

Green light reduces elongation when partially replacing sole blue light independently from cryptochrome 1a

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Abstract

Although green light is often neglected it can have several effects on plant growth and development. Green light is probably sensed by cryptochromes (crys), one of the blue light photoreceptor families. The aim of this study is to investigate the possible interaction between green and blue light and the involvement of crys in the green light response of plant photomorphogenesis. We hypothesize that green light effects on morphology only occur when crys are activated by the presence of blue light. Wild-type MoneyMaker (*MM*), *cry1a* mutant (*cry1a*) and two *CRY2* overexpressing transgenic lines (*CRY2-OX3* and *CRY2-OX8*) of tomato (*Solanum lycopersicum*) were grown in a climate chamber without or with green light ($30 \mu\text{mol m}^{-2} \text{s}^{-1}$) on backgrounds of sole red, sole blue and red/blue mixture, with all treatments having the same photosynthetic photon flux density of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. Green light showed no significant effect on biomass accumulation, nor on leaf photosynthesis and leaf characteristics such as leaf area, specific leaf area, and chlorophyll content. However, in all genotypes, green light significantly decreased stem length on a sole blue background, whereas green light did not affect stem length on sole red and red/blue mixture background. *MM*, *cry1a* and *CRY2-OX3/8* plants all exhibited similar responses of stem elongation to green light, indicating that *cry1a*, and probably *cry2*, is not involved in this green light effect. We conclude that partially replacing blue light by green light reduces elongation and that this is independent of *cry1a*.

Main Conclusion

Partially replacing sole blue light by green light reduced elongation independent of cryptochrome 1a. Replacing 20% of sole red, red/blue or sole blue light by green had no significant effect on biomass, leaf photosynthesis and leaf characteristics.

Introduction

Leaves reflect a relatively large part of green light causing the green appearance of plants. Green light was for a long time thought to be irrelevant for plant functioning. However, this perception is now fading (Smith et al., 2017). Although leaves appear green, the fraction of green light that is reflected is only about 10–15% (Smith, 1986; Paradiso et al., 2011), while the major share of about 75–80% is absorbed, and the rest transmitted. This suggests that there might very well be a role of green light in photomorphogenesis. Green light (G) may play a major role in controlling plant development in orchestration with red light (R) and blue light (B) (Folta and Maruhnich, 2007). Wang and Folta (2013) suggested that this role is likely more important at low light conditions found within a canopy in particular at high planting densities. On the other hand, Terashima et al. (2009) reported that at high photosynthetic photon flux (PPF), G drives leaf photosynthesis more efficiently than R and B. This is related to the fact that G can penetrate deep into the mesophyll layers at the single-leaf level (Smith et al., 2017).

There is increasing evidence for the ability of green light to regulate plant photomorphogenesis. Supplementing G to white light (W) or a mixture of R and B (RB) increased hypocotyl and petiole length in *Arabidopsis* (Folta, 2004; Zhang et al., 2011; Wang et al., 2015). Hypocotyls were longer when G:B ratio was higher (Sellaro et al., 2010). Higher G intensity also increased contents of photosynthetic pigments in *Arabidopsis* seedlings, and biomass and photosynthetic parameters in leaves of lettuce (Golovatskaya and Karnachuk, 2008; Efimova et al., 2013; Johkan et al., 2012; Muneer et al., 2014). Lettuce plants grown in a mixture of R, B and G (RBG) had larger specific leaf area (SLA) but lower stomatal conductance (g_s) compared with RB alone, where the total light intensity of RBG was higher than that of RB (Kim, 2005). Plant height and dry weight increased in cucumbers when adding 520 nm G to a mixture of B, R and far-red light (RBFrG) compared with RBFr alone of similar light intensity, whereas such effects were not found when adding 595 nm G (Brazaitytė et al., 2009). Growing lettuce plants at different combinations of G with RB showed that growth increased when the fraction green light was raised from 0 to 24%, but increasing its proportion from 24 to 86% decreased the growth of leaf area and shoot fresh weight and dry weight (Dougher and Bugbee, 2001; Kim et al., 2004).

The nature of the green light receptor remains controversial, although most researchers proposed that green light is sensed by cryptochromes (crys) (Banerjee et al., 2007; Bouly et al., 2007; Sato et al., 2015). In higher plants, three cryptochromes have been described to date: *CRY1* and *CRY2*, both localized predominantly in the nucleus and the cytoplasm (Lin and Shalitin, 2003), and *CRY3* in the organelles (Kleine et al., 2003). Two *CRY1* (*CRY1a* and *CRY1b*), one *CRY2* and one *CRY3* (*CRY-DASH*) genes have been isolated in tomato (Perrotta et al., 2000, 2001; Facella et al., 2006). It has been suggested that green light reverses the action of blue light on the level of active crys, making them inactive for blue light (Banerjee et al., 2007; Bouly et al., 2007). This antagonistic blue-green interaction was supposed to be mediated through the interconversion of flavin redox states of crys. The authors concluded that the fully oxidized chromophore (FAD) absorbs blue light and is then converted to a semi-reduced chromophore (FADH \cdot), which is the biologically active, green-absorbing form. However, there are some inconsistencies with this proposition. Wang et al. (2013) found that G cannot reverse the cry-mediated B inhibition of early stem elongation, and instead acts additively with B to drive cry-mediated inhibition. Sato et al. (2015) found that G stimulated hypocotyl elongation via cry2 in the absence of B. These findings indicate the specific mechanism underlying cry photo-excitation has not yet been identified. The carotenoid zeaxanthin has been suggested as a photoreceptor for the stomatal blue light response, which could be reversed when adding G to B, indicating that zeaxanthin might absorb G (Frechilla et al., 1999, 2000). Using different photoreceptor mutants of *Arabidopsis*, Zhang et al. (2011) concluded that the increased leaf inclination and petiole length induced by supplemental G to RB was not mediated by cryptochromes nor phytochrome A and B (Zhang et al., 2011). A yet unknown green light photoreceptor may exist in plants. While the cryptochrome family has been well studied in the model plant *Arabidopsis*, information about the crys is limited in crop plants, such as tomato, that have an architecture very different from that of *Arabidopsis* (Liu et al., 2018; Fantini et al., 2019).

The aim of this study is to investigate the interaction between green and blue light and the involvement of crys in the green light response of plant photomorphogenesis. We hypothesized that the effect of green

light on stem elongation only occurs when crys are activated by the presence of blue light. Experiments in climate rooms were conducted where the effects of 525 nm green light were studied by replacing 20% background light of sole blue, sole red as well as red/blue mixture. In contrast to many other studies on green light, we kept the photosynthetic photon flux density (PPFD) as well as the ratio of other colours the same when green light was added.

