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Inflorescence stems of *Arabidopsis thaliana* bend away from neighbours through a response controlled by phytochrome B

Escape al sombreado de inflorescencias de Arabidopsis thaliana *inducido por fitocromo B*

Alejandro Serrano[1, 2, 3]*, **M. Belén Maldonado**[2, 3], **and Hernán Boccalandro**[1]†
[1] Instituto de Biología Agrícola de Mendoza (IBAM)
Facultad de Ciencias Agrarias, Universidad Nacional de Cuyo
Alte Brown 500, 5505 Chacras de Coria, Mendoza, Argentina.
Consejo Nacional de Investigaciones Científicas y Técnicas (CCT-Mendoza CONICET)
Av. Ruiz Leal s/n, Parque Gral. San Martín, Casilla de Correo 507, 5500 Mendoza, Argentina.
[2] Consejo Nacional de Investigaciones Científicas y Técnicas (CCT-Mendoza)
Instituto Argentino de Zonas Áridas (IADIZA). Av. Ruiz Leal s/n Parque Gral. San Martín
Casilla de Correo 507. CP:5500, Mendoza, Argentina.
[3] PROBIOL UNCuyo, Mendoza, Argentina
*<aserrano@mendoza-conicet.gov.ar>
†Deceased on 10th December, 2011

ABSTRACT

Plants are able to detect the presence of neighbours by sensing the variation in the proportion of red and far red light (around 660 nm and 740 nm, respectively) in their surrounding ambient. The phytochromes are the main photoreceptors involved in neighbour detection, and regulate several physiological processes that modulate plant shape and architecture, enabling plants to compete for light by growing over the surrounding vegetation. The state of knowledge about this kind of responses in flowers and inflorescences is scarce compared to what is known in vegetative organs. In this context, we aimed to evaluate growth and bending responses of inflorescence stems induced by a unilateral signal of neighbours in *Arabidopsis thaliana* (L.). We carried out experiments employing natural canopies and artificial far red light sources inside a greenhouse where UV-Blue region of the sunlight spectrum was excluded. We report for the first time a lateral escape in terms of a bending response of the stem against the signal of neighbours, which is promoted by phytochrome B. Our results combined with pre-existing data may contribute to better understand the behaviour of flowering plants growing in patches, providing a new perspective of shade avoidance responses in terms of its effect in reproductive biology.

RESUMEN

Las plantas poseen la capacidad de detectar la presencia de otras plantas vecinas mediante la percepción de variaciones en la proporción de luz roja (660 nm) y roja lejana (740 nm) en el ambiente *lumínico circundante. Los fitocromos (phy) son los fotorreceptores que cumplen la función principal en la detección de plantas vecinas y su acción implica la regulación de varios procesos fisiológicos que modulan la morfología y arquitectura de la planta, derivando en respuestas de competencia por la luz como la promoción del crecimiento y el desarrollo de sus estructuras fotosintéticas por sobre la vegetación circundante. La mayor parte del conocimiento actual sobre las respuestas de escape al sombreado ha sido generada en estructuras vegetativas, mientras que los estudios sobre este tema en flores e inflorescencias son comparativamente escasos. En este contexto, planteamos como objetivo de nuestro estudio evaluar respuestas de crecimiento y de curvatura de las inflorescencias de la planta* Arabidopsis thaliana *(L.) inducidas por la señal unilateral de plantas vecinas. Para ello, llevamos a cabo experimentos utilizando cortinas de vegetación natural y fuentes de luz roja lejana artificial dentro de un invernáculo con filtros especiales para excluir la luz correspondiente a la región UV-azul del espectro lumínico natural. Reportamos por primera vez la existencia de un escape lateral manifestado como la curvatura de la inflorescencia hacia el lado opuesto a la señal de plantas vecinas. Esta respuesta es controlada principalmente por el fitocromo B (*phyB*). Nuestros resultados, en combinación con datos preexistentes son útiles para adquirir una mejor comprensión del comportamiento de las flores e inflorescencias de plantas que crecen en parches, proporcionando una nueva perspectiva de las respuestas de escape al sombreado que pueden tener una influencia importante sobre la biología reproductiva.*

