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Physiological responses of spring rapeseed (*Brassica napus* L.) to red/far-red ratios and light irradiance on pre and post flowering stages

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Abstract

Early shade signals promote the shade avoidance syndrome (SAS) such as petiole and shoot elongation and upward leaf position. In spite of the importance of light signals on plant performance, these photomorphogenic responses have not been studied deeply in rapeseed (*Brassica napus* L.). In opposition to other crops like maize and wheat, rapeseed has a dynamic phenotype with a complex developmental pattern in a primary rosette, followed by main stem elongation and indeterminate floral raceme growth. In this work, we analyzed i) morphological and physiological responses at individual plant level due to changes in red/far-red (R/FR) ratios during the vegetative and reproductive phases of development, and ii) changes in biomass allocation, grain yield and grain composition at crop level in response to light quality and quantity in two modern spring rapeseed genotypes. Four experiments were carried out under field conditions in pots and plots, modifying R/FR ratios and irradiance from vegetative or reproductive stages. In pot experiments, low R/FR ratio increased the upward leaf position and accelerated leaf senescence. Furthermore, shade signals affected reproductive performance, causing short main floral raceme and increasing floral branching with higher remobilization of soluble carbohydrates from the stems. In plot experiments, low irradiance during flowering reduced grain yield, harvest index and grain oil content, and high R/FR ratio reaching the crop partially alleviated such effects. We concluded that photomorphogenic

signals are integrated early during the vegetative growth in spring rapeseed, and light intensity signals are more important than light quality signals at crop level.

Keywords: shade-avoidance responses, red/far red ratio, irradiance, vegetative plasticity, branching, grain yield

Abbreviations: DAE, days after emergence; PAR, photosynthetically active radiation; PPFD, photosynthetic photon flux density; R/FR, red/far red ratio; SAS, shade-avoidance syndrome .

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Introduction

Rapeseed (*Brassica napus* L.) is the third most important oilseed in the world, following palm and soybeans. It is an excellent raw material for edible oil and biodiesel production (Velasco and Fernandez-Martinez 2002). Rapeseed crop can also replace winter cereals in crop rotations, allowing the incorporation of carbon into the soil through a great amount of crop residues, and early sowing of double-crop soybeans. As general rule, rapeseed yields are expected to reach 40-50% of wheat yields, but in low potential environments, rapeseed and wheat yields may match (Rondanini et al. 2012). Despite of the good comparative performance of rapeseed crops in non-optimal environments, it is perceived by the producers as a risky crop due to the high yield variability at global scale.

One source of yield variability in rapeseed may be associated with changes in the light environment explored by plants, affecting biomass production and allocation to harvestable grains. As the crop grows and develops, radiation and red/ far-red (R/FR) ratio reaching the green tissues decrease and modify the competitive relationships between individuals and consequently might produce changes in crop productivity (Casal 2013). Plants perceive the low R/FR ratio reflected by the proximity of neighboring plants responding morphologically before being shaded (Ballaré et al. 1987). Early shade signals promote the expression of the shade avoidance syndrome (SAS) that includes, among others, elongation of stems and petioles, changes in leaves orientation, acceleration of flowering and grain yield reduction (Casal 2013).

The expression of photomorphogenic plasticity is useful for plants to adapt to diverse light environments, but it may be an undesirable trait under crop production as it reduces yield productivity. Even though the importance of the *Brassicaceae* family, which includes several crops like rapeseed, broccoli (*Brassica oleracea*) and radish (*Raphanus sativus)*, the knowledge of developmental responses to light quantity and quality during their life cycle is scarce with the exception of the model plant *Arabidopsis thaliana*. However, pioneering studies of phytochrome B mutant of *Brassica rapa* demonstrate that phyB is the principal photoreceptor mediating SAS like in Arabidopsis (Devlin et al. 1992, Robson et al. 1993). Despite of the relevance of the phytochrome system defining the plant architecture of *Brassica* species, the SAS have not yet been described in hybrid modern genotypes of *B. napus* currently grown for edible oil production. We hypothesize that photomorphogenic responses could be particularly important in *B. napus* because, in opposition to other crops, it has a complex and dynamic pattern of leaf development in a primary rosette, followed by main stem elongation and floral racemes growth.

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The aims of this work are to analyze i) morphological and physiological responses at individual plant level in response to low R/FR ratios occurring during the vegetative and reproductive phases of development, and ii) changes in biomass allocation, grain yield and grain composition at crop level in response to changes in irradiance and increases of R/FR ratios in two modern spring rapeseed (*Brassica napus* L) genotypes.