Material And Methods

Plant materials and growth conditions

Tomato (*Solanum lycopersicum*) wild-type Moneymaker (*MM*) and two *CRY2* overexpressing transgenic lines (*CRY2-OX3* and *CRY2-OX8*, previously named line 52.3 and line 52.8 in Giliberto et al., 2005) seeds were kindly provided by Dr. Elio Fantini, ENEA Trisaia Research Center, Italy. Tomato *cry1a* mutant (*cry1a*) seeds were obtained from Tomato Genetic Resource Center, UC Davis from USA. Seeds were germinated in vermiculite under darkness for 3 days and then transferred to $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ white LED light. Day/night temperature was maintained at 22/18 °C with a photoperiod of 18 h. Relative air humidity was 70%.

Ten days after sowing, plants were transplanted in 11 × 11 × 12 cm black plastic pots filled with ~ 6 mm expanded clay grid (name and supplier) and light treatments started. The treatments consisted of sole blue, sole red, red/blue mixture (red/blue ratio = 3/1) with or without green. Total PPFD was kept at $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ in all treatments. When green was added the red/blue ratio was kept the same as the treatment without green (Table 1). Light was provided by monochromatic light-emitting diodes (LEDs) with peaks at 447 nm (blue; Philips, USA), 667 nm (red; Philips, USA), and 525 nm (green; Lumileds, USA) (Fig. 6). PPFD, phytochrome photostationary state (PSS) (Sager *et al.*, 1988), and the fraction of red (600 to 700 nm), blue (400 to 500 nm), and green (500 to 600 nm) light in all LED treatments were measured by an Apogee® Spectroradiometer SS-110. These measurements were performed at 45 cm distance from the light source, and the light source was kept at 40 ~ 50 cm distance from the top of plants during the growing period. The PPFD of each chamber was manipulated by adjusting the electrical current sourced to each LED.

Table 1

Total PPFD (photosynthetic photon flux density) and PPFD of red (R; 600–700 nm), blue (B; 400–500 nm) and green (G; 500–600 nm) for the six spectral treatments as well as the phytochrome photostationary state (PSS)

Spectral treatment	Light intensity ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				PSS
	Total	Red (R)	Blue (B)	Green (G)	
R	150	150			0.880
RG	150	120		30	0.884
B	150		150		0.505
BG	150		120	30	0.578
RB	150	112.5	37.5		0.881
RBG	150	90	30	30	0.877

Measurements

Plants were measured 21 days after transplanting. Stem length was measured up to the apex. Total leaf area was measured using a leaf area meter (model LI-3000; LI-COR, USA). Roots, stems and leaves were separated and dried in a ventilated oven at 105 °C for 24 h to determine the dry weight (DW). From the above, specific leaf area (m^2 of leaf area g^{-1} of leaf DW) was determined.

The following measurements were taken on the fourth leaf counted from the top. The photosynthesis was measured on at the center of the second leaflet. The net photosynthetic CO_2 fixation rates (P_n , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$) and transpiration rates (T_n , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) were measured with a portable gas-exchange system (LI-6400, Li-Cor, Lincoln, NE, USA) within a transparent cuvette. Air flow rate was $300 \mu\text{mol s}^{-1}$; CO_2 concentration in the sample chamber, was $400 \mu\text{mol mol}^{-1}$ and temperature of sample chamber was 22°C.

Photosynthetic pigments of fresh leaves were extracted in 100% *N,N*-Dimethylformamide (DMF) and then measured using Varian Cary 4000 spectrophotometer. The equations of Wellburn were used to determine concentrations of chlorophyll a (Chla) and b (Chlb) as well as total carotenoids (Car) in $\mu\text{g ml}^{-1}$ DMF.

$$\text{Chla} = 12 A_{663.8} - 3.11 A_{646.8}$$

$$\text{Chlb} = 20.78 A_{646.8} - 4.88 A_{663.8}$$

$$\text{Car} = (1000 A_{480} - 1.12 \text{Chla} - 34.07 \text{Chlb})/245$$

Statistical Set-up And Analysis

Five repetitions (taken as blocks in the analysis) of the six light treatments were conducted over time. Measurements were made on three individual plants in each light treatment for each genotype and repetition (nine plants for stem length and leaf number). Each repetition was randomised as a split-plot design (main factor light, subfactor genotype). Analysis of Variance (ANOVA) was conducted using Genstat 19.0 for Windows. Residuals were tested for normality (Sapiro-Wilk test at $P=0.05$). In case of non-normal residuals, the original data were log-transformed which always resulted in normal residuals. For mean separation, Fisher's unprotected LSD test at $P=0.05$ was used; unprotected, because we also applied this test for separating light treatment x genotype interaction means when the F-test for interaction was not significant at $P=0.05$.

Results

Green light reduced stem length when partially replacing sole blue light, but not when replacing sole red or red/blue mixture

Partially (20%) replacing sole B by G (BG) significantly reduced stem length in all four genotypes (Fig. 1). Partially replacing RB by G (RBG) did not change stem length in any of the genotypes compared to RB. Compared with sole R, green light replacement only significantly reduced stem length of genotypes overexpressing *CRY2* (*CRY2-OX3* and *CRY2-OX8*) (Fig. 1C and D).

Partially replacing sole R by B (i.e. comparing R with RB) reduced stem length except for the *cry1a* mutant (Fig. 1). The two *CRY2* overexpressors, *CRY2-OX3* and *CRY2-OX8*, did not differ in stem length (Fig. 1). Interestingly, *CRY1a*-deficient plants were remarkably higher than other genotypes, even under the 100% R background (Fig. 1).

Green light did not induce changes in specific leaf area but reduced shoot: root ratio of *CRY2* overexpression lines

No significant interaction between light treatment and genotype was observed in specific leaf area (SLA) (Fig. 8). Partially replacing sole blue and red/blue mixture by green light did not significantly change the leaf area, except that BG remarkably reduced leaf area of *CRY2-OX8* compared to B (Fig. 2). However, partially replacing sole R by G induced larger leaf area, though this was only significant in *cry1a* mutant and *CRY2-OX3* (Fig. 2). The *CRY2-OX3* and *CRY2-OX8* tended to have less leaf area than *MM* and *cry1a* mutant in all light treatments, though it was only significant when the background light contained blue light (B, BG, RB and RBG).

The shoot: root ratio of both wild-type and *cry1a* mutant did not respond to green light (Fig. 3a and b). However, G significantly reduced the shoot: root ratio of *CRY2* overexpressors under sole B background, as well as that of *CRY2-OX3* under sole R (Fig. 3c and d). In line with the results of stem length (Fig. 1),

cry1a mutant had the highest shoot: root ratios, though the effects were only significant under B and BG (Fig. 3).

