Physiologia Plantarum

An International Journal for Plant Biology

Physiologia Plantarum 152: 784–794. 2014

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

Deborah P. Rondanini[a,b], Maria del Pilar Vilariño[a], Marcos E. Roberts[a], Marina A. Polosa[a] and Javier F. Botto[a,c,*]

[a]Facultad de Agronomía, Universidad de Buenos Aires, C1417DSE, Buenos Aires, Argentina
[b]CONICET, C1033AAJ, Buenos Aires, Argentina
[c]IFEVA, Consejo Nacional de Investigaciones Científicas y Técnicas, Universidad de Buenos Aires, C1417DSE, Buenos Aires, Argentina

Correspondence
*Corresponding author,
e-mail: botto@agro.uba.ar

Received 16 January 2014;
revised 21 March 2014

doi:10.1111/ppl.12227

Early shade signals promote the shade avoidance syndrome (SAS) which causes, among others, petiole and shoot elongation and upward leaf position. In spite of its relevance, these photomorphogenic responses have not been deeply studied in rapeseed (*Brassica napus*). In contrast to other crops like maize and wheat, rapeseed has a complex developmental phenotypic pattern as it evolves from an initial rosette to the main stem elongation and an indeterminate growth of floral raceme. In this work, we analyzed (1) morphological and physiological responses at individual level due to low red/far-red (R/FR) ratio during plant development, and (2) changes in biomass allocation, grain yield and composition at crop level in response to high R/FR ratio and low irradiance in two modern spring rapeseed genotypes. We carried out pot and field experiments modifying R/FR ratios and irradiance at vegetative or reproductive stages. In pot experiments, low R/FR ratio increased the petiole and lamina length, upward leaf position and also accelerated leaf senescence. Furthermore, low R/FR ratio reduced main floral raceme and increased floral branching with higher remobilization of soluble carbohydrates from the stems. In field experiments, low irradiance during post-flowering reduced grain yield, harvest index and grain oil content, and high R/FR ratio reaching the crop partially alleviated such effects. We conclude that photomorphogenic signals are integrated early during the vegetative growth, and irradiance has stronger effects than R/FR signals at rapeseed crop level.

Introduction

Rapeseed (*Brassica napus*) is the third most important oilseed crop in the world, following palm and soybean. It is an excellent raw material for edible oil and biodiesel production (Velasco and Fernández-Martínez 2002). Rapeseed crop can also replace winter cereals in crop rotations, allowing the incorporation of carbon into the soil due to its great amount of crop residues, and the earlier sowing of double-crop soybean. Commonly, 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 crop

---

Abbreviations – DAE, days after emergence; DAF, days after flowering; PAR, photosynthetically active radiation; Post F, post-flowering; Pre F, pre-flowering; R/FR, red/far-red ratio; SAS, shade avoidance syndrome.

in non-optimal environments, it is perceived by the producers as a risky crop due to its high yield variability at a global scale (Rondanini et al. 2012).

One source of rapeseed yield variability may be associated with changes in the light environment experienced by plants that affect biomass production and its allocation to harvestable grains. As the crop grows and develops, plants begin to be shaded among each other. The optical properties of the leaves reduce blue and red photons and increase the proportion of far-red photons in shaded environments. As a consequence, irradiance and red/far-red (R/FR) ratio reaching the green tissues decrease, modifying the competitive relationships among individuals which can produce changes in crop productivity (Casal 2013). Plants perceive the low R/FR ratio reflected by neighboring plants and respond 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).

Furthermore, low radiance has profound consequences in plant growth and yield in rapeseed production. Radiation intercepted by the crop is a limiting factor for biomass production, especially during flowering and fruit set in rapeseed plants (Habekotté 1997a, 1997b). The leaves are the main source of photosynthesis until flowering and are replaced afterwards by the stems and green siliques. Excluding radiation in growing siliques reduced grain weight and oil content in chamber experiments (Fortescue and Turner 2007). In contrast, low radiation reaching rapeseed plants during the critical post-flowering period in field experiments reduced yield and number of grains, with no effects on grain weight (Rondanini et al. 2013).

Photomorphogenic plasticity allows plants to adapt in diverse light environments, but it may be an undesirable trait under crop production as it reduces yield. 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, although *Arabidopsis* is an ideal model to learn how plants work due to its simplicity, we need to validate and confirm the physiological and molecular mechanisms in other species in field conditions. Pioneering studies with mutants of *Brassica rapa* demonstrated that phytochrome B is the principal photoreceptor mediating SAS, such as in *Arabidopsis* (Devlin et al. 1992, Robson et al. 1993). Despite 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.