Materials and Methods

Vegetal material and experimental design

Four experiments, two on pots located outdoors and two at field plots, were carried out on 2010 and 2011 growing seasons at the Faculty of Agronomy, University of Buenos Aires experimental field (34º35'S, 58º29'W). In Exp.1 the spring rapeseed hybrid Hyola61 (Advanta Seeds) was sowed on August, 2 on plug trays filled with a mixture of sand: earth: perlite (50:25:25 v/v/v). Plantlet emergence was recorded 6 days after sowing and then homogeneous plantlets with 2 leaves were transplanted to individual pots of 10-L capacity filled with the same substrate. Pots were placed outdoors to solar (control) or low R/FR ratio during all their life cycle. In Exp. 2, the same hybrid was grown in identical conditions than Exp. 1 but the low R/FR treatment started at flowering. In this experiment, the plants were maintained outdoors under normal solar conditions up to the first flower open in the main floral raceme and then half of them were exposed to low R/FR ratio until harvest time. In Exp. 3 the spring rapeseed hybrid Jura (Don Atilio) was hand-sowed on April, 26 at field on a silty clay loam classified as Vertic Argiudoll according to the USDA taxonomy, in 2 x 1.5 m plots, 0.2 m row-spaced at a plant density of 80 pl m^{-2}. At first the first flower open in the main floral raceme the canopy was covered with different meshes to modify the light quality and quantity received up to crop harvest. In Exp. 4 the spring rapeseed hybrid Hyola61 (Advanta Seeds) was hand-sowed on May, 17 in the same way as in Exp. 3, and light treatments were also imposed from flowering to harvest. In all experiments, plants were irrigated, fertilized up to 60 kg sulphur and 150 kg nitrogen ha^{-1} and conducted free of weeds, pest and diseases (Supplementary Table 1).

Light treatments

In Exp. 1 and 2 pots were placed outdoors, in a single row leaving a distance of 30 cm between neighboring plants in front of photo-selective filters modifying R/FR ratio with lights turned on from 6:00 to 21:00, including the end-of-day period (from 18:00 to 21:00). Control treatment consisted of fluorescent tubes 60 W back (Growlux, Sylvania, Argentina) and a red acetate sheet (La Casa del Acetato,

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Buenos Aires, Argentina) plus a sheet of photo-selective film (Solatrol; BPI Agri, Stockton-on-Tees, UK) establishing a R/FR ratio= 1.3at the plant level. The low R/FR treatment consisted of 60 W internal incandescent lamp (Osram, Buenos Aires, Argentina) filtered with a blue acrylic sheet (Paolini 2031, Buenos Aires, Argentina) to generate a R/FR ratio= 0.3 at the plant level. In both experiments, plants were 0.30 m away from filters, so total light quantity was not significantly increased in the illuminated treatments and no temperatures effects due to the lights were recorded (data not shown). The light sources were East–West oriented, placed at plant height and towards the South side of the plants to avoid shading them. The light system to reduce the R/FR ratio was similar to that described earlier (Casal 1993, Crocco et al 2010). The light treatments started either at 23 days (Exp. 1) or at the beginning of flowering at 66 days after the emergence (Exp. 2). Pots with one plant each were randomly assigned to a given light condition in both experiments. Sixteen replicates for each light treatment and experiment were made.

The importance of the critical period after flowering for grain yield determination in rapeseed crop is well documented (Habekotté 1997). To assess the importance of light quality and intensity signals after flowering in realistic growing conditions, we designed Exp. 3 and 4 (supplementary table 1). Field plots were cultivated and, at the beginning of flowering, we established three light treatments: (a) solar high radiation with solar R/FR ratio (without filter), (b) low radiation with high R/FR ratio (Solatrol filter) and (c) low radiation with solar R/FR ratio (neutral filter). For (b) and (c) treatments, we build 2 x 2 x 1.5 m (height x width x length) micro-greenhouses over the plots using metallic structures covered with either a photo-selective film reducing between 27 and 50% (Solatrol; BPI Agri, Stockton-on-Tees, UK) or with black mesh film reducing between 40 and 60% of the solar radiation depending on the experiment, respectively (supplementary table 1). The Solatrol filter had been previously used and increased significantly the R/FR ratio reaching the plants (Mata and Botto 2009, 2011). Three plots for each light treatment and experiment were established.

Measurements

R, FR, and PPFD (photosynthetic photon flux density, μmol m^{-2} s^{-1}) were measured at the top, middle and bottom canopy (see Supplementary Figure 1) using a four-channel sensor SKR 1850A (Skye Instruments Ltd., Powys, UK) attached to a data logger LI-1400 (LI-COR Inc., Lincoln, NE). Local mean daily temperature (ºC) and daily global incident radiation (MJ m^{-2} d^{-1}) were obtained from a station of the National Weather Service placed 200 m from the experiments. In plot experiments, intercepted radiation by the canopy was measured at noon on clear days using a linear radiometer 1 m long (Cava-Rad, Cavadevices, Buenos Aires, Argentina). Phenological variables included sowing date, flowering date when 50% of the plants in the experiment had an open flower, harvest date, and the whole cycle length