No significant effect of green light on biomass accumulation nor photosynthesis rate

The total dry weight was not significantly affected by partially replacing the different colours by green light, nor was there a significant difference among various genotypes and other spectra (Fig. 4). The contents of chlorophylls (chls, chl a + b) and total carotenoids (car), as well as the ratio of chl a to chl b and chl a + b/car ratio were also mostly not influenced by the genotypes and light treatments (Fig. 9). However, partially replacing sole R by G significantly increased the chl a + b/car ratio of *cry1a* mutant. Consistent with these results, there were no significant effects of light treatment and genotype on leaf photosynthetic rate (Pn) (Fig. 5), transpiration rate (Tn), and stomatal conductance (gs) (Fig. 10).

Discussion

Partially replacing sole blue light by green light reduced elongation independent of *cry1a*

Tomato stem length was significantly reduced by partially (20%) replacing sole blue light by green light, whereas partially replacing sole red or red/blue mixture with G did not affect length (Fig. 1). These effects were due to elongation of internodes as leaf number was not affected (Fig. 7). *CRYs* were reported to mediate hypocotyl elongation inhibition driven by sole blue light or sole green light compared to darkness in *Arabidopsis*, and G acts additively with B to drive cryptochrome-mediated inhibition of elongation (Wang et al., 2013). However, in our study partially replacing sole B by green light, indicated that G reversed the B-induced stem elongation in tomato (Fig. 1). *CRY1a*-deficient and *CRY2* overexpressing lines (*CRY2-OX3* and *CRY2-OX8*) showed similar responses of stem length to partially replacing sole B by G as the wild-type MM (Fig. 1). This might be attributed to the overlapping role of *CRY1* and *CRY2* for the inhibition of elongation (Azari et al., 2010; Giliberto et al., 2005), suggesting that the role of *CRY2* is partially redundant with that of *CRY1* in the control of stem elongation (Giliberto et al., 2005). This indicated that this green light response was independent from *cry1a*, probably independent from *cry2* as well.

The stem length of the *cry1a* mutant was remarkably higher than for the other genotypes under the same light treatment (Fig. 1), confirming the involvement of *CRY1a* in the inhibition of internode elongation (Ninu et al., 1999). The overexpression of *CRY2* in *CRY2-OX3* and *CRY2-OX8* induced shorter stems (Fig. 1), confirming also the involvement of *cry2* under all light treatments (Yang et al., 2017).

Through blue light the neutral FAD chromophore in crys is converted into a photoexcited state (FADH[•]), absorbing green light which converts the cryptochromes into a fully reduced and inactive state (Lin, 2003; Banerjee et al., 2007; Bouly et al., 2007). Green light partially inhibits *cry2* oxidation by blue light (Banerjee et al., 2007; Zeugner et al., 2005; Bouly et al., 2007; Frechilla et al., 2000), contributing to reduced levels of

FADH. However, this photocycle model could not explain all interactions between blue and green light on stem length, like the finding that G acts additively not reversely with B to drive cryptochrome-mediated stem growth inhibition in *Arabidopsis* (Wang et al., 2013). Green light did not inhibit blue light-mediated cry2 degradation and the expression of the *FLOWERING LOCUS T* gene (Li et al., 2011), and it induced similar response on stem elongation in *CRY2* overexpressing lines to wild-type (Fig. 1). These results suggest that this photocycle between two FAD protein forms cannot explain the photoperception of crys in plant cells, and the specific mechanism underlying crys photo-excitation has not been identified (for review, see Yang et al., 2017).

The involvement of CRY2 in regulating plant photomorphogenesis

In contrast with *MM* and *cry1a* mutant, in *CRY2-OX3* and *CRY2-OX8* stem length was reduced when partly replacing sole R by G (Fig. 1), while partly replacing B by G induced a lower shoot: root ratio and smaller leaf area (not significant in *CRY2-OX3*) (Figs. 2 and 3). These results indicate the involvement of *CRY2* in green light effects on stem length, shoot: root ratio and leaf area.

Comparing tomato transgenic *CRY2* overexpressing lines with wild-type plants, *CRY2* may control vegetative development and photosynthesis as suggested by high throughput transcriptomic and proteomic analyses by Lopez et al. (2012), and by the overproduction of chlorophylls in *CRY2* overexpressors (Giliberto et al. 2005). However, we did not observe significant differences between *CRY2-OX3/OX8* and *MM* on SLA, photosynthesis, and chlorophyll content (Fig. 9, 5, and 10). We conclude that effects of *CRY2* on phenotype are limited, which might result from its redundant role with *CRY1a*.

PHYs play role in blue light effects on elongation

Besides mediation by *CRYs*, the blue light effects might also be mediated by *PHYs*. The PSS value which is an indicator of phytochrome status, was lower under sole blue than that under all other light treatments; green light had little effect on the PSS value (Table 1). *CRYs* and *PHYs* converge blue and red light signals at different levels to co-regulate physiological responses, such as root greening, de-etiolation, shade avoidance symptoms, photoperiodic flowering, etc (Su et al., 2017). In contrast to the expectation that blue light triggered shorter plants due to involvement of cry, stems under sole B were not shortest (Fig. 1), indicating elongation might be counteracted by phytochrome action. The phytochrome effect may dominate stem elongation of cry, therefore longer plants were observed under sole blue resulting from less reduction in elongation due to cryptochromes.

Strikingly, similar to 100% B, 100% R also induced significantly longer *cry1a* mutant plants compared to *MM* (Fig. 1), consistent with the results of Fantini et al. (2019). On the contrary, Ninu et al. (1999) found that 8-days old *CRY1a* antisense tomato plants did not show an elongated hypocotyl under red light but under blue light (both approximately $8 \mu\text{mol m}^{-2} \text{s}^{-1}$). These different results might be caused by the fact that *CRY1a* gene is not knocked out but only downregulated in *CRY1a* antisense plants, or by differences in development stage or light intensity. Accumulating evidence in the model plant *Arabidopsis* has revealed that *CRYs* and *PHYs* share two mechanistically distinct pathways that coordinately regulate

transcriptional changes in response to light. However, the role of photoreceptor interactions and the mechanism responsible for the direct convergence of *CRYs* and *PHYs* signals on the *COP1/SPA* complex or phytochrome-interacting factors (*PIFs*) remain elusive (Su et al., 2017).

Arabidopsis cryptochrome 1, phytochromes A, B1 and B2 are all capable of mediating responses to B under some circumstances (Weller *et al.*, 2000). *CRYs* may act in a blue-light independent manner to affect *PHY* regulation of gene expression and development, resulting in different protein expression between the *WT* and *cry1cry2* mutant *Arabidopsis* in red light as well as in blue light (Yang et al. 2008; Lopez et al., 2012). *Arabidopsis CRY1* interacts directly with *PIF4* in a blue light-dependent manner to repress the transcription activity of *PIF4* (Ma et al., 2016). This indicates that stem elongation in *cry1a* mutants under sole R could be mediated by downstream genes shared by *CRYs* and *PHYs* (Facella et al., 2012; Su et al., 2017). However, the extent and relative importance of their individual contributions differ depending on irradiance, on which other photoreceptors are present, and on which plant process is examined.