In this work, we hypothesize that photomorphogenic responses could be particularly important in *B. napus* depending on the developmental phase involved because rapeseed plants have a complex and dynamic pattern of leaf development in a primary rosette, followed by the production of photosynthetic structures after flowering. The objectives were to study (1) the effects of low R/FR ratio on vegetative, reproductive and yield traits at individual plant level in pot experiments, and (2) the effects of low irradiance and high R/FR ratio after flowering on the productivity in field experiments using two modern spring rapeseed (*B. napus* L.) genotypes extensively cultivated in Argentina.

Materials and methods

Vegetal material and experimental design

Four experiments, two on pots located outdoors and two at field plots, were carried out in 2010 and 2011 growing seasons at the Faculty of Agronomy, University of Buenos Aires experimental field (34°35′S, 58°29′W). In our experiments, we selected two short cycle cultivars used in rapeseed crops cultivated in winter-spring at several locations in the humid Pampas around Buenos Aires, Argentina (Iriarte and Valetti 2008). In experiment 1, the spring rapeseed hybrid Hyola61 (Advanta Seeds, Venado Tuerto, Argentina) was sown 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 two leaves were transplanted to individual pots of 10 l capacity filled with the same substrate. Pots were placed in the field exposed to solar irradiance (control) or low R/FR ratio by adding FR light next to the plants until the end of the experiment. In experiment 2, the same hybrid was grown in identical conditions as in experiment 1, but the low R/FR treatment started at flowering. In this experiment, the plants were maintained outdoors under normal solar conditions until the first flower opened in the main floral raceme and then half of them were exposed to low R/FR ratio until harvest time. In experiment 3, the spring rapeseed hybrid Jura (Don Atilio, Venado Tuerto, Argentina) was hand-sown on April 26 at field on a silty clay loam classified as Vertic Argiudoll according to the USDA taxonomy, in 2 × 1.5 m plots, 0.2 m row-spaced at a plant density of 80 $\mathrm{pl\,m^{-2}}$. After the first flower opened in the main floral raceme, the canopy was covered with different meshes until harvest to modify the light quality and quantity received by the crop. In experiment 4 the spring rapeseed hybrid Hyola61 (Advanta Seeds) was hand-sown on May 17 in the same way as in experiment 3, and light treatments were also imposed from flowering to harvest. In all experiments, plants were irrigated, fertilized up to reach 60 kg sulfur and 150 kg nitrogen ha^{-1} and conducted free of weeds, pest and diseases (Fig. S1; Table S1, Supporting Information).

Light treatments

In experiments 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. R/FR ratio was modified with lights turned on from 6:00 to 21:00 h, including the end-of-day period (from 18:00 to 21:00 h). Control treatment consisted of fluorescent tubes 60 W (Growlux; Havells Sylvania, Buenos Aires, Argentina) behind a red acetate sheet (La Casa del Acetato, Buenos Aires, Argentina) plus a sheet of photo-selective film (Solatrol; BPI Agri, Stockton-on-Tees, UK) establishing a R/FR ratio = 1.3 at the plant level. The low R/FR treatment consisted of 60 W incandescent lamps (Osram, Buenos Aires, Argentina) filtered with a red acetate sheet and 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 temperature effects due to the lights were recorded (data not shown). The light sources were East–West oriented, placed at plant height and toward the South side of the plants to avoid shading them. The light system to reduce the R/FR ratio was described earlier (Casal 1993, Crocco et al. 2010). The light treatments started either at 23 days (experiment 1) or at the beginning of flowering, 66 days after emergence (DAE) (experiment 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.

To assess the importance of light quality and intensity signals after flowering in realistic growing conditions, we designed experiments 3 and 4 (Fig. S1). Field plots were cultivated and, at the beginning of flowering, three light treatments were established: (1) solar high irradiance with solar R/FR ratio (without filter), (2) low irradiance with high R/FR ratio (Solatrol filter) and (3) low irradiance with solar R/FR ratio (neutral filter). For (2) and (3) treatments, we built 2 × 2 × 1.5 m (height × width × 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 irradiance depending on the experiment (Fig. S1). The Solatrol filter has 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 photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) were measured at the top, middle and bottom canopy (Fig. S4) 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 (°C) and daily global incident irradiance (MJ m^{-2} day^{-1}) were obtained from a station of the National Weather Service placed 200 m from the experiments. In field experiments, intercepted irradiance by the canopy was measured at noon on clear days using a 1-m long linear radiometer (Cava-Rad, Cavadevices, Buenos Aires, Argentina).

Table S2 shows plant traits measured throughout the four experiments. Phenological variables included sowing date, flowering date (50% of the plants in the experiment with an open flower), harvest date, and the whole cycle length (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 but this attribute was not recorded due to the difficulty in determining its exact timing visually. 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 leaves facing the light filter and opposite to the 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 70°C for 72 h, and the harvest index was calculated as the grain/total biomass ratio. Grain yield from pot experiments (g pl^{-1} expressed on a dry basis) was determined by harvesting each plant and threshing main raceme grains apart from floral branch grains. Grain was dried in an air-forced oven at 70°C for 72 h and then weighed. Grain yield from field experiments (kg ha^{-1} expressed on a 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

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 experiments 1 and 2, incident solar radiation was computed as isolated plants were considered, whereas in experiments 3 and 4, solar radiation intercepted by the crop canopy was considered. In experiment 1, isolated plants were exposed to low R/FR ratio during all the life cycle while plants of experiment 2 were cultivated under control condition until flowering and then half of them were exposed to low R/FR ratio until harvest.