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(days from sowing to harvest) which was divided into the stages of pre-flowering (between sowing and flowering, Pre F) and post-flowering (from flowering to harvest, Post F). Harvest time was not necessarily associated with physiological maturity as this attribute was not recorded due to the difficulty in determining its exact timing with visual methods. Vegetative measurements included plant height, length and width of leaves, foliar angle (angle formed by an imaginary vertical axis and the petiole) of the most recently fully expanded leaf faced to the light filter and opposite to filter, and the number of senesced leaves per plant. Reproductive measurements included length of main floral raceme, number of floral branches, and number of siliques. Aboveground biomass at harvest was separated into grain and non-grain, dried in an air-forced oven at 70ºC for 72 h, and harvest index was calculated as the grain/total biomass ratio. Grain yield from pots experiments (g pl^{-1} expressed in dry basis) was determined by harvesting each plant and threshing main raceme grains apart from the floral branches grains. Grain was dried in an air-forced oven at 70ºC for 72 h and then weighted. Grain yield from plots experiments (kg ha^{-1} expressed in dry basis) was determined by harvesting three central rows in each plot, and weighing the dry seed threshed. Plant density was determined in each plot at the beginning of flowering and at harvest. Grain oil content was determined by Soxhlet extraction (IUPAC method 1.122) and protein content by micro-Kjeldahl (Nelson and Sommer 1973). Soluble carbohydrates content in the vegetative stem at harvest was determined by Antrona technique (Scott and Melvin 1966).

Statistical analyses

The experimental design of Exp. 1 and 2 was completely randomized with 16 replicates for each light treatment, where the experimental unit was an individual plant in each pot. In Exps. 3 and 4, the design was complete randomized blocks with 3 blocks (replicates) for each light treatment and the experimental unit was the individual plot. Means of light treatments were analyzed with ANOVA and Tukey's test with 5% of level of significance. Simple linear regressions were also fitted to data. Angular transformation was applied to data expressed percentage in percentage to get homogeneity of variance. Statistical package INFOSTAT was used (www.infostat.com.ar).

Results

Plant cycle and environment explored by spring rapeseed plants

Whole life cycle duration ranged between 130 and 168 d for Hyola61 (Exp. 1 and 2, respectively), and between 180 and 183 d for Jura and Hyola61 genotypes (Exp 3 and 4, respectively). Frost days did not

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occur in any experiment, and mean temperature for vegetative and reproductive periods was within the range usually explored by spring genotypes at this latitude. Light and temperature conditions in Exp. 1 to 4 are shown in Table 1. It was about 14 and 21 ºC for vegetative and reproductive periods, respectively in the pot experiments (Exp. 1and 2), and 12 and 16.5 ºC, respectively, in the plot experiments (Exp. 3 and 4, Table 1). In pot experiments, accumulated intercepted solar radiation was about 600 and 900 MJ m^{-2} during vegetative and reproductive periods, respectively (Table 1). In plot experiments, Solatrol and neutral filters reduced the accumulated solar radiation (respect the treatment without filter) during the reproductive period between 27 and 39% (Exp. 3), and between 50 and 60% (Exp. 4), respectively (Table 1).

Low R/FR ratio increases upward leaf position and leaf senescence in isolated plants cultivated in pots

Shade avoidance responses are induced during the life cycle of plants. To have a better understanding about how photomorphogenic signals affect the architecture of rapeseed plants, we measured the angle of leaves, the length and width of the leaf blade at 50 and 60 DAE (day after emergence) corresponding to leaf number 7-9 completely expanded at the rosette stage in plants exposed to low or solar R/FR ratios. The foliar angle was affected by the R/FR ratio, DAE, and the leaf position to filter (Table 2). Leaves faced to the filter were more erected than leaves opposite to the filter in both R/FR treatments. Low R/FR ratio caused more erected leaves compared with solar R/FR ratio, with a significant effect for leaves opposite to the filter at 50 DAE. Over time, the leaves become more horizontal and no differences between light treatments were observed (Table 2). No interaction effects were found among light and leaf position respect to filter at 50 ($P=0.082$) and 60 DAE ($P=0.146$). Furthermore, R/FR treatments did not affect the length and the width of the leaf blade (data not shown).

Rapeseed plants showed a differential pattern of leaf senescence when they were cultivated since the beginning of the experiment at solar or low R/FR ratios (Fig. 1, $P<0.0001$). In the Exp. 1, leaf senescence was similar between different R/FR ratio treatments at the early rosette developmental stage, but afterwards a highest rate of senescence was observed at the post-flowering stage when plants were cultivated at low R/FR ratio (Fig. 1). By contrast, when rapeseed plants were exposed to low R/FR ratio only from flowering, the differential pattern of leaf senescence between R/FR conditions was not observed (Exp. 2, data not shown).

Low R/FR ratio induces shorter and more flowering racemes with a higher mobilization of soluble carbohydrates from vegetative stems