Replacing 20% of red, red/blue or blue light by green had no significant effect on biomass production

McCree (1972) measured the instantaneous response of leaf photosynthesis to different spectra, finding that the quantum yield of photosynthesis of green photons (525 nm) can be about 25–30% less than that of red photons (675 nm), while the quantum yield of green is comparable to that of blue photons (450 nm). However, this may not be representative of whole plants or plant communities grown at high PPFD under mixed colors of light. Green light could drive carbon fixation deep within leaves (Sun et al., 1998), even more efficient than R or B (Nishio, 2000), because it could penetrate deep into the mesophyll layers (Smith et al., 2017). In our study where the light contained 0 or 20% green, the leaf photosynthesis rate, stomatal conductance and transpiration rate were not significantly affected by green light (Figs. 5 and 10). Similarly, the contents of chlorophyll a and b and carotenoids as well as their ratios, were hardly affected by green light (Fig. 9).

Partially replacing sole R or B or R/B mixture by green light did not cause differences in leaf area, SLA, shoot: root ratio and biomass of *MM* and *cry1a* mutant. This contradicts previous findings on green light responses, but in those studies PPFD also increased when adding G (Kim, 2005; Samuolienė et al., 2012; Novičkovas *et al.*, 2012). Zhang et al. (2011) reported that 40% green light induced a shade avoidance response in *Arabidopsis* seedlings, whereas 10% did not. Too much G (51%) or too little (0%) decreased lettuce growth, while about 24% resulted in the highest growth rate (Kim et al., 2004). However, in our study 20% G did not induce much effects, which is comparable to the study of Hernández and Kubota (2015) who analyzed the effect of 28% G in cucumber. Kaiser et al. (2019) found that replacing 32% of a red/blue mixture spectrum by green light significantly increased plant biomass and yield. These different observations among studies suggest that G effects might be genotype-specific and dependent on and/or interact with other environmental conditions.

Conclusions

Tomato stem elongation was significantly reduced by green light only when it partially replaced sole blue light, which as such might have suggested a role for cryptochrome. However, *cry1a* mutant and *CRY2* overexpressing plants showed similar trends on stem length as the wild-type. This indicates that this response to green light is probably independent from *cry1a* and *cry2*. Moreover, *cry1a* mutants were significantly taller than other genotypes under all spectra, whereas *CRY2* overexpressing plants had a much shorter stem. We conclude that *cry1a*, and probably *cry2*, are not involved in green light effects on elongation.

Abbreviations

B, blue light; Car, carotenoids; Chl, chlorophyll; CRY, cryptochrome; DMF, *N,N*-Dimethylformamide; DW, dry weight; FAD, a fully oxidized chromophore; FADH[•], a semi-reduced chromophore; G, green light; gs, stomata conductance; MM, moneymaker; Pn, net photosynthesis rate; PPF, photosynthetic photon flux; PPFD, photosynthetic photon flux density; PSS, phytochrome photostationary state; R, red light; SLA, specific leaf area; Tn, transpiration rate; W, white light

Declarations

Compliance with ethical standards

Conflict of interest:

We state no conflict of interest with others.

Ethical statement:

Our work complies with the ethical rules applicable for this journal.

Author contributions

XZ, EH, LM, WJ conceived and designed the experiment. XZ and MB conducted the experiment and analyzed the data. XZ wrote the first draft of the manuscript and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures

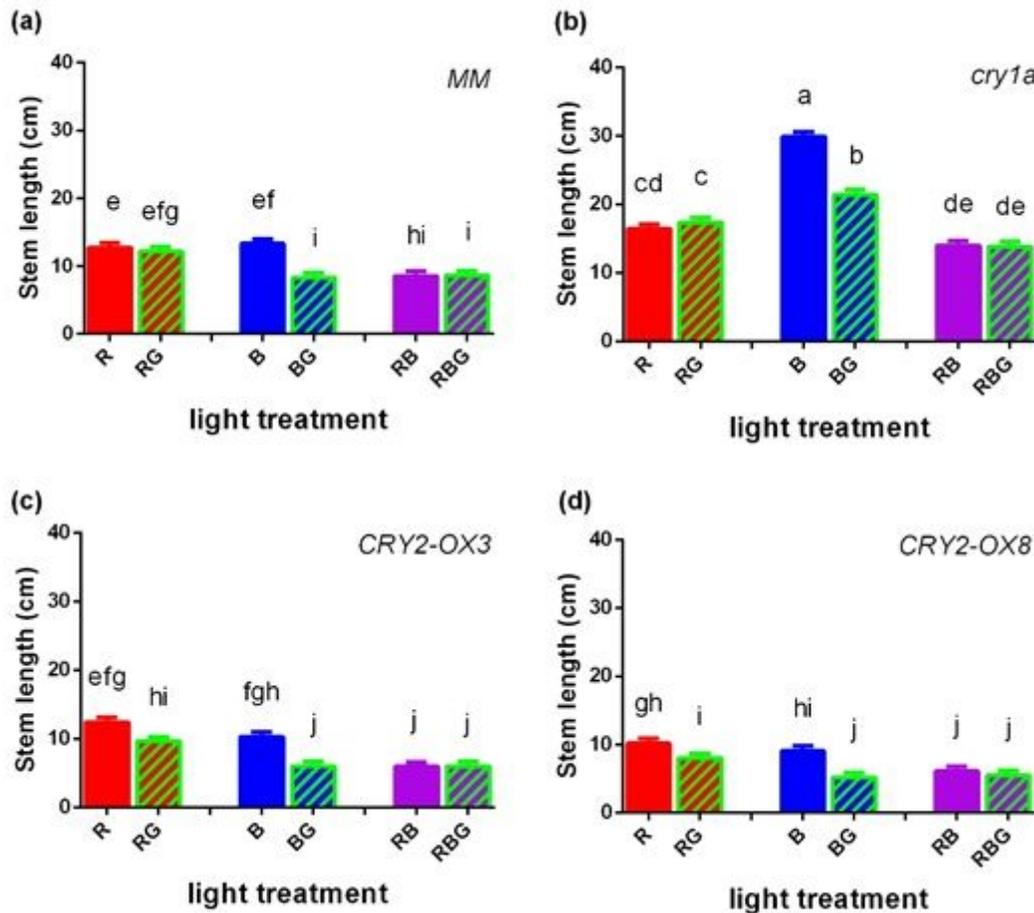


Figure 1

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on stem length on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) cry1a (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). There was significant interaction between light treatment and genotype (log-transformed data; $P < 0.001$). Different letters above bars indicate significant differences among light treatment x genotype interaction means ($P = 0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n = 5$) each based on 9 replicate plants.