			Mean temperature (°C)		Cumulative solar radiation (MJ m^{-2})	
Exp	Treatments	R/FR ratio	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

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 the anthrone method (Scott and Melvin 1953).

Statistical analyses

The experimental design of experiments 1 and 2 was completely randomized with 16 replicates for each light treatment; the experimental unit was an individual plant in each pot. For experiments 3 and 4, the design was complete randomized block with three 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 at 5% level of significance. Simple linear regressions were also fitted to data. Angular transformations were applied to data expressed 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 days for Hyola61 (experiments 1 and 2, respectively), and between 180 and 183 days for Jura and Hyola61 genotypes (experiments 3 and 4, respectively). Frost days did not occur in any experiment, and mean temperatures for vegetative and reproductive periods were within the range usually explored by spring genotypes at this latitude. Light and temperature conditions in experiments 1–4 are presented in Table 1. Mean temperatures were about 14 and 21°C for vegetative and reproductive periods, respectively, in the pot experiments (experiments 1 and 2), and about 12 and 16.5°C, respectively, in the field experiments (experiments 3 and 4, Table 1). In pot experiments, accumulated intercepted solar irradiance was about 600 and 900 MJ m^{-2} during vegetative and reproductive periods, respectively (Table 1). In the reproductive period of field experiments, Solatrol and neutral filters reduced the accumulated solar radiation between 27 and 39% (experiment 3), and between 50 and 60% (experiment 4), respectively (Table 1).

Low R/FR ratio increases upward leaf position, leaf and petiole length 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 corresponding to leaf number 7–9 completely expanded at the rosette stage in plants exposed to low or control R/FR ratios. The foliar angle was affected by the R/FR ratio, DAE and the leaf position to filter (Table 2). Leaves facing the filter were more erect than leaves opposite to the filter in both R/FR treatments. Low R/FR ratio caused more erect leaves compared with control 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 interactions were found among light and

Table 2. R/FR ratio effects on foliar insertion angle for the last expanded leaves in the rapeseed rosette facing filter and opposite to filter (see scheme below), at two dates (50 and 60 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 experiment 1.

	50 DAE		60 DAE	
Leaf position	Facing filter	Opposite to filter	Facing filter	Opposite to filter
Control	30°05′ ab	64°25′ c	41°50′ a	61°00′ b
Low R/FR	20°43′ a	38°86′ b	40°07′ a	50°43′ ab

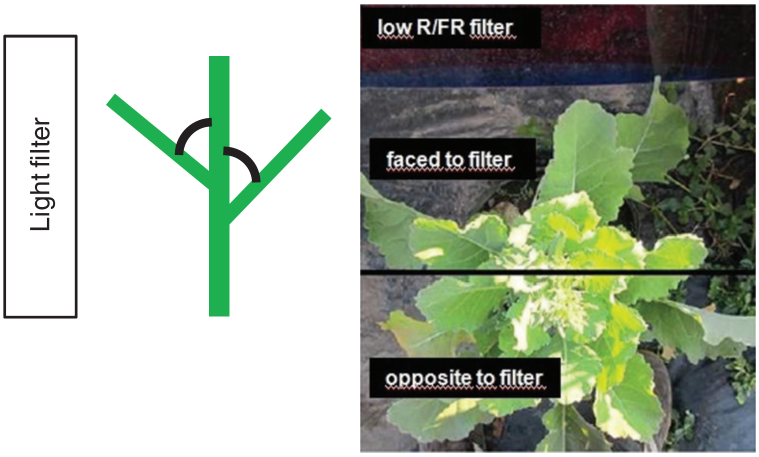

Table 3. R/FR ratio effects on petiole length and leaf blade dimensions for the last expanded leaf in the rapeseed rosette at 50 DAE. Different letters indicate significant differences ($P < 0.05$) between light treatments. Data are from experiment 1.

