth under negative (13 °C/21 °C DT/NT) or positive DIF (21 °C/13 °C DT/NT). Presented are the mean of 3–4 independent samples, where each sample contained leaves from 20 plants, ±SE. Different letters denote significantly different values.

Table 1. Dry weight (g) parameters, leaf area (cm^2), and specific leaf area (cm^2 g^{-1}) of WT and *la cry*^{s} mutant pea seedlings after 20 d of growth, of which the last 14 d were under negative (13 °C/21 °C DT/NT) and positive DIF (21 °/13 °C DT/NT)

Presented are means ±SE with n=19–20. Data on significant effects of replicates are not shown.

Treatment and genotype	Leaf dry weight (g)	Stem dry weight (g)	Root dry weight (g)	Leaf area (cm^{-2})	Specific leaf area (cm^{-2} g^{-1})
Positive DIF WT	0.229±0.012	0.165±0.009	0.170±0.008	139±5	615±21
Negative DIF WT	0.191±0.011	0.109±0.006	0.161±0.009	127±5	670±21
Positive DIF *la cry*^{s}	0.184±0.008	0.221±0.006	0.129±0.009	114±4	624±12
Negative DIF *la cry*^{s}	0.159±0.008	0.182±0.003	0.111±0.007	110±5	696±17
Statistical analysis (ANOVA: GLM)					
DIF treatment	0.000	0.000	0.001	0.02	0.000
Genotype	0.000	0.000	0.000	0.000	NS
Treatment×genotype	NS	NS	NS	NS	NS

(on average 27% less root dry weight, P <0.01; Table 1). *la cry*^{s} had 6% lower leaf area than the WT (P <0.01). However, the mutant was similar to the WT regarding SLA, and negative DIF increased SLA by ~10% in both genotypes. Thus, with no significant difference between the genotypes regarding SLA, this indicates that GA signalling through DELLAs does not affect SLA.

In order to get a picture of how a negative DIF treatment affected plant architecture, stem mass ratio (SMR; stem mass per unit plant mass), root mass ratio (RMR; root mass per unit plant mass), and leaf mass ratio (LMR; leaf mass per unit plant mass) were calculated. On average, the *la cry*^{s} mutant had a significantly higher SMR, and lower RMR and LMR than the WT under both positive and negative DIF (P <0.01; Fig. 4), reflecting the higher amount of stem tissue, and less root and leaf tissue in the mutant as compared with the WT (Table 1). In the WT, a negative DIF treatment resulted in a reduction of SMR from 0.29 to 0.24 (P <0.01) and an increase in the RMR from 0.30 to 0.34 (P <0.01). There was also a less pronounced effect of DIF on LMR in the WT, with a reduction from 0.421 to 0.405 (P=0.01). In contrast to the WT, *la cry*^{s} did not show any changes in RMR, LMR, or SMR. Taken together, these data indicate that DIF regulation of GA levels contributes to mediate these changes in dry matter allocation, while a saturated GA response to a large extent prevents these changes (Figs 3, 4, and Table 1).

There was a tendency for *la cry*^{s} plants to develop more leaves than the WT in the time frame of the experiment. After 12 d of DIF treatment (and 18 d of total growth), WT plants had unfolded leaf 5 while *la cry*^{s} plants were unfolding leaf 6. When grown for 1 month, *la cry*^{s} developed 15.5 leaf pairs as compared with 12.6 leaf pairs for the WT (G Grindal Patil, unpublished results), demonstrating that DELLA proteins affect the leaf unfolding rate.

Negative DIF reduces chlorophyll content in both the WT and the DELLA mutant

Plants grown at negative DIF are reported to be paler green due to lower chlorophyll content than plants grown at positive DIF (Moe and Heins, 2000), and this was also found in our experiment (Fig. 5). In the youngest leaves there was more than a 50% reduction of relative chlorophyll content in both genotypes in plants grown at negative DIF compared with plants grown at positive DIF, while the reduction in older leaves was less pronounced. Furthermore, in young leaves of *la cry*^{s} and the WT there were no

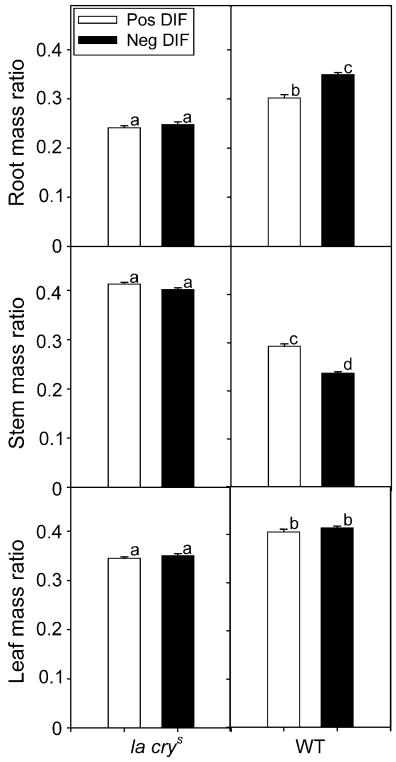

Fig. 4. Morphological responses to negative and positive DIF. Leaf mass ratio is the leaf mass per unit plant mass in WT and *la cry*^{s} mutant pea seedlings after 20 d of growth. Root mass ratio is the root mass per unit plant mass. Stem mass ratio is the shoot mass per unit plant mass. DIF treatments were applied for the last 14 d. Positive DIF was 21 °C/13 °C. Negative DIF was 13 °C/21 °C. Results are the mean ±SE of 20 plants. Different letters denote significantly different values.