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Pots experiments did not show significant effects on aboveground biomass nor grain yield per plant but main raceme length and floral branching were affected by low R/FR ratio depending on the experiment (Table 3).When light treatments were applied from the vegetative stage, the number of floral branches per plant was significantly increased by low R/FR ratio, resulting in a higher ratio among biomass branches to main raceme (Table 3, Exp. 1). However, when light treatments were applied from flowering, the length of main raceme was 8 cm shorter under low R/FR than control (Table 3, Exp. 2, P= 0.02). Furthermore, the length of main raceme was related positively to the number of siliques in both Exp. 1 and 2 (Fig. 2). Soluble carbohydrate concentration in the vegetative stem was measured at harvest to assess the degree of reserves remobilization to reproductive sinks. In Exp. 1, plants grown in low R/FR ratio showed less soluble carbohydrates concentration in stems than those cultivated at solar R/FR ratio suggesting a greater degree of remobilization (P=0.017, Fig. 3). However, soluble carbohydrate concentration in stems was similar for plants exposed to different R/FR ratios since flowering (P=0.59, Fig. 3, Exp. 2). The higher soluble remobilization for plants cultivated in low R/FR ratio in the Exp.1 compared with those of the Exp. 2 may be related to the higher strong sink demanding (grain yield= 25.5 *versus* 15.8 g pl^{-1}, Table 3) and/or the higher proportion of reproductive branches (branches/ main raceme biomass= 8.1 *versus* 4.5, Table 3).

Patterns of quantity and quality of canopy light after flowering for plants cultivated in plots

To evaluate the effects of irradiance and R/FR ratio on the development and yield parameters in rapeseed plants, we designed plots experiments with three light treatments: i) without filter, with high radiation and solar R/FR ratio, b) Solatrol filter with low radiation and high R/FR ratio, and c) neutral filter with low radiation and solar R/FR ratio (Supplementary Table 1). Light treatments modified PPFD and R/FR ratio profiles throughout the canopy according to the stratum considered (Fig. 4). At the 5 DAE, the R/FR ratio in the control condition without filter was 1.07 ± 0.03 at the top canopy, falling sharply to 0.10 ± 0.01 and 0.06 ± 0.01 at middle and bottom canopy, respectively. The significant reduction of the R/FR ratio at lower positions of the rapeseed crop was caused by the dense layer of yellow flowers and green peduncle from floral racemes at the middle canopy, and green leaves at the bottom canopy. As expected, the neutral filter did not change the R/FR ratio with respect to without filter condition (Fig. 4). However, the Solatrol filter strongly increased the R/FR ratio throughout all canopy strata, from 4.6 ± 1.3 in the top to 0.9 ± 0.3 in the middle and 0.32 ± 0.07 in the bottom canopy (Fig. 4). At the day 25 DAE, R/FR ratio at middle canopy was 0.4, 0.09 and 1.6 for control, neutral and Solatrol treatments and <0.2 at the bottom position in the canopy with significant differences among light treatments (Fig. 4). The range of R/FR ratios between

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treatments at middle canopy was greater than those at 5 DAE possibly due to the differential fall of yellow petals and growth of green siliques in plants grown under each light condition.

PPFD profiles inside rapeseed canopy were modified by light treatments, according to the canopy stratum considered (Fig. 4). As expected, irradiance at the top canopy was reduced below Solatrol and neutral filters causing a drop in the cumulative radiation during the whole post-flowering period (Table 1). At middle canopy, PPFD was 48, 27 and 39 mol m^{-2} s^{-1} for control, neutral and Solatrol filters, respectively at 5 DAE, whereas PPFD rose up to 129 mol m^{-2} s^{-1} for all treatments at 25 DAE (Fig. 4). Thus, PPFD increased 3 or 4 times over the time, when the canopy stratum changed from yellow flowers to green siliques (Fig. 4). At bottom canopy, very low PPFD < 20 mol μm^{-2} s^{-1} was measured in both dates, without significant differences among light treatments (Fig. 4).

In both plot experiments a consistent 'self-thinning' effect was observed as plant density dropped between crop implantation (80 plants m^{-2}) and harvest time, with no statistical differences between light treatments within each experiment (Table 4). Self-thinning ranged between 15 and 35% in the Exp. 3 and between 48 and 60% in the Exp. 4.

Low radiation reduces harvest index, grain yield and oil content in the seeds

Quantity and quality light since flowering affected reproductive parameters at harvest (Table 4). In Exp. 3, harvest index (i.e., the proportion of total biomass allocated into grains) decreased significantly in plants cultivated under neutral and Solatrol filters with respect to the control treatment suggesting a relevant importance of irradiance affecting this trait. Nevertheless, aboveground biomass and grain yield did not differ among light treatments (Table 4). By contrast, in Exp. 4, plants grown under neutral filters had lower grain yield with respect to untreated control plants (Table 4). Plants cultivated under Solatrol filters showed intermediate grain yield, but not statistical differences with the control (Table 4).

Oil content in grains was significantly lower in plants cultivated under neutral and Solatrol filters respect to untreated control plants, and the opposite behavior was true for grain protein content. A negative relationship between oil and protein content was observed, with a slope of -1.4 indicating that the drop of oil percentage was more than proportional respect to the increase of protein percentage (Fig. 5). By contrast, a positive linear relationship was observed between oil content and grain yield which was statistically significant for plants of the Exp. 4 but not for those of the Exp. 3 (Fig. 5). Our data suggest that the correlation is significant when grain yield shows a broad range of variation as observed in the Exp. 4.