	Petiole length (cm)	Leaf blade length (cm)	Leaf blade width (cm)
Control	13.37 b	18.10 b	16.15 a
Low R/FR	15.17 a	19.53 a	16.01 a

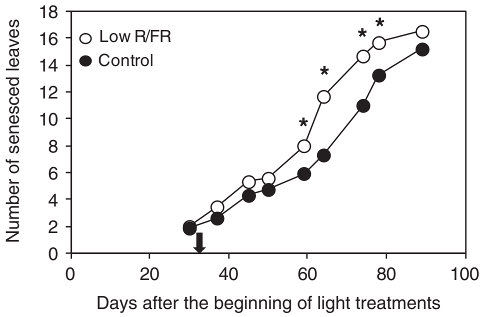

Fig. 1. Dynamics of leaf senescence in isolated rapeseed plants under contrasting R/FR ratios (experiment 1). Arrow indicates the time of flowering (identical in both treatments). Asterisks indicate significant differences among light treatments.

leaf position respect to filter at 50 ($P = 0.082$) and 60 DAE ($P = 0.146$). Furthermore, low R/FR increased petiole and leaf length, but not leaf width at 50 DAE (Table 3).

Rapeseed plants showed a differential pattern of leaf senescence when they were cultivated since the beginning of the experiment at control or low R/FR ratios (Fig. 1, $P < 0.0001$). In experiment 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). In 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 (experiment 2, data not shown).

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

Pot 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 4).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

Table 4. 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 (experiments 1 and 2).

Experiment	Light treatments	Length of main raceme (m)	Floral branches (N° 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

branches to main raceme (Table 4, experiment 1). However, when light treatments were applied from flowering, the main raceme was 8 cm shorter under low R/FR than for controls (Table 4, experiment 2, $P = 0.02$). Furthermore, the length of the main raceme was positively related to the number of siliques in experiments 1 and 2 (Fig. 2). Interestingly, the association between main raceme length and number of siliques was stronger when the low R/FR condition was imposed at pre-flowering than at post-flowering ($R^2 = 0.63$ vs 0.35, respectively, Fig. 2).

Soluble carbohydrates concentration in the vegetative stem was measured at harvest to assess the degree of reserves remobilization to reproductive sinks. In experiment 1, plants grown in low R/FR ratio showed less soluble carbohydrates concentration in stems than those cultivated at control R/FR ratio suggesting a greater degree of remobilization ($P = 0.017$, Fig. 3). However, soluble carbohydrates concentration in stems was similar for plants exposed to different R/FR ratios since flowering ($P = 0.59$, experiment 2, Fig. 3). The higher soluble remobilization for plants cultivated in low R/FR ratio in experiment 1 compared with those of experiment 2 may be related to a higher strong sink demand in the former (grain yield = 25.5 vs 15.8 g pl^{-1}, Table 4) and/or the higher proportion of reproductive branches (branches/main raceme biomass = 8.1 vs 4.5, Table 4).

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

To evaluate the effects of irradiance and R/FR ratio on the development and yield parameters in rapeseed plants, we designed field experiments with three light treatments: (1) without filter, with high irradiance and solar R/FR ratio, (2) Solatrol filter with low irradiance and high R/FR ratio and (3) neutral filter with low irradiance and solar R/FR ratio (Fig. S1). Light treatments modified PAR and R/FR ratio profiles throughout the canopy according to the stratum considered (Fig. 4). Five days after flowering (DAF), the R/FR ratio for the controls 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 peduncles of 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

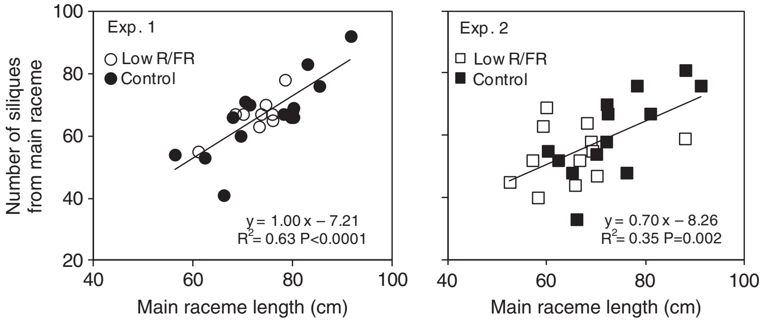

Fig. 2. Relationship between the length and the number of siliques from main raceme in rapeseed plants under contrasting R/FR ratios (experiments 1 and 2). Linear adjust to data is also shown.

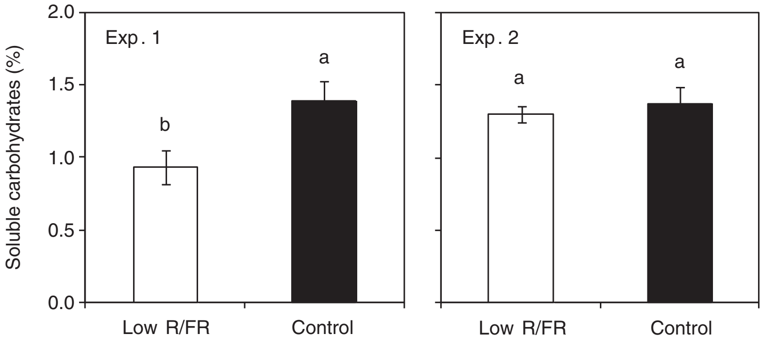

Fig. 3. Soluble carbohydrates concentration (%, g glucose per 100 g dry mass) in vegetative stem at harvest of rapeseed plants under contrasting R/FR ratios applied to the whole cycle (experiment 1) or since flowering until harvest (experiment 2).

canopy strata, from 4.6 ± 1.3 at the top to 0.9 ± 0.3 at the middle and 0.32 ± 0.07 at the bottom canopy (Fig. 4). At 25 DAF, 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 treatments at middle canopy was greater than those at 5 DAF possibly due to a different fall of yellow petals and growth of green siliques in plants grown under each light condition.