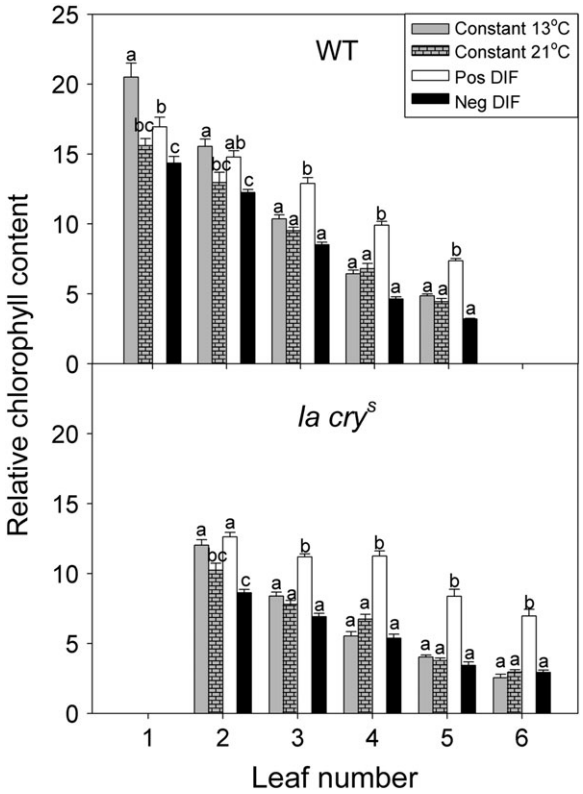

Fig. 5. Effect of constant day and night temperatures (13 °C/13 °C and 21 °C/21 °C DT/NT) and of positive and negative DIF (21 °C/13 °C and 13 °C/21 °C DT/NT) on chlorophyll content of leaves of WT and the *la cry*^{s} mutant of pea. Leaf number 1, which represents the oldest leaf, the epicotyledons, were too small to be measured in *la cry*^{s}. Results are the mean ±SE of 10 plants. Different letters denote a significant difference.

or only small differences in relative chlorophyll content, while the oldest leaves of the WT contained more chlorophyll than those of *la cry*^{s}. In order to try to separate the effects of different day and night temperatures from the DIF effect, plants grown at constant 13 C or 21 °C were included in the experiment. WT plants grown under constant temperatures contained intermediate levels of chlorophyll as compared with plants grown under positive and negative DIF, but clearly age was a factor as the oldest leaves of plants grown under constant 13 °C had the highest chlorophyll content (Fig. 5). We analysed the genotypes separately and, for both genotypes, the main factors and the interaction factor were significant (temperature treatment, P <0.0001; leaf number, P <0.0001; temperature treatment and leaf, P <0.01 for both genotypes).

Negative DIF reduces F_v/F_m and electron transport rate in young, expanding leaves

In order to study in more detail the effect of a negative DIF treatment and impaired GA signalling on photochemistry of the leaves, a complete chlorophyll fluorescence quenching analysis by the saturation pulse method was performed in dark-adapted leaves (Table 2). A GLM analysis revealed that negative DIF slightly reduced F_v/F_m, especially in expanding leaves [significant interaction between leaf and DIF treatment (P <0.01)]. For the expanding leaves, F_v/F_m was slightly higher in the mutant leaves than in the WT, whereas there was almost no difference for the expanded leaves [significant interaction between leaf and genotype (P <0.01)]. However, as mentioned above, there was a slight difference in developmental stage between the mutant and WT, and this might have had an effect on the measurements. The ETR at 210 μmol m^{-2} s^{-1} was higher in expanded leaves than in young leaves. This effect was greater for the WT than for *la cry*^{s} [significant interaction between leaf and genotype (P <0.01)]. ETR was also higher at positive DIF for young leaves, whereas there was no difference for expanded leaves [significant interaction between leaf and DIF treatment (P <0.01)]. The expanding leaves had higher NPQ than the fully expanded leaves (P=0.02). This higher capacity to dissipate excess energy may be an acclimation to higher irradiance because they are closer to the fluorescent tubes, or it may be a result of a lower photosynthetic capacity in the youngest leaves (Fig. 6). There were only small differences in the proportion of open PSII centres (Q_p) (Table 2).

Impaired DELLA signalling does not affect photosynthetic capacity or respiration

To try to explain the observed growth/biomass responses of pea plants to the DIF treatments, and to evaluate the role of GA signalling through DELLA on photosynthetic capacity, net CO_2 gas exchange was measured in young, expanding (leaf 5 and 4) and fully expanded leaves (leaf 3), during a 24 h night/day cycle using a portable leaf cuvette gas exchange system set to ambient growth conditions.