Key words: shade avoidance, inflorescence, far red, phytochromes

Palabras clave: *escape al sombreado, inflorescencia, rojo lejano, fitocromo*

Abbreviations: Ultraviolet Radiation B = UV-B; Ultraviolet B Resistance Locus 8 = UVR8; Ultraviolet Radiation A = UV-A; Phototropin 1 = PHOT1; Phototropin 2 = PHOT2; Cryptochrome 1 = CRY1; Cryptochrome 2 = CRY2; ZEITLUPE = ZTL, FLAVIN-BINDING KELCH REPEAT F-BOX = FKF1; LOV KELCH PROTEIN2 = LKP2; Phytochrome A = PHYA; Phytochrome B = *PHYB*; Phytochrome C = PHYC; Phytochrome D = PHYD; Phytochrome E = PHYE; Wild Type = WT. Mutant plants are indicated by the name of the mutated allele *phyB*.

INTRODUCTION

Plants have the ability to detect and respond to light of different wavelengths through a complex network of photoperceptive systems (Galvão & Fankhauser, 2015).

While blue and red light is highly absorbed by leaves, far red is reflected and transmitted through green tissues. Therefore, the section of the light spectrum corresponding to red and far red light provide important information to plants, especially about the presence or absence of neighbours, since the perception of light conditions characterized by a low red:far red ratio (R:FR) indicates the proximity of a dense shaded area, as opposed to an open landscape, which is characterized by high R:FR light conditions (Taylorson & Borthwick, 1969; Holmes & Smith, 1977).

The detection of the variation in R:FR is important particularly in shade-intolerant plants. Therefore, a series of developmental responses is triggered by the plants as soon as they perceive the presence of neighbours anticipating the shading and competing for light, which leads to an improvement of photosynthetical efficiency. This series of responses has received the name of Shade Avoidance Syndrome (SAS) and includes processes as petiole elongation (Hisamatsu et al., 2005; Casal, 2012) and enhanced growth of stems and internodes (Ballaré et al., 1991; Devlin et al., 1998), both responses that typically contribute to escape from neighbours shade in terms of height. Few cases of lateral escaping (or negative phototropism) have been reported in response to far red light. Such are the cases of cucumber seedlings, which bend against a far red-reflecting canopy (Ballaré et al., 1992) and leaves of some maize cultivars, which also escape from the low R:FR signal of surrounding plants (Maddonni et al., 2002). In addition, spatial distribution of *A. thaliana* rosette leaves was also found to be affected by the presence of neighbours (Crepy & Casal, 2014).

Most of the studies about these growth responses leading plants to escape away (or in height) from neighbours are focused in vegetative organs. Conversely, shade avoidance responses in flowers or inflorescences are usually understood only in temporal or developmental terms but not in spatial terms. For example, low R:FR ratio light conditions promote early flowering in *A. thaliana* (Halliday et al., 1994; Pigliucci & Schmitt, 1999), a strategy typically associated with stress avoidance (Stanton et al., 2000). The signal of neighbouring plants also affects floral display in *A. thaliana* by reducing lateral buds' development (Holalu & Finlayson, 2017). In addition, the development of taller inflorescence stems was described by Finlayson et al. (2010). Although it is possible that such a response might have a positive effect in pollination of plants growing in dense populated patches, it also has been reported that the promotion of stem growth by low R:FR conditions reduces grain yield in crops (Liebenson et al., 2002). Therefore it is suggested that there is a trade-off between resources allocated to the promotion of stem growth and those destined to yields, especially in densely-populated agricultural scenarios (Kebrom & Brutnell, 2007).

Plant responses to the variations in light ambient are controlled by a photoperceptive system, consisting on a complex network of photoreceptors. In the case of the model plant *Arabidopsis thaliana* (L.), five families of photoreceptors are known to date: UV-B radiation (315 nm) is perceived by the protein UVR8, UV-A and Blue light (320-500 nm) is signalized by three families of proteins: the phototropin family (PHOT1 and PHOT2), the cryptochrome family (CRY1 and CRY2) and the zeitlupe family (ZTL, FKF1 and LKP2). Finally, the phytochrome family, comprising five members (PHYA to PHYE) mediates responses to red and far red light (660 nm and 740 nm, respectively). The responses to the shade of surrounding vegetation are mainly controlled by the phytochrome family of photoreceptors. Phytochromes alternate into an active (Pfr) and an inactive (Pr) state when exposed to red or far red light respectively. Upon red light exposure, phytochrome B in its Pr form undergo a reaction of photoconversion to its Pfr form and translocate into the nucleus (Whitelam et al., 1998; Staiger, 2008), initiating a signaling pathway that ends in hypocotyl, petiole and stem growth inhibition (Ruberti et al., 2012). Conversely, after far red irradiation, the inverse reaction takes place, meaning that the Pfr form of *phyB* changes to Pr and emigrates from the nucleus to the cytosol; as a consequence, growth takes place. Mutant plants lacking one or more functional phytochromes show constitutive shade avoidance responses, e.g. they grow taller and have elongated petioles even in open landscapes (Whitelam et al., 1998).