PAR 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,

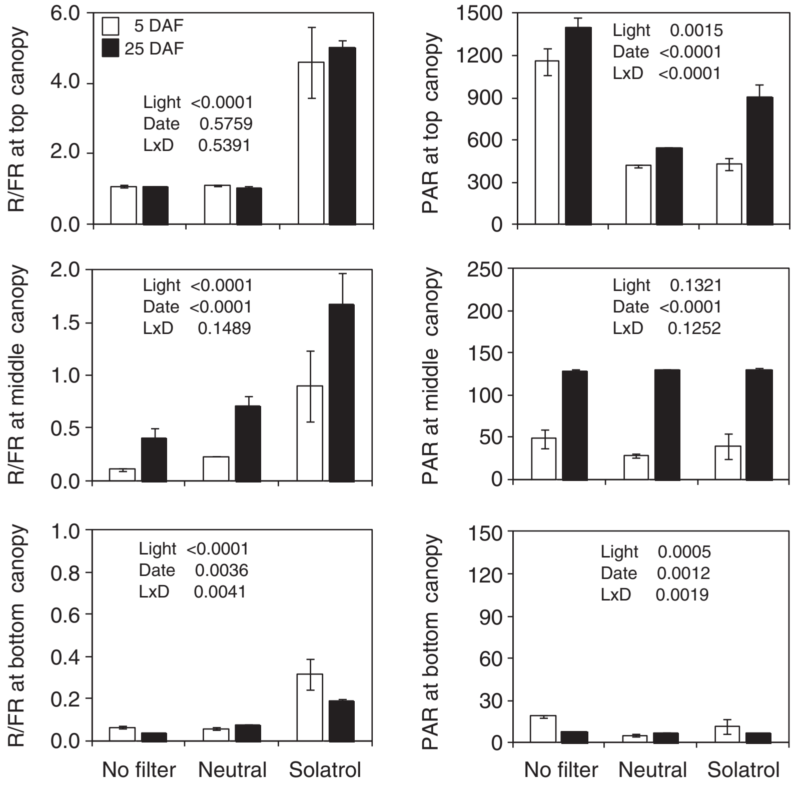

Fig. 4. R/FR ratio (left panels) and PAR (µmol m^{-2} s^{-1}, right panels) profiles at top, middle and bottom positions of rapeseed canopy at 5 and 25 DAF under neutral and Solatrol filters or without filter. Notice different scales among canopy strata. *P* values for Anova test are shown. Data are from experiment 4.

Table 5. Light effects on biomass allocation and reproductive output for spring rapeseed field experiments (experiments 3 and 4). Different letters indicate significant differences ($P < 0.05$) among treatments. nd, no data.

Experiment	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	nd	709.1 a	0.29 b	205.1 a
	Neutral filter	54.2 a	nd	758.3 a	0.26 b	196.6 a
	Without filter	71.9 a	nd	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

PAR was 48, 27 and 39 mol m^{-2} s^{-1} for control, neutral and Solatrol filters, respectively, at 5 DAF, whereas PAR rose up to 129 mol m^{-2} s^{-1} for all treatments at 25 DAF (Fig. 4). Thus, PAR increased 3 or 4 times over the time, when the canopy stratum changed from yellow flowers to green siliques (Fig. 4). Very low radiation reached the bottom of canopy (PAR<20 μmol m^{-2} s^{-1}) and slightly but significant interaction between light × date factors were observed (Fig. 4).

In both field 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 5). Self-thinning ranged between 15 and 35% for experiment 3 and between 48 and 60% for experiment 4.

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

The quantity and quality of the light perceived after flowering affected reproductive parameters at harvest (Table 5). In experiment 3, the harvest index (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 on this trait. Nevertheless, aboveground biomass and grain yield did not differ among light treatments (Table 5). In contrast, in experiment 4, plants grown under neutral filters had lower grain yield with respect to untreated control plants (Table 5). Plants cultivated under Solatrol filters showed intermediate grain yield, without statistical differences with the control (Table 5).