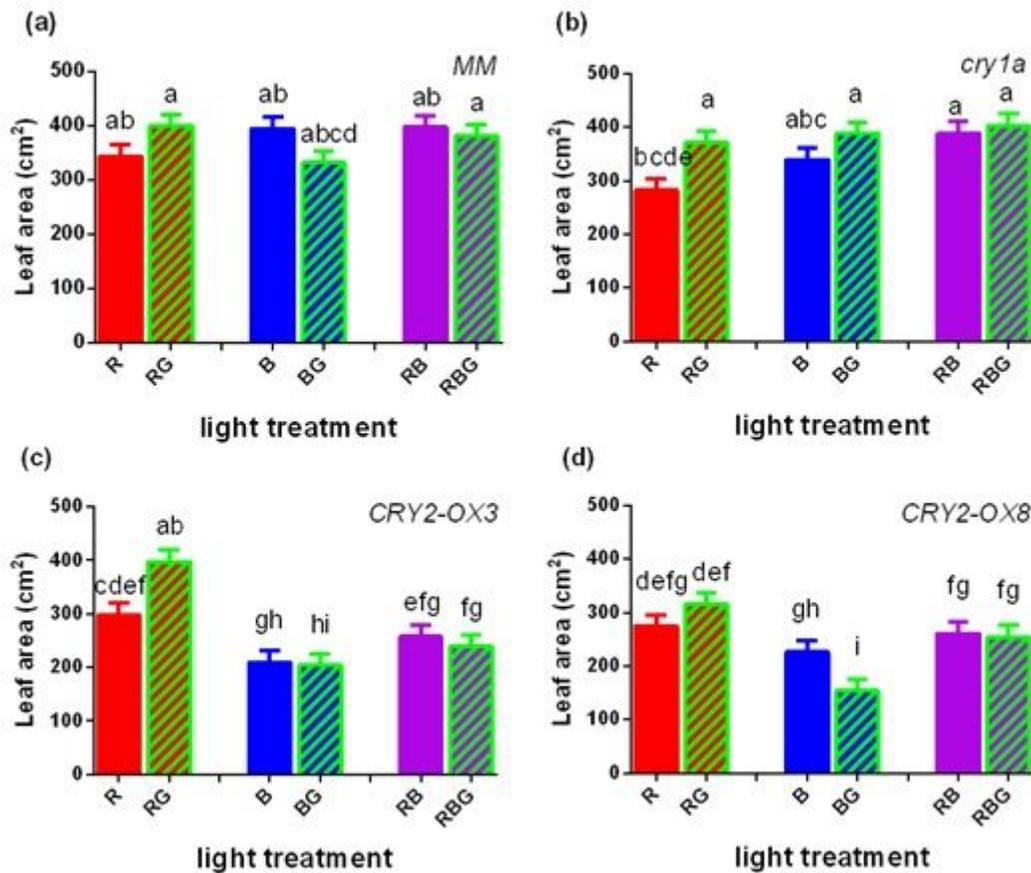


Figure 2

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on leaf area on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) cry1a (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). Interaction between light treatment and genotype was significant (log-transformed data; $P < 0.001$). Different letters above bars indicate significant differences between light treatments x genotype interaction means ($P = 0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n = 5$) each based on 3 replicate plants.

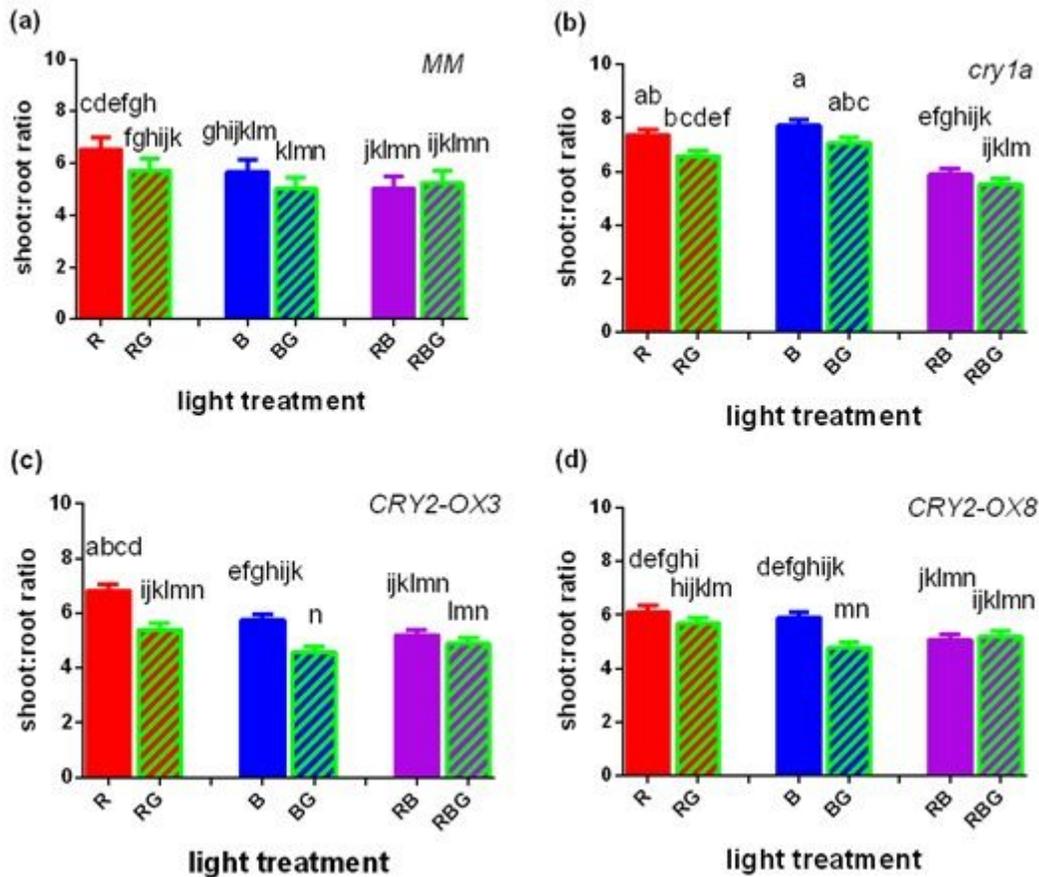


Figure 3

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on shoot: root ratio on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) cry1a (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). A significant interaction between light treatment and genotype was observed ($P=0.013$). Different letters above bars indicate significant differences between light treatment x genotype interaction means ($P=0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n=5$) each based on 3 replicate plants.