In this article, the escape from a unidirectional signal of natural and simulated canopies is evaluated in inflorescence stems of *A. thaliana*. We hypothesized that inflorescences exposed to the unilateral signal of neighbours evoke an escape in height as well as a directional escape, which are controlled by *phyB*. The results we expect to find given that this hypothesis is correct are that plants grown beside natural and simulated canopies, compared to isolated plants, will exhibit: i) taller stems, ii) a bending response of the stem away from neighbours or iii) an asymmetric distribution of lateral branches (i.e. developed predominantly on the open side of the stem). In addition, if these effects are controlled by *phyB* we predict that these responses will be absent in *phyB* mutants lacking functional *phyB* protein.

MATERIALS AND METHODS

In order to assess the above mentioned hypotheses we conducted two types of experiments, by which we attempted to determine; i) the effect of a natural canopy on inflorescence architecture and orientation ("natural canopy experiment", see details below), and ii) if a light ambient enriched in the far red portion of the spectrum is the main stimulus that promotes the responses of plants to their neighbours ("artificial far red light experiment", see details below). In both cases, the behaviour of mutant plants lacking functional phytochrome B (*phyB*) was compared with wild type (WT) plants in order to evaluate the role of phytochrome B in the responses.

For both kinds of experiments the growth conditions of the plant material were as follows. Seeds of *A. thaliana* plants were sown on 0.8% agar, stratified for 3-5 days at 4 °C in darkness and then exposed to white light to induce germination. Then, seedlings were transferred to 180 cm^3 pots filled with two parts of perlite (Bio-Organic S.R.L, Mendoza, Argentine), two parts of peat moss *Sphagnum* (Bio-Organic S.R.L, Mendoza, Argentine) and one part of sand (Casa Forconi, Mendoza, Argentine), and watered each day with a solution containing 0.7 g/L of Hakaphos Red (COMPO, Spain).

Plants grew until flowering stage inside growth chambers under 12 hours light/ 12 hours dark at 200 $\mu mol \times m^{-2} \times s^{-1}$ of white light at 25 °C. Once the inflorescence primordia were observable, the plants were transferred to a greenhouse and placed inside ventilated transparent closures of 1x3x1.5 meters covered by a UV-blue light-excluding filter (LEE Filters N° 767-Oklahoma Yellow) to avoid a potential phototropic effect of blue and ultraviolet light. Ventilation was achieved by leaving the lower part of the closures opened to enable renewed air income and by installing a series of coolers on the south upper side to extract warm air.

For the natural canopy experiment, a 50 cm tall column of grass (Raygrass) was placed 5 cm to the south side of 8 WT and 8 *phyB* plants. An equivalent group of 8 WT and *phyB* isolated plants (i.e. plants inside the same type of closures but without surrounding grass) was used as a control (n=8). After one week, the height and the deviation angle of the inflorescence stem against the canopy was measured with a caliper and a clinometer respectively (**Figure 1**). Additionally, we registered the number and the compass orientation of branches emerged from the inflorescence stem.