Oil content in grains was significantly lower in plants cultivated under neutral and Solatrol filters with 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 with respect to the increase of protein percentage (Fig. 5). In contrast, a positive linear relationship was observed between oil content and grain yield which was statistically significant in experiment 4 but not in experiment 3 (Fig. 5). Furthermore, the variation of grain yield was higher in experiment 4 than in experiment 3.

Soluble carbohydrates concentration in vegetative stems was measured at flowering and harvest in experiment 4 (Table S3). A significant reduction of soluble carbohydrates was detected from vegetative stems among dates ($P < 0.0001$) suggesting an intense remobilization of reserves to the sinks (>90%). Although the light factor was not significant ($P = 0.20$), plants cultivated under high R/FR ratio showed a tendency to have a higher soluble carbohydrates concentration than those cultivated under lower R/FR ratios, suggesting that higher R/FR ratio reduces the remobilization of resources to the grains (compare Solatrol vs. neutral or without filters, Table S3).

Discussion

Plants are able to perceive quantity and quality light signals that provide information to adjust the canopy architecture (Casal 2013). Some of these signals included low irradiance and reduction in the R/FR ratio typical of dense stands (Erwin et al. 2006). Here, we showed that a modern spring rapeseed hybrid displays strong photomorphogenic responses to low R/FR ratio 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 field conditions. Interestingly, the increase of R/FR ratio after flowering might partially alleviate yield reduction under low irradiance. Taken together, these results suggest that photomorphogenic signals are integrated early during the vegetative growth and irradiance has stronger effects than R/FR signals at rapeseed crop level.

The main effects of low R/FR ratio in isolated rapeseed plants included the erect position of leaves at the early rosette stage (Table 2), an increased leaf and petiole length (Table 3) and the acceleration of leaf senescence when plants perceive low R/FR ratio since early stages

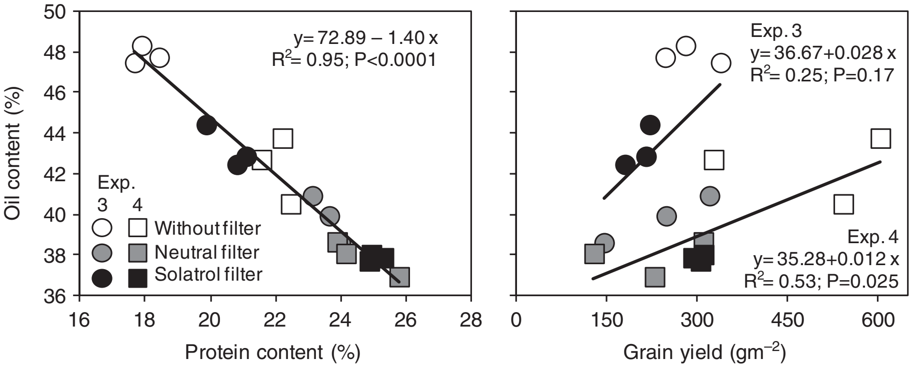

Fig. 5. Relationships between grain oil and protein content (left panel) and between oil content and grain yield (right panel) in rapeseed plants grown under different light treatments from flowering. Linear adjustments are shown in each graph.

of development (Fig. 1). The ability to reorient leaves to the light signals typical of dense canopies has also been 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 is cultivar specific and appears early in the ontogeny. Isolated maize plants grown in the field next to filters reflecting FR photons placed their leaves more perpendicular than control plants exposed to 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 to these results, the increase of R/FR ratio with red-light emitting diodes on basal leaves delayed senescence compared to non-irradiated controls (Rousseaux et al. 2000).

Shade signals also altered reproductive performance in isolated rapeseed plants grown with low R/FR ratio. When low R/FR ratio was perceived since the vegetative stage, rapeseed plants increased floral branching and remobilization of soluble carbohydrates from stem (see experiment 1, Fig. 3, Table 4). However, when plants were exposed to low R/FR ratio since flowering, these differences disappeared suggesting that shade signals are integrated early during plant development. Furthermore when low R/FR was done after flowering, rapeseed plants have shorter main floral shoots and consequently lower number of siliques, but not necessary less grain yield (See experiment 2, Fig. 2; Table 4). The increase of floral branching in isolated rapeseed plants exposed to low R/FR ratio was unexpected. Previous evidences showed 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 and at flowering reproductive leafless shoots appear downwards from the main floral shoot. This pattern of growth differs from tillering of cereals, and branching in soybean or tomato. Furthermore, the increase of branching might be compatible with a loss of apical dominance when plants grow under non-stress conditions like those established in the pot experiments. In agreement with the results of our work, the low R/FR ratio promoted floral branching under non-resource-limiting conditions in maize plants because apical bud dominance is broken activating the growth of axillary buds (Whipple et al. 2011).