Younger leaves had significantly higher night respiration (R_{night}) than older leaves in both genotypes, and plants grown at negative DIF had higher R_{night} than plants grown at positive DIF (Fig. 6). The effect of DIF on R_{night} was most pronounced in expanded leaves (leaf 3) where the relative difference was 3-fold, with R_{night} values of ~0.35 μmol m^{-2} s^{-1} and 1.05 μmol m^{-2} s^{-1} under negative and positive DIF, respectively, for both genotypes. In expanding leaves of WT plants (leaf 5), R_{night} was 1.2 μmol m^{-2} s^{-1} and 1.8 μmol m^{-2} s^{-1} under negative DIF and positive DIF, respectively. Corresponding R_{night} values for *la cry*^{s} were 0.9 μmol m^{-2} s^{-1} and 1.4 μmol m^{-2} s^{-1}. There was no

Table 2. Chlorophyll fluorescence parameters of young expanding and fully expanded leaves of WT and *la cry*^{s} mutant of 18-day-old pea seedlings grown under negative (13 °C/21 °C DT/NT) and positive DIF (21 °C/13 °C DT/NT)

F_v/F_m represent maximal dark-adapted PSII efficiency, ETR is the electron transport rate through PSII at 210 μmol m^{-2} s^{-1} irradiance, NPQ is non-photochemical quenching, and Q_p is the proportion of open PSII centres. Presented are means ±SE with n=7.

Treatment/leaf/genotype	F_v/F_m	ETR (μmol e^{-} m^{-2} s^{-1})	NPQ	Qp
Positive DIF/leaf 5/WT	0.801±0.003	53.0±2.2	1.16±0.13	0.807±0.010
Negative DIF/leaf 5/WT	0.776±0.002	50.4±1.4	1.00±0.06	0.789±0.011
Positive DIF/leaf 3/WT	0.829±0.001	58.2±0.8	0.87±0.03	0.789±0.010
Negative DIF/leaf 3/WT	0.820±0.002	62.2±1.0	0.66±0.03	0.826±0.090
Positive DIF/leaf 5/*la cry*^{s}	0.821±0.001	60.5±0.4	0.86±0.98	0.832±0.002
Negative DIF/leaf 5/*la cry*^{s}	0.795±0.006	53.1±1.3	0.98±0.03	0.787±0.010
Positive DIF/leaf 3/*la cry*^{s}	0.834±0.001	58.1±1.5	1.06±0.08	0.792±0.011
Negative DIF/leaf 3/*la cry*^{s}	0.827±0.001	57.2±0.9	0.96±0.05	0.797±0.008
Statistical analysis (ANOVA: GLM)				
DIF treatment	0.000	NS	NS	NS
Genotype	0.000	NS	NS	NS
Leaf	0.000	0.000	0.017	NS
DIF×genotype	NS	0.010	0.039	0.027
DIF×leaf	0.000	0.001	NS	0.000
Genotype×leaf	0.001	0.000	0.000	NS
Genotype×leaf×DIF treatment	NS	NS	NS	NS

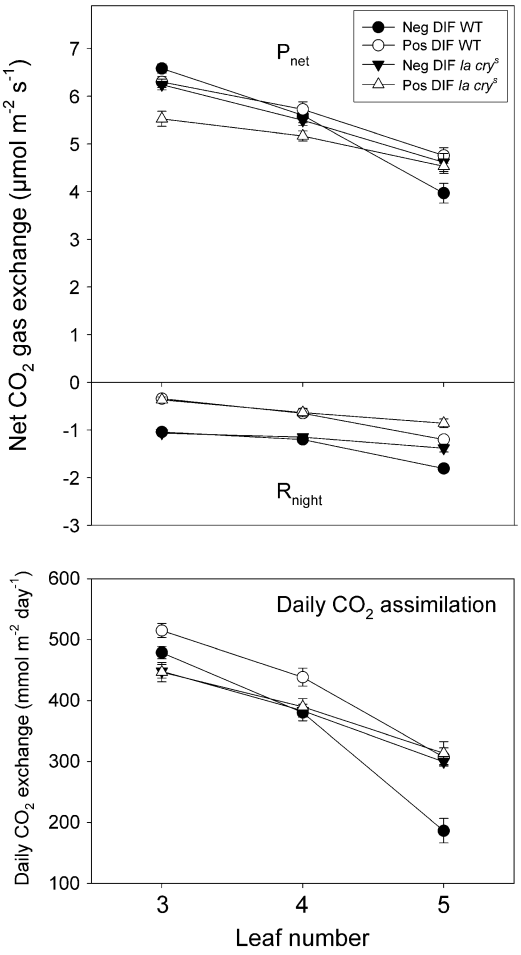

Fig. 6. Gas exchange under ambient growth temperatures in leaves of WT and the *la cry*^{s} mutant of pea grown under positive and negative DIF. The upper diagram is night respiration (R_{night}), the middle diagram is net photosynthesis (P_{net}), and the lowest diagram is diurnal net assimilation of CO_2 (diurnal CO_2 assimilation= P_{net}–R_{night}). The temperature regimes were: negative DIF 13 °C/ 21 °C day temperature/night temperature and positive DIF 21 °C/ 13 °C. Each value is the mean ±SE of six measurements. The measurements were done at ambient leaf temperature (15 °C under negative DIF and 23 °C under positive DIF). Measurements were done on days 12–13 after the start of the DIF treatments.

significant difference in R_{night} between leaves of WT and *la cry*^{s}, except for the youngest leaf (leaf 5). The difference in CO_2 exchange for these young leaves is most probably due to the fact that leaf number 5 in *la cry*^{s} had developed further than leaf number 5 of the WT, and probably releases less CO_2 related to growth respiration. The temperature response R_{night} was also tested in leaves from plants grown under negative and positive DIF, and, as expected, R_{night} increased almost 2-fold from 13 °C to 21 °C (Supplementary Fig. S1 aavaailable at *JXB* online). Thus, temperature and developmental state determines R_{night}, while a saturated GA response has no effect on R_{night}.