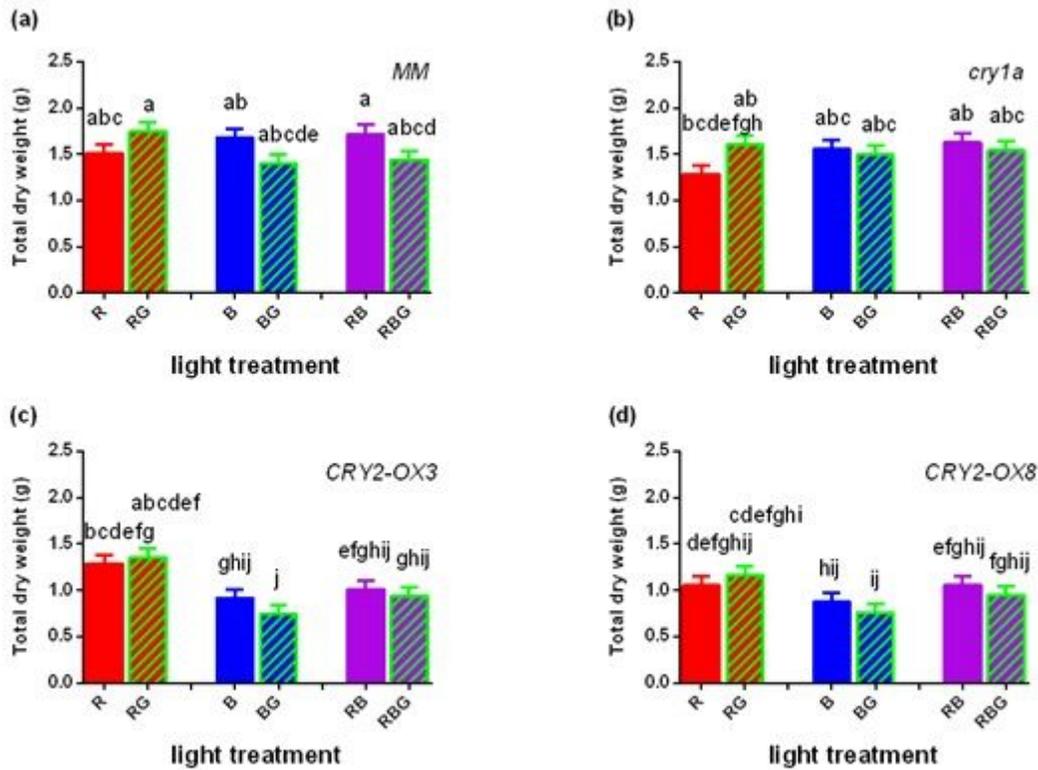


Figure 4

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on total dry weight on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) cry1a (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). No significant interaction between light treatment and genotype was found ($P=0.686$), but effects of light treatment ($P=0.04$) and genotype ($P<0.001$) were significant. Different letters above bars indicate significant differences between light treatment x genotype interaction means ($P=0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n=5$) each based on 3 replicate plants.

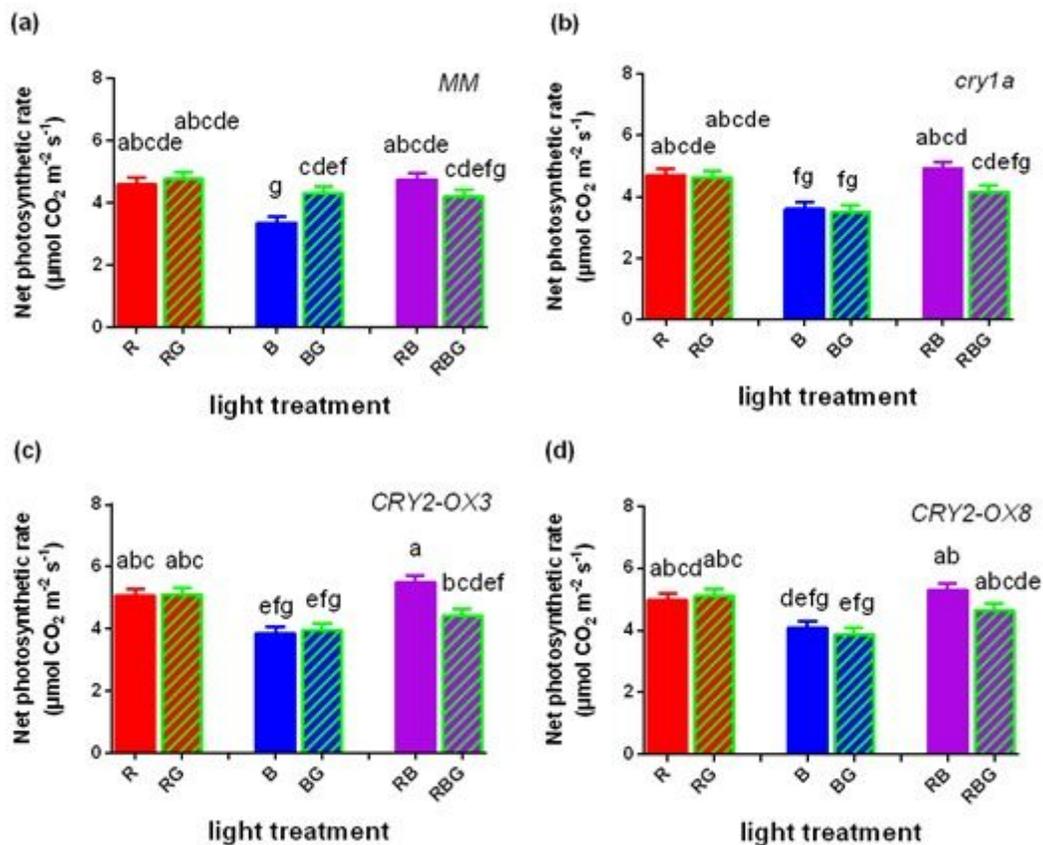


Figure 5

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on photosynthesis rate on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) cry1a (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). No significant interaction between light treatment and genotype was observed ($P=0.968$), but effect of light treatment was significant ($P<0.001$). Different letters above bars indicate significant differences between light treatment x genotype interaction means ($P=0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n=5$) each based on 3 replicate plants.

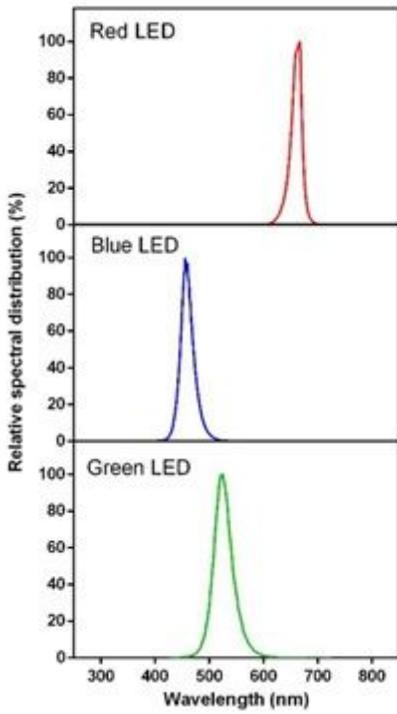


Figure 6

Relative spectral distributions of the red, blue and green monochromatic LEDs.