In field conditions, irradiance was more relevant than light quality signals after flowering and determined yield components in the two modern spring rapeseed genotypes. Exposing rapeseed plants to low irradiance after flowering reduced grain yield, harvest index and grain oil content (Table 5; Fig. 5). These results indicate a great sensitivity of the reproductive output to the amount of solar irradiance captured by the canopy after flowering. It also agrees with recent results on shading effects in winter rapeseed (Brunel-Muguet et al. 2013). In this work, the authors demonstrate that a reduction of PAR applied at the early flowering stage delayed leaf senescence, optimizing light capture and avoiding biomass allocation to reproductive organs. It is noteworthy that plants exposed to high R/FR ratio apparently reduced the detrimental effects of low irradiance on rapeseed yield parameters (Table 5). At harvest, a higher concentration of soluble carbohydrates, though not statistically significant, was detected in vegetative stems of plants grown under high R/FR ratio (compare Solatrol vs. neutral filters in Table

S3). Furthermore, a tendency to increase the aboveground biomass at harvest was found for plants grown under Solatrol filter compared with those cultivated under neutral filter (Table 5). These results agree with the higher mobilization of soluble carbohydrates from vegetative stems documented for isolated plants grown under low R/FR ratio in the pot experiments (Fig. 3).

Shading conditions caused a reduction of the plant stand at field (Table 5). 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 has not been considered yet. In other species as maize, intra-specific competition generates early plant hierarchies with dominant and dominated 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.

We conclude that irradiance and quality of light have significant consequences on rapeseed plants performance under field conditions. First, even though low R/FR ratio has effects on vegetative, reproductive and yield traits, this light quality signal does not reduce seed yield or aboveground biomass. These results suggest that plants have the capacity to integrate other relevant environmental signals during their life cycle that affect yield parameters such as biomass and seed productivity. Second, the irradiance is a driving force more important than the R/FR ratio controlling plant productivity when plants are cultivated at crop level. In our experiments using two rapeseed hybrids, harvest index and grain yield increased significantly when plants were cultivated with high irradiance and strong variations between genotypes were found for both traits. In fact, genotype by environment interactions should be considered in future experiments to have a better comprehension on rapeseed productivity implications in different light environments.

Authors' contributions

D. P. R., M. P. V. and J. F. B. designed the experiments and wrote the paper. D. P. R. and M. P. V. performed field experiments and M. E. R., M. A. P. and J. F. B. performed pot experiments.

Acknowledgements – We thank Don Atilio and Advanta Seeds for kindly providing rapeseed seeds, Mirta Tinaro and Luis Pedace for technical assistance at field experiments, and Dr Daniel Miralles for useful comments to an early version of the manuscript. This work was supported by funds from University of Buenos Aires (UBACyT 2011-2014: 20020100100774) and the National Agency of Scientific and Technological Promotion from Argentina (PICT 2010-2489).

References

Ballaré CL, Scopel AL (1997) Phytochrome signalling in plant canopies: testing its population-level implications with photoreceptor mutants of *Arabidopsis*. Funct Ecol 11: 441–450

Ballaré CL, Sánchez RA, Scopel AL, Casal JJ, Ghersa CM (1987) Early detection of neighbour plants by phytochrome perception of spectral changes in reflected sunlight. Plant Cell Environ 10: 551–557

Brunel-Muguet S, Beauclair P, Bataille MP, Avice JC, Trouverie J, Etienne P, Ourry A (2013) Light restriction delays leaf senescence in winter oilseed rape (*Brassica napus* L.). J Plant Growth Regul 32: 506–518

Canola Council (2013). Crop establishment. Available at http://www.canolacouncil.org/crop-production/canola-grower's-manual-contents (accessed 10 July 2014)

Casal JJ (1988) Light quality effects on the appearance of tillers of different order in wheat (*Triticum aestivum*). Ann Appl Biol 112: 167–173

Casal JJ (1993) Novel effects of phytochrome status on reproductive shoot growth in *Triticum aestivum* L. New Phytol 123: 45–51

Casal JJ (2013) Canopy light signals and crop yield in sickness and in health. ISRN Agron 2013: 650439

Crocco CD, Holm M, Yanovsky MJ, Botto JF (2010) AtBBX21 and COP1 genetically interact in the regulation of shade avoidance. Plant J 64: 551–565

Devlin PF, Rood SB, Somers DE, Quail PH, Whitelam GC (1992) Photophysiology of the elongated internode (ein) mutant of *Brassica rapa*: ein mutant lacks a detectable phytochrome B-like polypeptide. Plant Physiol 100: 1442–1447

Djakovic-Petrovic T, Wit MD, Voesenek LACJ, Pierik R (2007) DELLA protein function in growth responses to canopy signals. Plant J 51: 117–126

Erwin JE, Rohwer C, Gesick E (2006) Red:far red and photosynthetically active radiation filtering by leaves differs with species. Acta Hortic 711: 195–199

Evers JB, Vos J, Andrieu B, Struik PC (2006) Cessation of tillering in spring wheat in relation to light interception and red: far-red ratio. Ann Bot 97: 649–658