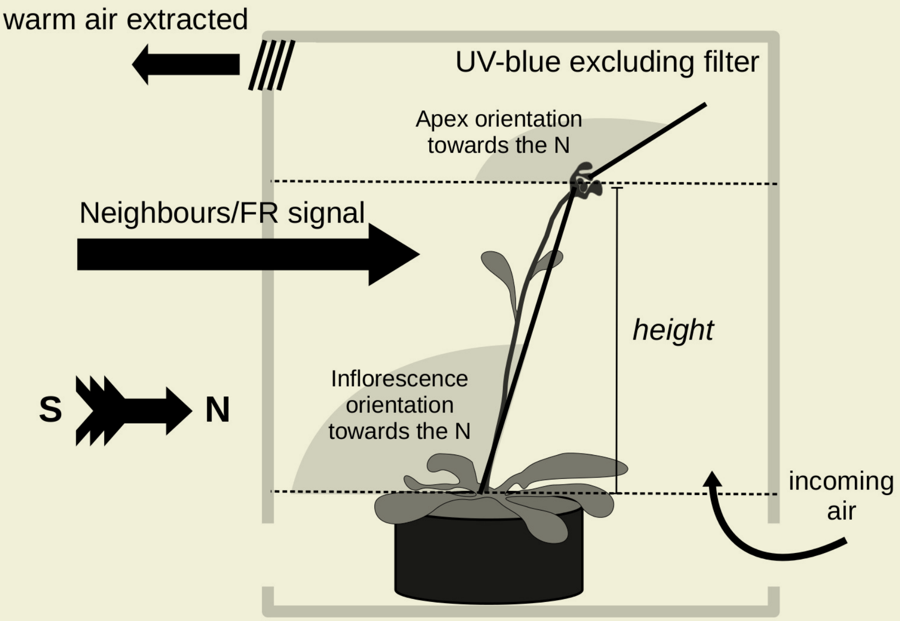

Figure 1. Diagram of the experimental set up and the variables measured, showing an *Arabidopsis thaliana* plant viewed from the side, the orientation of the apex and the whole inflorescence towards the north (i.e. deviation angle from the neighbours or the far red signal, indicated by the gray semicircles) and the height, measured from the rosette base to the apex of the inflorescence

Figura 1. Diagrama de las condiciones experimentales y las variables medidas, mostrando una planta de Arabidopsis thaliana *vista de perfil, la orientación del ápice y del escapo floral completo hacia el norte (i.e. ángulo de desviación en contra de las vecinas o la señal de rojo lejano indicados mediante semicírculos grises) y la altura, medida desde la base de la roseta hasta el ápice de la inflorescencia*

Expected results indicative of a putative shade avoidance response would be:

- Taller inflorescences or higher angles exhibited by plants placed next to the canopy compared with control plants.
- Higher proportion of branches developed on the northern (open) side of the stem than in the southern (canopy) side in plants exposed to the canopy, compared with control plants.

Expected results indicative of a predominant role of *phyB* in the response would be:

- Random inflorescence orientation (angle values around 90°) in *phyB* mutants regardless of the presence or absence of neighbours, compared with a northern orientation (angle values significantly higher than 90°) in WT plants exposed to the unilateral presence of neighbours.

For the artificial far red light experiment, a far red light source (peak wavelength = 740 nm, irradiance = 25 $\mu mol \times m^{-2} \times s^{-1}$) was placed 60 cm to the south side of 14 WT and 14 *phyB* plants, with a running column of water between the plants and the source to dissipate heat. The same setting was used as a control with the lights turned off (n=14 replicates for each genotype). After one week, the height and deviation angle of the inflorescence stem against the far red source, as well as the number and compass side of emergence of lateral branches was measured in the same way as for the canopy experiments.

Expected results indicative of a putative shade avoidance response would be:

- Taller inflorescences or higher angles exhibited by plants exposed to unilateral far red light compared with control plants.
- Higher proportion of branches developed on the northern (open) side of the stem than in the southern (far red-illuminated) side in plants exposed to unilateral far red light, compared with control plants.

Expected results indicative of a predominant role of *phyB* in the response would be:

- Random inflorescence orientation (angle values around 90°) in *phyB* mutants in both control and far red light treatments, compared with a northern orientation (angle values significantly higher than 90°) in WT plants exposed to unilateral far red light.

Light conditions were measured using a UV-VIS spectrorradiometer (Ocean Optics USB4000) and processed with Spectra Suite software. The light gradients achieved for each treatment are shown in **Figure 2**.

All the experiments were conducted in the IBAM-CONICET institute, Faculty of Agronomical Sciences of the National University of Cuyo (33°00'S, 68°52'W, Mendoza, Argentine) in April 2013. At this latitude and time of the year, the sun shines predominantly from the north.