Soluble carbohydrates concentration in vegetative stems was measured at flowering and harvest (Supplementary Table 2). A significant reduction of soluble carbohydrates was detected from vegetative

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stems among dates ($P<0.0001$) suggesting an intense remobilization of reserves to the sinks (> 90%). Although the light factor was not significant ($P=0.20$), a tendency to a higher soluble carbohydrates concentration at crop harvest was found for plants grown under Solatrol filter compared with those cultivated under neutral or without filters indicating a lower degree of remobilization of resources to the grains (Supplementary Table 2).

Discussion

Rapeseed plants are able to perceive quantity and quality light signals that provide information about the dynamic status of canopy architecture. Some of these signals included low irradiances and reduction in the R/FR ratios typical of dense stands. Here we showed that a modern spring rapeseed hybrid displays strong photomorphogenic responses to low R/FR ratios in vegetative and reproductive phases of development when isolated plants are cultivated in pots. However, in two rapeseed genotypes, low irradiance perceived by plants after flowering is more relevant than light quality signals determining reductions in grain yield and other harvest traits under crop conditions. Interestingly, the increase of R/FR ratios after flowering might partially alleviate yield reduction under low irradiance. Taken together, these results suggest that photomorphogenic signals are integrated very early during the vegetative growth in rapeseed genotypes.

The main effects of low R/FR ratios in isolated rapeseed plants included the erect position of leaves at early rosette stage (Table 2) and the acceleration of leaf senescence when plants perceived low R/FR ratio since early stages of development (Fig.1). The ability to reorient leaves according to the light signals typical of dense canopies have been also observed in Arabidopsis (Ballaré and Scopel 1997, Djakovic-Petrovic et al. 2007) and maize (Maddonni et al. 2001, 2002) as a strategy to reduce mutual shading among leaves and increase the efficiency to intercept PAR. In maize, the ability to reorient leaf growth according to the light signals is cultivar specific and appear early in the ontogeny. Isolated maize plants grown in the field next to filters reflecting FR light placed their leaves more perpendicular to the direction of the incoming reflected FR than control plants with filters that do not alter solar R/FR ratio (Maddonni et al. 2002). Furthermore, accelerated leaf senescence for plants cultivated with low R/FR is well documented for sunflower plants (Rousseaux et al. 1996). The senescence of target leaves was advanced when isolated sunflower plants were grown in the field with the aid of mirrors placed beneath the leaves to selectively reflect FR light (Rousseaux et al. 1996). According with these results, the increase of R/FR ratios received by basal leaves grown with red-light emitting diodes delayed senescence compared to non-irradiated controls (Rousseaux et al. 2000).

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Shade signals also altered reproductive performance in isolated rapeseed plants grown with low R/FR ratio. The reduction of R/FR ratio by filters placed next to the plants caused short main floral shoot and increased floral branching with higher soluble carbohydrates remobilization from stem if the treatment began at the vegetative stage (Fig. 3, Table 3). However, when plants were exposed to low R/FR ratio since flowering these differences disappeared suggesting that shade photomorphogenic signals are integrated early during rapeseed development. Increased floral branching in isolated rapeseed plants exposed to low R/RF ratio was unexpected, as it is common to observe that low R/FR ratios reduce tillering or branching in wheat (Casal 1988, Evers et al. 2006, Ugarte et al. 2010), soybean (Green-Tracewicz et al. 2011), barley (Skinner and Simmons 1993) and maize (Maddonni et al. 2002). Unlike cereals, rapeseed plants have a vegetative rosette habitat and then flowering develops reproductive leafless shoots appearing basipetally from the main floral shoot. This pattern of growth differs from tillering of cereals, and branching in soybean or tomato. Interestingly, in a study with 80 accessions of Arabidopsis, a cruciferae as rapeseed with the same pattern of growth, we found that plants growth under shade developed shorter main floral shoots than plants cultivated under natural radiation (unpublished data). Furthermore, reduced floral length and increased branching might be compatible with a loss of apical dominance when plants grown under well and non-stress conditions. It has been suggested that low R/FR ratio promotes floral branching under non–resource-limiting conditions because apical bud dominance is broken activating the growth of axillary buds in maize plants (Whipple et al. 2011).

In field conditions, radiation was more relevant than light quality signals after flowering, defining yield trait components in two modern spring rapeseed genotypes. Low irradiance from flowering reduced grain yield, harvest index and grain oil content (Table 4 and Fig. 5). These results indicate a great sensitivity of reproductive output to the amount of solar radiation captured by the canopy after flowering. It also agrees with shading effects studied recently in winter rapeseed by Brunel-Muguet et al. (2013), where a 43% reduction of PAR applied at the early flowering stage delayed leaf senescence, optimizing light capture and reducing biomass allocation to reproductive organs. It is noteworthy that a possible relieving effect of high R/FR ratio could reduce the detrimental effects of low radiation on the rapeseed yield parameters (Table 4). We found a higher, but not statistically significant, difference on soluble carbohydrates concentration at harvest for plants grown under Solatrol filter compared with those cultivated under neutral or without filters (Supplementary Table 2). Furthermore, a tendency to increase the aboveground biomass at harvest in plants grown under Solatrol filters compared to neutral filters was observed (Table 4). These results agree with the higher mobilization of soluble carbohydrates from vegetative stems in isolated rapeseed plants growth with low R/FR ratio (Fig. 3).