Net photosynthesis (P_{net}) in the daytime was measured at ambient leaf temperature (2 °C higher than air temperature). In both genotypes, young leaves had significantly lower P_{net} than older leaves, and thus followed the opposite pattern to R_{night} (Fig. 6). P_{net} of the WT was similar at negative and positive DIF for leaf 3 and 4, but was reduced in leaf 5 at negative DIF. *la cry*^{s} behaved differently from the WT, with less P_{net} under positive than negative DIF for leaf 3, but with similar P_{net} under negative and positive DIF in leaf 4 and 5. Differences in P_{net} between leaves could not be explained by differences in g_s (data not shown).

Daily CO_2 assimilation (P_{net}–R_{night}) was higher in expanded leaves than in young, expanding leaves of both genotypes (Fig. 6). In the WT, daily CO_2 assimilation was significantly higher in leaves grown at positive DIF compared with negative DIF. The difference was most pronounced in the youngest leaves. A negative DIF treatment reduced diurnal CO_2 assimilation by 39% in leaf 5, 13% in leaf 4, and 8% in leaf 3. In contrast to the WT, there was no difference in diurnal net CO_2 assimilation of the *la cry*^{s} mutant grown at positive DIF or negative DIF.

In order to get an indication of the effect of the DIF treatments on true photosynthesis (P; P=P_{net}+R_{day}), the rate of day respiration (R_{day}) was measured using two different methods. The first method involved reducing the light intensity from ambient light level to a photon flux density of 1–2 µmol photons m^{-2} s^{-1} PAR for 2 min and then recording R_{day}. The second method involved fitting Equation 1 (see Materials and methods) for the net CO_2 exchange versus internal CO_2 partial pressure over the low partial pressure range according to von Caemmerer and Farquhar (1981). The absolute values of R_{day} depended on which method was used (data not shown). Despite variation in absolute values in the data sets, both methods significantly indicated that R_{day} was 1.2–2.0 times higher in leaves at positive DIF compared with corresponding leaves at negative DIF in both genotypes. Thus an attempt to calculate P revealed a significantly higher true photosynthesis in plants grown at positive DIF, with the largest difference in expanding leaves (data not shown). This suggests that 8 °C lower day temperature under negative DIF might reduce photosynthetic performance. This is further supported by the finding that at saturating light and CO_2 conditions, P_{net} is positively correlated with temperature and increases by almost 1.5-fold from 15 °C to 23 °C (Supplementary Fig. S2 at *JXB* online).

Respiration in leaves (R_{day} or R_{night}) correlated with growth (as measured by stem elongation rate; Fig. 2C) and ambient temperature. It was also found that there was a strong positive correlation between temperature and R_{night} (Supplementary Fig. S2 at *JXB* online). The response of R_{night} to a temperature change was rapid and occurred within a few minutes (data not shown). Stavang *et al.* (2007) showed that stem elongation responds within minutes to a drop in temperature, and thus growth rate, temperature, and respiration are closely correlated.

Initial studies on the WT indicated the following: (i) as maximum photosynthetic performance is temperature dependent (Supplementary Fig. S2 at *JXB* online), it is crucial to measure photosynthetic performance at the same measurement temperature; and (ii) young expanding leaves of

plants grown under negative DIF performed less well than corresponding leaves developing under positive DIF under saturated light and CO_2 conditions (Supplementary Fig. S3). Therefore, further measurements were done at the same measurement temperature (23 °C) on fully expanded (leaf 3) and young, expanding leaves (leaf 5).

To study further the kinetics of photosynthetic capacity of both genotypes and to compare leaves developed under negative or positive DIF conditions, CO_2 response curves (10–1000 ppb) at saturating light conditions (1000 μmol m^{-2} s^{-1}) were recorded for expanded (leaf 3) and young, expanding leaves (leaf 5) at the same measurement temperature (23 °C). From the *A*/*Ci* curves, the maximum carboxylation velocity of Rubisco (V_{cmax}) and the maximum electron rate contributing to RuBP regeneration (J_{max}) were estimated as described by von Caemmerer and Farquhar (1981).

Expanding leaves had lower V_{cmax} values than fully expanded leaves (Table 3). Negative DIF reduced V_{cmax} in both expanding and fully expanded leaves, but the reduction was more pronounced in expanding leaves (significant interaction for DIF×leaf number; P=0.04). However, this difference was significant only for young leaves of *la cry*^{s}. There was no effect of genotype on V_{cmax}. Whether the leaves were expanded or not accounted for most of the variation regarding J_{max}, with expanding leaves having lower values than expanded leaves. A negative DIF treatment reduced the J_{max} of expanding leaves even more in both genotypes (significant interaction between DIF treatment and leaf number). There was quite a good correlation between electron transport measured with chlorophyll fluorescence (ETR: Table 2) and J_{max} estimated from gas analysis measurements (r^2=0.72). With *la cry*^{s} being GA saturated, it appears that GA signalling through DELLA cannot be the cause of the reduction of J_{max} and V_{cmax} observed in expanding leaves under negative DIF.