Fortescue JA, Turner DW (2007) Changes in seed size and oil accumulation in *Brassica napus* L. by manipulating the source–sink ratio and excluding light from the developing siliques. Aust J Agric Res 58: 413–424

Green-Tracewicz E, Page ER, Swanton CJ (2011) Shade avoidance in soybean reduces branching and increases plant-to-plant variability in biomass and yield per plant. Weed Sci 59: 43–49

Habekotté B (1997a) Identification of strong and weak yield determining components of winter oilseed rape compared with winter wheat. Eur J Agron 7: 315–321

Habekotté B (1997b) Evaluation of seed yield determining factors of winter oilseed rape (*Brassica napus* L.) by means of crop growth modelling. Field Crops Res 54: 137–151

Iriarte LB, Valetti O (2008) Cultivo de colza. Editorial INTA, Buenos Aires (in Spanish)

Maddonni GA, Otegui ME (2006) Intra-specific competition in maize: contribution of extreme plant hierarchies to grain yield, grain yield components and kernel composition. Field Crops Res 97: 155–166

Maddonni GA, Otegui ME, Cirilo AG (2001) Plant population density, row spacing and hybrid effects on maize canopy architecture and light attenuation. Field Crops Res 71: 183–193

Maddonni GA, Otegui ME, Andrieu B, Chelle M, Casal JJ (2002) Maize leaves turn away from neighbors. Plant Physiol 130: 1181–1189

Mata DA, Botto JF (2009) Manipulation of light environment to produce high-quality poinsettia plants. HortScience 44: 702–706

Mata DA, Botto JF (2011) Photoperiod, light and temperature requirements for the control of plant architecture and flowering time in *Salvia exserta*. J Hortic Sci Biotech 86: 408–414

Nelson DW, Sommer LE (1973) Determination of total nitrogen in plant material. Agron J 65: 109–112

Pagano E, Maddonni GA (2007) Intra-specific competition in maize: early established hierarchies differ in plant growth and biomass partitioning to the ear around silking. Field Crops Res 101: 306–320

Robson PRH, Whitelam GC, Smith H (1993) Selected components of the shade-avoidance syndrome are displayed in a normal manner in mutants of *Arabidopsis thaliana* and *Brassica rapa* deficient in phytochrome B. Plant Physiol 102: 1179–1184

Rondanini DP, Gomez NV, Agosti MB, Miralles DJ (2012) Global trends of rapeseed grain yield stability and rapeseed-to-wheat yield ratio in the last four decades. Eur J Agron 37: 56–65

Rondanini DP, Vilariño MP, Gomez NV, Miralles DJ (2013) Contenido y composición del aceite de colza (*Brassica napus* L.) en respuesta a la temperatura y radiación post-floración. Proceedings of II Workshop Internacional Red Raíces Ecofisiología, 26–27 Agosto 2013, Mar del Plata, Argentina.

Rousseaux MC, Hall AJ, Sánchez RA (1996) Far-red enrichment and photosynthetically active radiation level influence leaf senescence in field-grown sunflower. Physiol Plant 96: 217–224

Rousseaux MC, Hall AJ, Sánchez RA (2000) Basal leaf senescence in a sunflower (*Helianthus annuus*) canopy: responses to increased R/FR ratio. Physiol Plant 110: 477–482

Scott TA, Melvin EH (1953) Determination of Dextran with Anthrone. Anal Chem 25: 1656–1661

Skinner RH, Simmons SR (1993) Modulation of leaf elongation, tiller appearance and tiller senescence in spring barley by far-red light. Plant Cell Environ 16: 555–562

Ugarte CC, Trupkin SA, Ghiglione H, Slafer G, Casal JJ (2010) Low red/far-red ratios delay spike and stem growth in wheat. J Exp Bot 61: 3151–3162

Velasco L, Fernández-Martínez JM (2002) Breeding oilseed crops for improved oil quality. In: Basra AS, Randhawa LS (eds) Quality Improvement in Field Crops. Food Products Press, The Haworth Press, Binghamton, NY, pp 309–344

Whipple CJ, Kebrom TH, Weber AL (2011) Grassy tillers1 promotes apical dominance in maize and responds to shade signals in the grasses. Proc Natl Acad Sci USA 108: E506–E512

Supporting Information

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

Table S1. Rapeseed genotypes and sowing and flowering dates of pot and field experiments.

Table S2. Overview of traits measured in pot and field experiments.

Table S3. 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 experiment 4.

Fig. S1. Overview of pot and field experiments modifying R/FR ratios and irradiance prior or after flowering in two spring rapeseed cultivars.

Fig. S2. Photographs from field experiment (experiment 4) at 5 and 25 days after flowering (DAF) indicating the three zones of light measurements, at top, middle and bottom canopy.

Edited by T. Vogelmann