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Shading conditions caused a reduction of plant stand at field conditions (Table 4). A 'self-thinning' effect has been addressed for dense rapeseed canopies (Canola Council 2013), but the influence of irradiance and R/FR ratios on the competitive relationship among plants have not been yet considered. In other species as maize, intra-specific competition generates early plant hierarchies with dominant and dominates plants (Maddonni and Otegui 2006) affecting maize productivity (Pagano and Maddonni 2007). Considering that early low R/FR ratio and low irradiance have relevant effects on self-thinning, it will be valuable to design new experiments at different plant densities to evaluate the self-thinning consequences on reproductive outputs in modern spring rapeseed hybrids.

Author contributions

DPR, MPV and JFB designed the experiments and wrote the paper. DPR and MPV performed plots experiments and MER, MAP and JFB performed pots experiments.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S.1. Light treatments applied to spring rapeseed in pot and plot experiments.

Table S.2. Soluble carbohydrates concentration (%, g glucose per 100 g dry mass) in vegetative stem of rapeseed plants cultivated under Solatrol and neutral filters or without filter measured at flowering and harvest. Different letters indicate significant differences ($P<0.05$) among flowering and harvest times within each light treatment. Data are from the Exp. 4.

Fig. S.1. Photographs from plot experiment (Exp. 4) at 5 and 25 days after flowering (DAF) indicating the three zones of measurements, top, middle and bottom canopy.

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Figure legends

Figure 1. Dynamics of leaf senescence in isolated rapeseed plants under contrasting R/FR ratios (Exp. 1). Arrow indicates the time of flowering (identical in both treatments). Asterisks indicate significant differences among light treatments.

Figure 2. Relationship between the length and the number of siliques from main raceme in rapeseed plants under contrasting R/FR ratios (Exp. 1 and 2). Linear adjust to data is also shown.

Figure 3. Soluble carbohydrates concentration (%, g glucose per 100 g dry mass) in vegetative stem at harvest of rapeseed plants under contrasting R/FR ratios applied to the whole cycle (Exp. 1) or since flowering until harvest (Exp. 2).

Figure 4. R/FR ratio (left panels) and PPFD (mol µm^{-2} s^{-1}, right panels) profiles at top, middle and bottom positions of rapeseed canopy at 5 and 25 days after flowering (DAF) under neutral and Solatrol filters or without filter. Notice different scales among canopy strata. Different letters into each panel indicate significant differences among light treatments, and respective P-values are also shown. Data are from Exp. 4.

Figure 5. Relationships between grain oil and protein content (left panel) and between oil content and grain yield (right panel) in rapeseed plants grown under different light treatments from flowering. Linear adjustments are shown into each graph.

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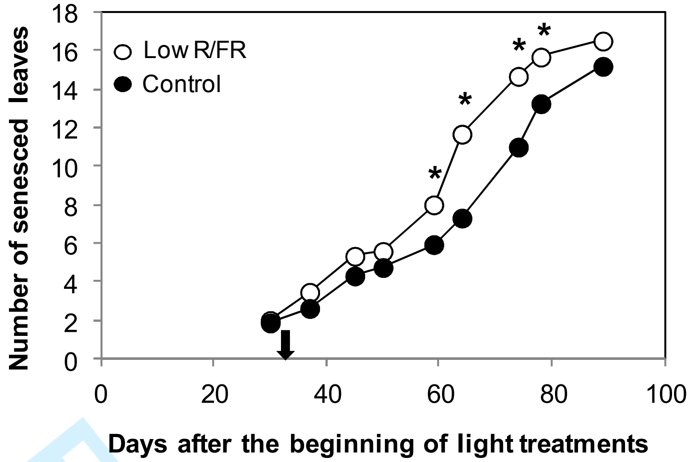

Figure 1.

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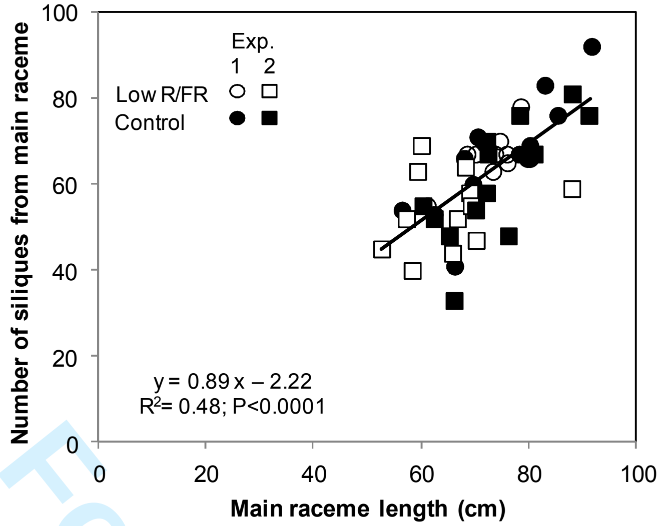

Figure 2.