Calculations of stomatal limitation from the CO_2 response curves revealed that the WT and *la cry*^{s} behaved similarly regarding S% (Table 3). Under positive DIF there were no significant differences between expanding and fully expanded leaves regarding S% in either the WT or *la cry*^{s}. However, under negative DIF, S% was higher in expanded leaves of both genotypes [even though the difference was not statistically significant for the WT (P=0.10)]. A_{sat} values were also calculated from the CO_2 response curves, and the highest numbers were found in expanded leaves under negative DIF in both the WT and *la cry*^{s}, even though stomatal limitation was higher in these leaves. In expanding leaves A_{sat} was significantly reduced as compared with expanded leaves. Furthermore, A_{sat} of young expanding leaves grown under negative DIF was significantly lower than A_{sat} of expanding leaves in plants grown under positive DIF.

In conclusion, negative DIF decreased the photosynthetic capacity of young expanding pea leaves. However, based on the observation that *la cry*^{s} behaves as the WT regarding respiration and photosynthetic capacity of pea, GA signalling through DELLA appears to have no or only a minor role in determining these capacities.

Discussion

WT pea plants grown under negative DIF contain less active GA_1 in developing stem and leaf tissue than WT plants grown at positive DIF (Fig. 3; Grindal *et al.*, 1998;

Table 3. Photosynthetic parameters of leaves of 18-day-old WT and *la cry*^{s} mutant pea seedlings grown under negative (DT13 °C/NT21 °C) and positive DIF (DT13 °C/NT21 °C)

Leaf 3 represents a fully expanded leaf and leaf 5 had just been unfolded and was expanding. Maximum carboxylation velocity of Rubisco, V_{cmax}, maximum electron transport rate contributing to RuBP regeneration, J_{max}, and relative effect of stomatal limitation of photosynthesis, S%, were calculated from *A*/*Ci* curves performed at 23 °C, and as described by von Caemmerer and Farquhar (1981) and Farquhar and Sharkey (1982). A_{sat} is maximum CO_2 assimilation at an irradiance of 1000 μmol m^{-2} s^{-1} and CO_2 of 1000 ppb. Presented are means ±SE with n=6–7.

Treatment/leaf/genotype	V_{cmax} (μmol CO_2 m^{-2} s^{-1})	J_{max} (μmol e^- m^{-2} s^{-1})	S%	A_{sat} (μmol CO_2 m^{-2} s^{-1})
Positive DIF/leaf 5/WT	56.3±1.3	102.4±1.5	30.2±2.0	20.5±0.3
Negative DIF/leaf 5/WT	49.9±2.8	87.0±2.9	29.3±2.5	16.6±0.6
Positive DIF/leaf 3/WT	74.9±2.3	125.0±2.6	29.4±1.0	25.3±0.6
Negative DIF/leaf 3/WT	72.5±1.6	129.8±1.3	38.3±1.9	26.0±0.3
Positive DIF/leaf 5/*la cry*^{s}	64.5±2.3	108.5±2.1	31.3±1.8	20.9±0.6
Negative DIF/leaf 5/*la cry*^{s}	46.3±2.1	86.1±2.8	28.2±2.5	17.4±0.7
Positive DIF/leaf 3/*la cry*^{s}	70.5±3.1	113.0±3.0	34.5±2.1	21.7±0.5
Negative DIF/leaf 3/*la cry*^{s}	63.4±3.3	118.3±3.0	41.1±3.0	24.4±0.6
Statistical analysis (ANOVA: GLM)				
DIF	0.000	0.000	0.04	0.03
Genotype	NS	0.01	NS	0.03
Leaf	0.000	0.000	0.000	0.000
DIF×genotype	0.03	NS	NS	NS
DIF×leaf	0.04	0.000	0.005	0.000
Genotype×leaf	0.02	0.000	NS	0.000
DIF×genotype×leaf	NS	NS	NS	NS

Stavang *et al.*, 2005). There are reports indicating that a negative DIF treatment reduces photosynthesis (Berghage *et al.*, 1990; Agrawal *et al.*, 1993) and also recent studies report a potential connection between GA levels and photosynthesis (Biemelt *et al.*, 2004; Huerta *et al.*, 2008). The purpose of this study was to separate thermoperiodic growth responses mediated by regulation of GA levels from those independent of GA signalling through DELLAs, and thereby also to investigate a potential connection between GA, growth, and photosynthetic and respiratory capacity in pea.