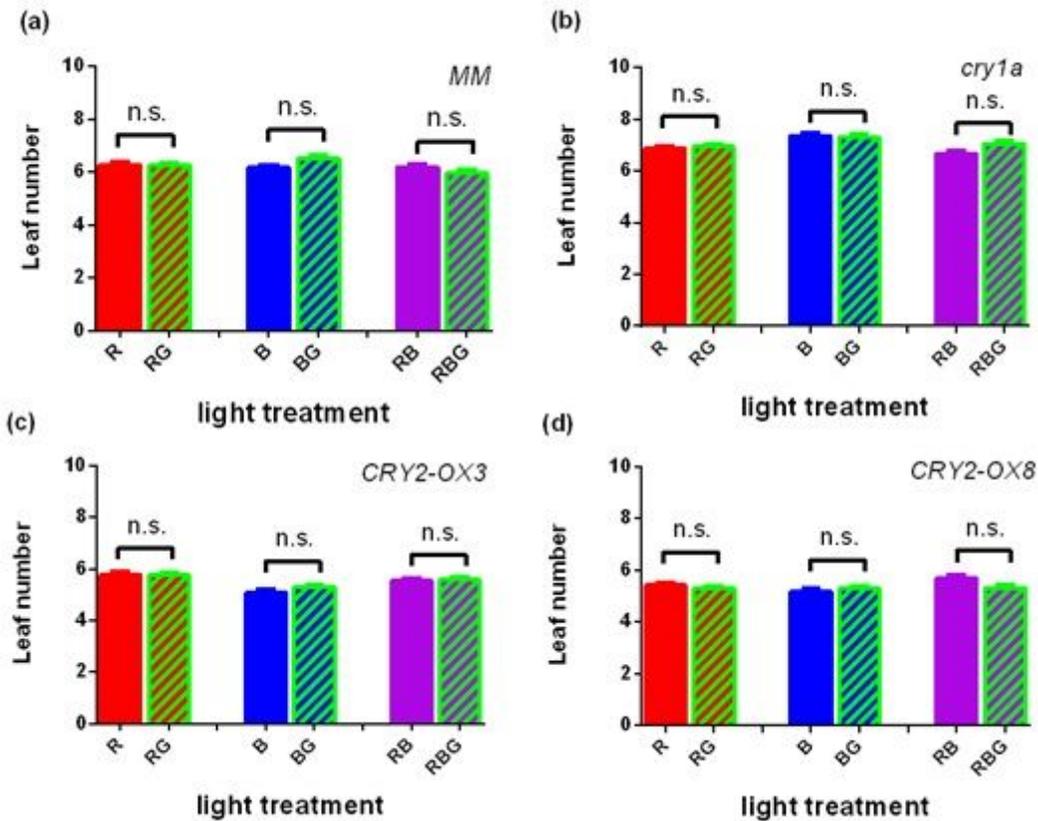


Figure 7

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on leaf number on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) *cry1a* (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). No significant (n.s.) effects of green light were found. Vertical bars indicate \pm SE of the mean of 3 blocks (n=3) each based on 9 replicate plants.

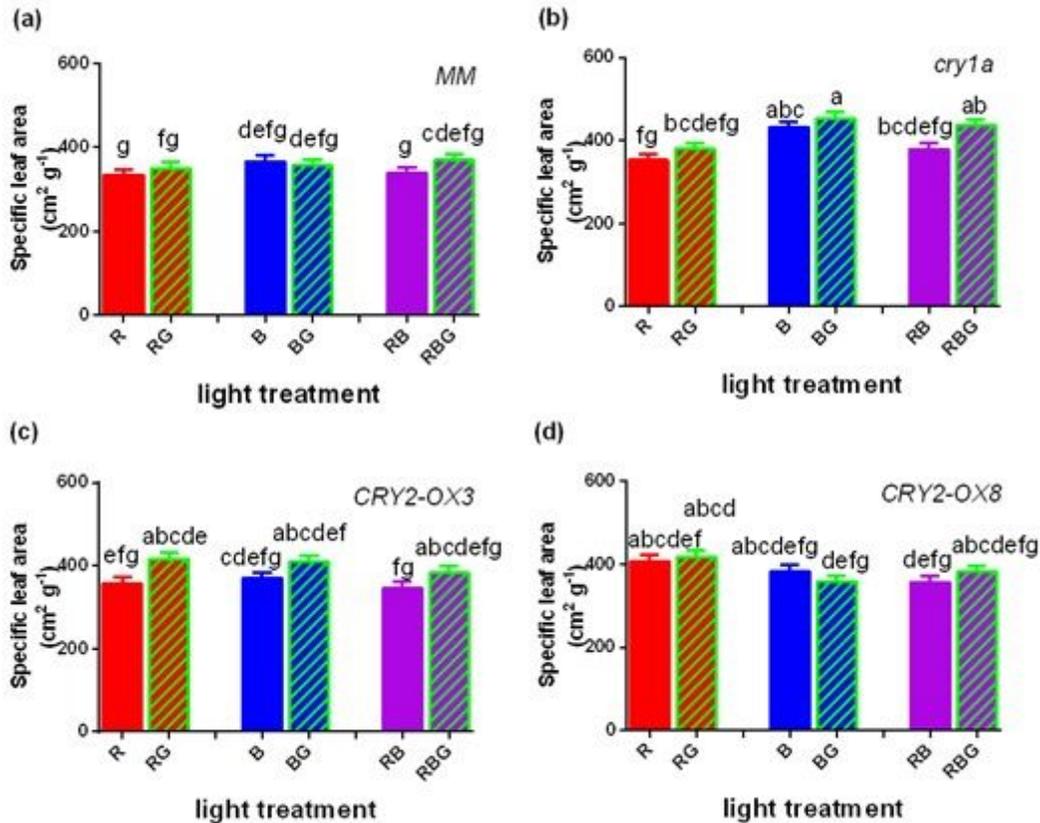


Figure 8

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on specific leaf area on day 21 after transplanting of four tomato genotypes, (a) MM (Moneymaker, wild-type), (b) *cry1a* (CRY1a deficient), (c) CRY2-OX3 (CRY2 overexpressing, line 52.3) and (d) CRY2-OX8 (CRY2 overexpressing, line 52.8). No significant interaction between light treatment and genotype was found (log-transformed data; P=0.283) but effects of light treatment (log-transformed data; P=0.049) and genotype (log-transformed data; P=0.002) were significant. Different letters above bars indicate significant differences among light treatment x genotype interaction means (P=0.05). Vertical bars indicate \pm SE of the mean of 5 blocks (n=5) each based on 3 replicate plants.