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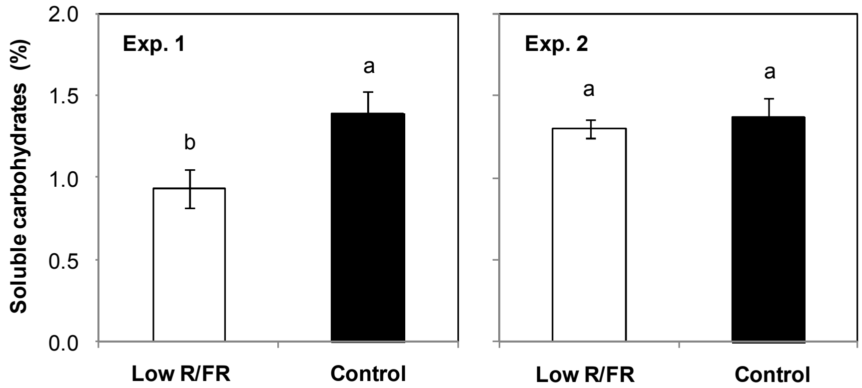

Figure 3.

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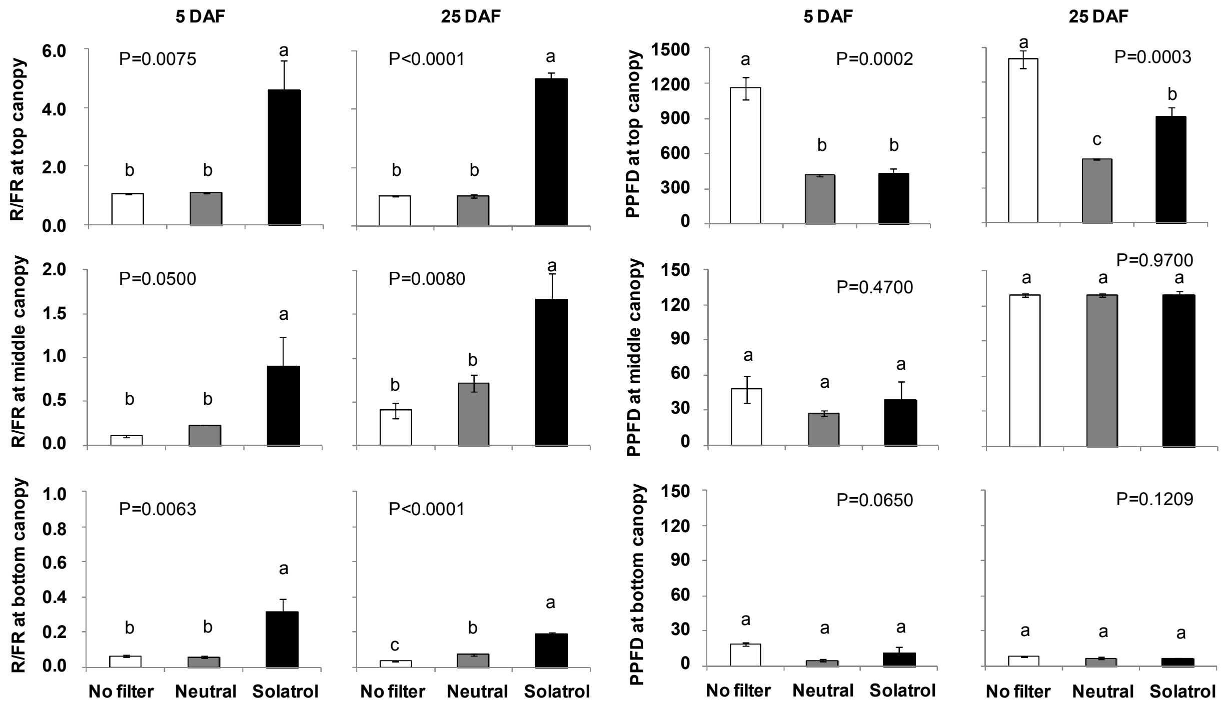

Figure 4.

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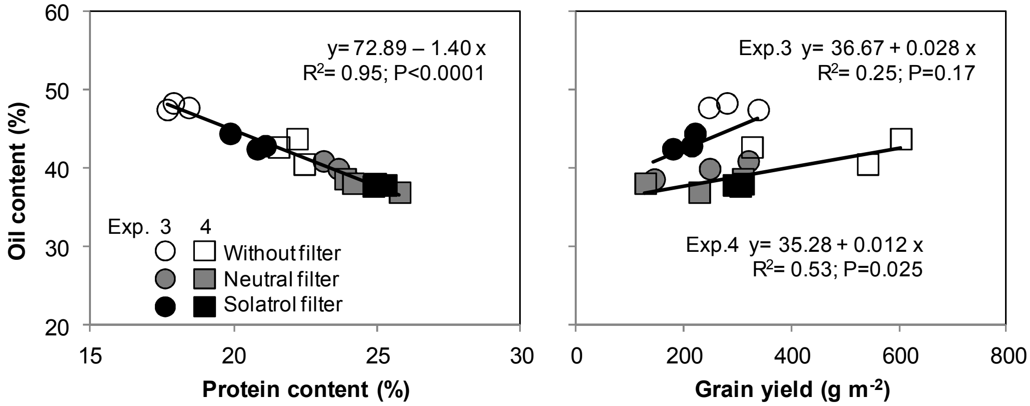

Figure 5.