Expanding leaves of WT pea grown under negative DIF had a significantly lower photosynthetic capacity measured as both net CO_2 uptake (Fig. 7) and ETR (Table 2) than expanding leaves of plants grown under positive DIF, where GA_1 levels were 60% higher (Fig. 3). Thus, at first glance there appeared to be a correlation between GA_1 levels and photosynthetic capacity. This correlation was still valid in fully expanded leaves; there was no significant difference in GA_1 levels between DIF treatments and no significant difference regarding photosynthetic capacity, even though expanded leaves of plants grown under negative DIF tended to have a slightly higher photosynthetic capacity than expanded leaves of plants grown under positive DIF (ETR, Table 2, Fig. 7; and J_{max} and V_{cmax}, Table 3). However, a correlation between photosynthetic performance and GA_1 signalling through DELLAs falls apart when the double DELLA mutant *la cry*^{s} is taken into consideration. Being almost twice as tall as the WT and growing as if saturated with GA independently of temperature treatment (Fig. 1) and GA_1 levels (Fig. 3), no effect was found of non-functional DELLAs on photosynthetic parameters such as ETR (Table 2) and A_{sat}, J_{max}, and V_{cmax} (Fig 7, Table 3).

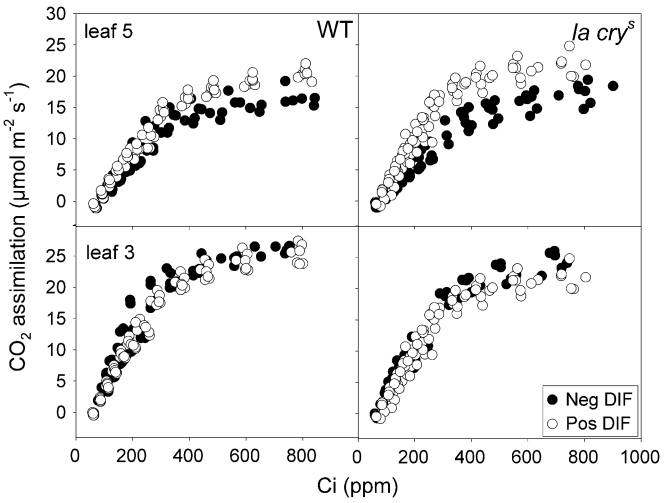

Fig. 7. CO_2 assimilation/internal CO_2 (*A*/*Ci*) curves of plants of WT and the *la cry*^{s} mutant of pea, grown under positive and negative DIF. Before the measurements the plants were grown at negative DIF (13 °C/21 °C DT/NT) and positive DIF (21 °C/13 °C) for 12 d. The measurements were performed at a leaf temperature of 23 °C for both DIF treatments, and 6–7 measurements are plotted for each leaf and DIF treatment.

Furthermore, *la cry*^{s} was affected by the DIF treatments in a similar way to the WT regarding photosynthetic capacity, indicating that regulation of these processes is independent of GA signalling through DELLAs. Based on these observations, it is unlikely that GA is a main determinant of a plant's photosynthetic capacity on a leaf area basis, as could be expected from earlier results in GA application studies (Khan, 1996; Hayat *et al.*, 2001; Yuan and Xu, 2001). Thus, the present results are in line with work performed on tomato, where it was concluded that the rate of photosynthesis per unit leaf area was similar for GA-deficient plants and WT plants (Nagel and Lambers, 2002). Interestingly, in transgenic tobacco (*Nicotiana tabacum*), light-saturated photosynthesis per leaf area was actually higher in transgenic plants with low GA content as compared with the WT and plants with a high content of GA (Biemelt *et al.*, 2004). This is comparable with the finding that application of paclobutrazol, a GA biosynthesis inhibitor, to *Catharanthus roseus* plants enhanced photosynthesis (Jaleel *et al.*, 2007). The existence of GA signalling pathways other than through the DELLA pathway cannot yet be excluded (see for instance Maymon *et al.*, 2009) but, based on the published and contradictory reports, it seems unlikely that there exists a simple control of photosynthetic capacity by GAs. Furthermore, no effect of a saturated GA signalling response on respiration was found, and R_{night} of the DELLA mutant *la cry*^{s} was identical to that of the WT. In addition R_{night} of the mutant and WT was affected similarly by different night temperatures (Fig. 6).

The present study, as well as earlier studies, has shown that a negative DIF treatment reduces the levels of chlorophyll as compared with a positive DIF treatment (Fig. 4; Erwin and Heins, 1995; Grindal, 1997; Vaagen *et al.*, 2003). The negative DIF treatment reduced chlorophyll content in both genotypes and in all leaves of the pea plants. It is reported that chlorophyll content increases from a negative to a positive DIF (Erwin and Heinz, 1995). This observation supports a very simple hypothesis; low temperature in DT inhibits production of chlorophyll, while high NT stimulates degradation processes of chlorophyll. Thus, such a shift in equilibrium could explain the connection between DIF and chlorophyll content of plants grown under different DIF regimes. If this is the case, plants grown at constant temperatures should contain intermediate amounts of chlorophyll. The data from this experiment partly support such a hypothesis. However, it is also possible that a negative DIF temperature regime (the opposite of common temperature oscillations in nature) might disturb or delay the synchronizing of chlorophyll- and/or chlorophyll-binding protein (CAB) production, resulting in reduced chlorophyll accumulation under negative DIF. It has been shown that temperature alterations influence the oscillation patterns of CAB mRNAs (Piechulla and Riesselmann, 1990). The chlorophyll content was very low in the shoot tip (Fig. 5), and it could be that a reduced amount of chlorophyll under negative DIF is part of the reason for reduced ETR and J_{max}. However, a negative DIF treatment only slightly reduced the maximal quantum

efficiency (F_v/F_m) of PSII (Table 2). Also, even though the amount of chlorophyll was reduced in expanded leaves of WT plants grown under negative DIF, these leaves tended to have a higher photosynthetic capacity than corresponding expanded leaves of WT plants grown under positive DIF (Fig. 7, Tables 2, 3). Thus, at least at this stage of development, a lower chlorophyll level has no effect on photosynthetic capacity or quantum efficiency of PSII. A change from negative to positive DIF makes the leaves green within a few day/night cycles (Moe and Heins, 2000), and chlorosis as a result of negative DIF does not represent a practical problem in commercial pot and bedding plant production.