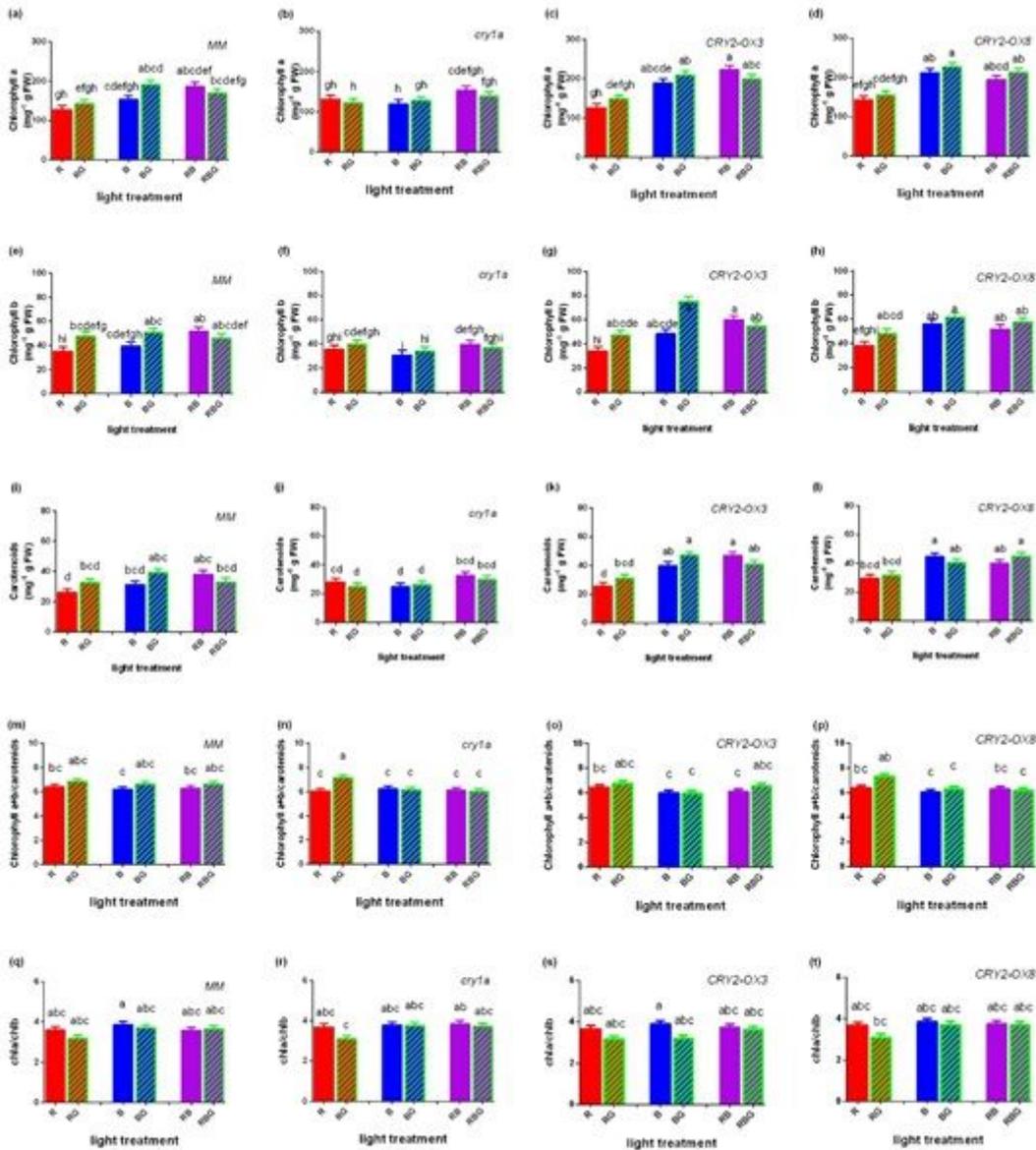


Figure 9

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on chlorophyll content on day 21 after transplanting of four tomato genotypes, MM (Moneymaker, wild-type), cry1a (CRY1a deficient), CRY2-OX3 (CRY2 overexpressing, line 52.3) and CRY2-OX8 (CRY2 overexpressing, line 52.8). (a~d) chlorophyll a (chl a) content; (e~h) chlorophyll b (chl b) content; (i~l) carotenoids (car) content; (m~p) chl a+b/car ratio; (q~t) chl a/b ratio. A significant interaction between light treatment and genotype was found on chl a/b ratio ($P=0.044$) and chl a+b/car ratio (log-transformed data; $P=0.02$). No significant interaction between light treatment and genotype was found on chl a ($P=0.229$), chl b (log-transformed data; $P=0.234$), and car content ($P=0.36$), but effects of light treatment ($P=0.001$; $P<0.001$; $P=0.002$) and genotype ($P<0.001$; $P<0.001$; $P<0.001$) were significant. Different letters above bars indicate significant differences among light treatment x genotype interaction means ($P=0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n=5$) each based on 3 replicate plants.

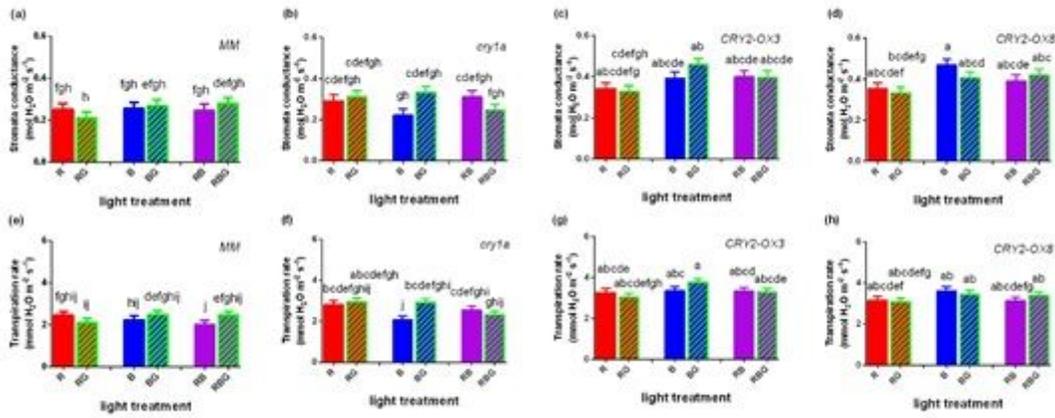


Figure 10

Effect of partially (20%) replacing sole red (R), sole blue (B) or red/blue (RB; ratio 3:1) by green (G) light on stomatal conductance (a~d) and transpiration rate (e~h) on day 21 after transplanting of four tomato genotypes, MM (Moneymaker, wild-type), cry1a (CRY1a deficient), CRY2-OX3 (CRY2 overexpressing, line 52.3) and CRY2-OX8 (CRY2 overexpressing, line 52.8). No significant interaction between light treatment and genotype was found on stomatal conductance ($P=0.617$) and transpiration rate ($P=0.657$). Different letters above bars indicate significant differences among light treatment x genotype interaction means ($P=0.05$). Vertical bars indicate \pm SE of the mean of 5 blocks ($n=5$) each based on 3 replicate plants.