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Table 1. R/FR ratios, mean temperature and cumulative intercepted solar radiation during pre and post-flowering periods in the four experiments. Data are mean ± 1 SD. In Exp. 1 and 2, incident solar radiation was computed as isolated plants were considered, whereas in Exp. 3 and 4, solar radiation intercepted by the crop canopy was considered. In the Exp. 1, isolated plants were exposed to low R/FR ratio during all the life cycle meanwhile plants of Exp. 2 were cultivated under control condition until flowering and then half of them were exposed to low R/FR ratio until harvest.

		R/FR ratio	Mean temperature (ºC)		Cumulative solar radiation (MJ m^{-2})	
Exp	Treatments		Pre F	Post F	Pre F	Post F
1	Control	1.08	14.1 ± 3.5	20.6 ± 3.9	546	1025
	Low R/FR	0.36	14.1 ± 3.5	20.6 ± 3.9	546	1025
2	Control	1.07	14.9 ± 3.5	21.9 ± 3.8	713	854
	Low R/FR	0.38	14.9 ± 3.5	21.9 ± 3.8	713	854
3	Solatrol filter	4.50	12.9 ± 3.6	16.4 ± 2.3	680 ± 48	528 ± 52
	Neutral filter	1.09	12.9 ± 3.6	16.4 ± 2.3	680 ± 48	441 ± 44
	Without filter	1.10	12.9 ± 3.6	16.5 ± 2.3	680 ± 34	723 ± 36
4	Solatrol filter	5.03	11.6 ± 3.0	16.9 ± 2.4	815 ± 82	452 ± 36
	Neutral filter	1.03	11.6 ± 3.0	16.9 ± 2.4	815 ± 82	362 ± 29
	Without filter	1.05	11.6 ± 3.0	16.9 ± 2.4	815 ± 41	906 ± 45

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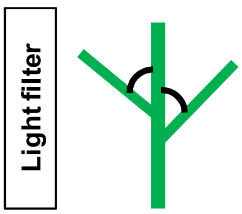
Table 2. R/FR ratio effects on foliar insertion angle for the last expanded leaves in the rapeseed rosette faced to filter and opposite to filter (see scheme below), at two dates (50 and 60 days after emergence, DAE). Different letters indicate significant differences (P<0.05) between light treatments and filter position within each date. A photograph top view of the foliar arrangement in a plant laterally illuminated with low R/FR light is also shown. Horizontal line in the photograph indicates the symmetry axis. Data are from Exp. 1.

	50 DAE		60 DAE	
Leaf position	faced to filter	opposite to filter	faced to filter	opposite to filter
Control	30º 05'ab	64º 25'c	41º 50'a	61º 00'b
Low R/FR	20º 43'a	38º 86'b	40º 07'a	50º 43'ab



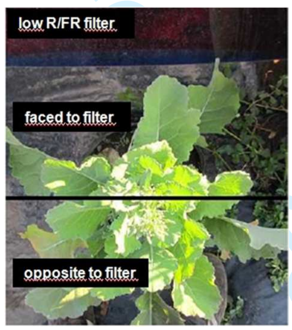


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Table 3. Yield components of spring rapeseed isolated plants grown under different light treatments. Different letters indicate significant differences (P<0.05) between light treatments within each experiment (Exp 1 and 2).

Exp	Light treatments	Length of main raceme (m)	Floral branches (Nº pl^{-1})	Aboveground biomass (g pl^{-1})	Branches/main raceme biomass (g pl^{-1})	Grain yield (g pl^{-1})
1	Control	0.75 a	7.0 b	89.3 a	4.7 b	23.9 a
	Low R/FR	0.72 a	8.6 a	89.7 a	8.1 a	25.5 a
2	Control	0.73 a	6.5 a	54.6 a	6.0 a	14.7 a
	Low R/FR	0.65 b	7.0 a	53.3 a	4.5 a	15.8 a

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Table 4. Light effects on biomass allocation and reproductive output for spring rapeseed plot experiments (Exp 3 and 4). Different letters indicate significant differences ($P<0.05$) among treatments. [a] not available

Exp.	Treatments	Plant density (pl m^{-2})	Plant height (m)	Aboveground biomass (g m^{-2})	Harvest index	Grain yield (g m^{-2})
3	Solatrol filter	71.0 a	---[na]	709.1 a	0.29 b	205.1 a
	Neutral filter	54.2 a	---	758.3 a	0.26 b	196.6 a
	Without filter	71.9 a	---	848.3 a	0.34 a	288.3 a
4	Solatrol filter	31.7 a	1.30 a	1135.6 a	0.27 a	302.6 ab
	Neutral filter	37.1 a	1.27 a	724.9 a	0.30 a	222.4 b
	Without filter	42.9 a	1.22 a	1478.6 a	0.33 a	490.4 a

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