The reason for reduced dry weight accumulation in WT plants grown at negative DIF in this experiment does seem to be partly an effect of reduced area of photosynthetic tissue (leaf and stem), especially leaf area (Table 2), and partly an effect of ambient temperature on plant photosynthesis. The reduction of leaf area under negative DIF in the WT is probably related to the down-regulation of GA_1 levels, as there was no significant reduction of leaf area in *la cry^s*. The negative DIF treatment in our study resulted in 8 °C cooler temperatures in the photoperiod and 8 °C higher temperatures in the dark period as compared with the positive DIF temperature regime. This resulted in significantly higher R_{night} under negative DIF (Fig. 6). The temperature responsiveness of R_{night} in leaves from plants grown under negative and positive DIF was tested and, as expected, R_{night} increased with temperature (Supplementary Fig. S1 at *JXB* online). Photosynthesis is temperature dependent as well (Hikosaka *et al.*, 2006), and the temperature response of maximum photosynthetic capacity from 13 °C to 21 °C demonstrated clearly that maximum photosynthetic capacity increased with temperature (Supplementary Fig. S2). However, this does not need to be the case at ambient CO_2 concentrations and light levels where photorespiration will also influence the temperature response of photosynthesis.

P_{net} was quite similar in WT plants under positive and negative DIF. However, attempts to measure R_{day} indicated that R_{day} was affected by temperature similarly to R_{night}. Consequently, since R_{day} is lower under negative DIF, so also is true photosynthesis. True photosynthesis thus depends on the magnitude of R_{day}, and different methods gave different answers, but this suggest that true photosynthesis is higher under positive DIF. Probably, part of the R_{day} and R_{night} is related to growth, and thus a correlation with growth as measured by stem elongation and temperature was expected. Indeed, plants under positive DIF grow more during daytime than plants under negative DIF (Fig. 2). R_{night} subtracted from P_{net} also resulted in a reduced average CO_2 accumulation on a daily basis under negative DIF (Fig. 6).

Dry weight accumulation in the *la cry^s* mutant was only half of that of the WT (Table 1). One of the main reasons for this is probably that the two first leaf pairs developed were very small, often malformed, and most probably did not contribute much to a positive net CO_2 assimilation on a whole-plant basis. Therefore, growth during the first 8–10 d for the mutant probably occurred very much at the expense of stored reserves in the seed. The third, fourth, fifth, and sixth leaf pair developed properly, but total leaf area (Table 1) was lower in *la cry^s* despite more rapid leaf unfolding. Due to less root development in the *la cry^s* mutant, one should expect that it had less root respiration than the WT, and that further growth might improve dry weight accumulation compared with the WT, if there were free access to nutrients. Higher specific respiratory costs for stem growth in *la cry^s* could be expected if the stem biomass of the mutants contained a larger fraction of compounds such as lipids or lignin, for which biosynthesis is highly energy demanding, compared with the WT. If so, higher energy costs for stem growth could contribute to the low dry weight accumulation in the *la cry^s* mutant in this study. Supporting such an idea is the finding that tobacco plants overexpressing a GA biosynthesis gene increased their content of lignin in stem tissue (Biemelt *et al.*, 2004). There was no difference in diurnal net assimilation rate between negative and positive DIF treatments in the *la cry^s* mutant (Fig. 4), and in this regard it differed from the WT.

In the present study, reduced levels of active GA in expanding shoot tissue in the WT caused by a negative DIF treatment reduced dry matter accumulation in stem tissue more than in root and leaves (Table 1). As a consequence, the SMR decreased while the RMR increased (Table 2). DIF did not affect RMR or LMR and only slightly reduced SMR in the *la cry^s* mutant. The root growth of *la cry^s* was limited as compared with the WT, and the shoot/root ratio was significantly higher. A reduction in the relative growth rate of GA-deficient tomato plants was mainly caused by reduced investments of carbon in stem and leaf tissues, and higher allocation of carbon to root growth and root respiration (Nagel *et al.*, 2001). Thus, it appears that regulation of active GA in shoot tissue plays a major role in controlling root growth by controlling how much of the totally assimilated carbon in the shoot should be allocated to growth of stem tissue. Growth of root tissue in transgenic *Populus* with high GA content was reduced compared with the WT, and this caused problems with rooting of the transplants (Eriksson *et al.*, 2000). However, root growth improved at later stages.

Stem elongation is largely controlled by GAs, and there is a log linear relationship between stem elongation and the amount of GA_1 (Ross *et al.*, 1989; Weller *et al.*, 1994; Grindal *et al.*, 1998). Our study shows that *la cry^s* grown at negative DIF can compensate for reduced stem elongation rate in the daytime in the night, and thus DIF did not affect stem elongation of the mutant within the experimental period (Figs 1, 2C). However, the WT did not compensate for reduced stem growth in the daytime during the night when grown at negative DIF, and this is probably due to a direct effect of a down-regulation of GA_1 levels in developing tissue. A saturated GA response in *la cry^s* thus makes the mutant unable to respond to DIF treatments, at least in a short-term experiment, and further supports the conclusions that GA is an important mediator of

thermoperiodic stem elongation (Grindal *et al.*, 1998; Stavang *et al.*, 2005). The inhibition of the stem elongation rate by light (Fig. 2) in both the WT and *la cry*^{s} is likely to be mediated by phyB (Weller *et al.*, 1994), phytochrome-interacting factors (Nozue *et al.*, 2007), and circadian clock regulation (Michael *et al.*, 2008).

Even if the DELLA proteins are non-functional in *la cry*^{s}, GA_1 levels drop in response to a negative DIF treatment as they do in the WT (Fig 3), indicating that the DELLAs are not involved in the temperature regulation of GA_1 levels by feedback mechanisms. High levels of the GA precursors GA_{44}, GA_{20}, and GA_{19}, as well as high levels of the inactivate GA_{29}, might indicate that GA 3-oxidation is inhibited in *la cry*^{s} (Fig. 3). This is probably a sign of feedback regulation of GA biosynthesis, and Weston *et al.* (2008) showed that *PsGA20ox1*, *PsGA3ox1*, and *PsGA3ox2* were down-regulated and that *PsGA2ox1* and *PsGA2ox2* were up-regulated in the whole shoot tissue of the *la cry*^{s} mutant. These expression patterns indicate that GA_1 levels are low in *la cry*^{s}. However, the measurements of GA_1 in leaves revealed that there is no difference between *la cry*^{s} and the WT (Fig. 3). This apparent discrepancy might be explained by tissue-specific GA regulation in pea. Such an explanation is supported by the fact that even though it is GA saturated, *la cry*$_s$ has smaller leaves than the WT.

A negative DIF treatment reduced the level of GA_{19} and increased the level of GA_{29} in *la cry*^{s}. Also the ratio of GA_1/GA_8 increased, indicating that temperature-mediated GA inactivation resulting from increased transcript levels of *GA2ox1* and *GA2ox2* observed in WT pea under negative DIF also is functional in *la cry*^{s} (Stavang *et al.*, 2005). This regulation of GA_1, however, is of no use for the mutant, as a typical DIF response on stem elongation is absent within the time frame of the experiment. Given that GAs act through DELLAs only, this suggests that GA is not involved in determining the photosynthetic and respiratory capacity of pea, but affects plant architecture only. However, the role of GA in leaf development is not as clear as in the stem, and other pathways may exist. In an interesting study by Huerta *et al.* (2008), a transgenic line of citrus with elevated levels of GAs was found to have a higher photosynthetic capacity, stomatal conductance, and expression of genes related to photosynthesis than control plants. However, when GA was applied in a short-term experiment, no such positive effect on genes related to photosynthesis was observed, indicating that structural and architectural changes (more light entering into the canopy, etc.) mediated by GAs indirectly can result in increased photosynthetic performance and growth. Support for such a hypothesis is the finding that tall transgenic *Populus* with elevated GA levels had a higher RGR than control plants (Eriksson *et al.*, 2000).

Thermoperiodic growth involves regulation of GA_1 levels by GA 2-oxidation in pea (Grindal *et al.*, 1998; Stavang *et al.*, 2005), but other plant hormones might contribute as well. For instance, high temperature-stimulated hypocotyl elongation in *Arabidopsis* seedlings is under hormonal control and involves GA, auxin, and brassinosteroids as well as the PIF4 protein that interacts with phytochrome (Stavang *et al.*, 2009). Whether similar mechanisms are acting in pea remains to be investigated.

The present study shows that the effect of negative DIF on plant biomass production in WT pea is a result of the net effect of reduced growth and photosynthesis during daytime, increased respiration during the night and altered plant morphology. A saturated GA response in the *la cry*^{s} mutant neither resulted in increased photosynthetic capacity, as measured on a leaf area basis, nor increased dry weight accumulation. Instead the saturated GA response mutant allocated more dry matter to stem tissue and less to root and leaf tissue. Furthermore, it did not respond to DIF with changes in biomass allocations or changes in stem elongation as did the WT. In conclusion, higher GA levels or a saturated GA response do not appear to be major determinants of photosynthetic capacity on a leaf area basis in pea, and the primary effect of different GA levels appears to be a change in plant morphology.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Responsiveness of R_{night} to temperature of young expanding (leaf 5) and fully expanded (leaf 3) leaves of plants grown under negative or positive temperature. The 95% confidence intervals were calculated by Sigmaplot 11.0.

Figure S2. Responsiveness of light- and CO_2-saturated photosynthesis of young expanding (leaf 5) and fully expanded (leaf 3) leaves of plants grown under negative or positive temperature. The 95% confidence intervals were calculated by Sigmaplot 11.0.

Figure S3. Light- and CO_2-saturated photosynthesis of young (leaf 4–5) and fully expanded (leaf 2–3) leaves of WT pea measured under ambient growth temperatures in plants grown under negative (13 °C/21 °C DT/NT) and positive DIF (21 °C/13 °C DT/NT).

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