Statistical analysis

Non parametric analysis was performed since data variability was not homogeneously distributed among treatments, particularly the data corresponding to angles. This was absolutely expected due to the nature of the questions addressed, since a positive effect of canopy or far red light might be expressed as a deviation from the randomness that is likely to be observed in control plants. An unpaired two-samples Mann-Whitney test with a unilateral alternative hypothesis was carried out to test the difference in height. To test for differences in inflorescence orientation between experimental conditions and genotypes we analysed the data through one-sample Wilcoxon tests with a unilateral alternative hypothesis (the angle is significantly greater than 90°) for each sample. To compare between proportions of branches developed at different sides of the stems we used a Chi-squared analysis for two-samples. Significance level was set to $\alpha=0.05$. All the statistical tests were carried out using R software, version 3.4.0 (R Development Core Team, 2017).

RESULTS

Escape in height

Unexpectedly, no difference in inflorescence height was appreciable between control plants and those placed next to the grass column after one week of ex-

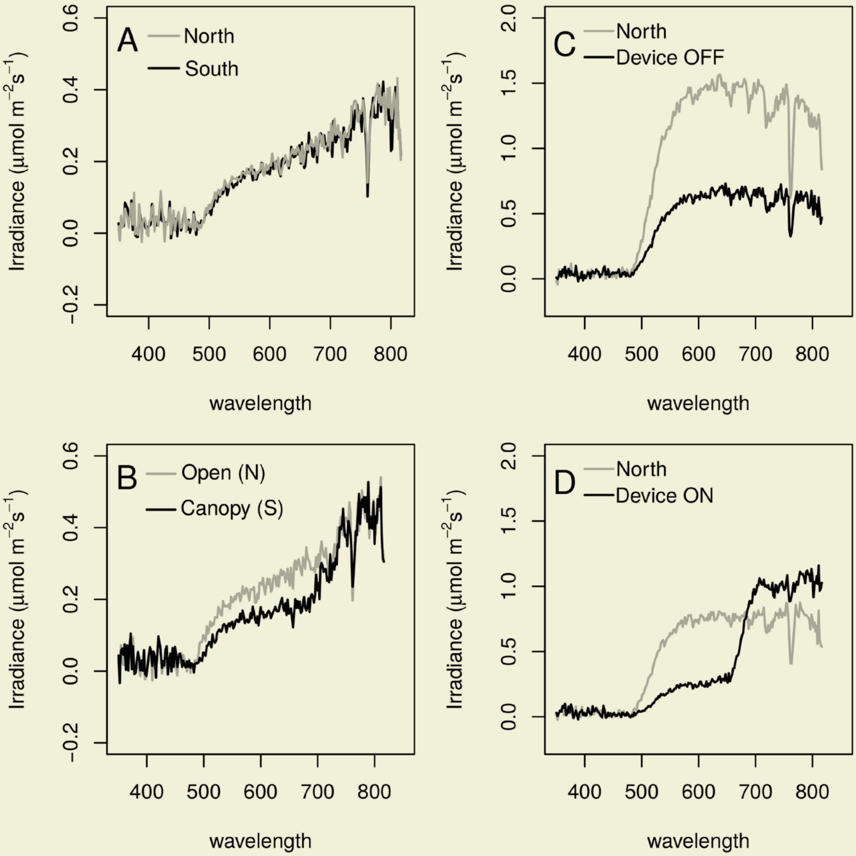

Figure 2. Light gradients achieved through the use of natural and artificial approaches to manipulate the R:FR ratio in natural canopy experiments (A and B) and artificial far red experiments (C and D). A. Light irradiance throughout the UV-visible spectrum reaching the isolated plants from the north and the south. B. Light irradiance reflected from the canopy placed at the south of *Arabidopsis* plants and coming from the open side at the north. C. Light irradiance reaching the plants from the open side at the north and from the south, thus shaded by the structure of the light device turned OFF used as control. D. Light irradiance reaching the plants from the far red light source placed at the south and the opossite open side, at the north. Irradiance units are µmol of photons per square meter per second

Figura 2. Gradientes de luz generados bajo las diferentes condiciones experimentales donde se manipuló la relación R:RL. A. Irradiancia de luz en el rango UV-visible alcanzada a nivel de la inflorescencia en las plantas aisladas desde el norte y desde el sur. B. Irradiancia de luz reflejada desde el canopeo ubicado al sur de plantas de Arabidopsis*, y proveniente del lado abierto al norte. C. Irradiancia de luz alcanzada a nivel de las inflorescencias proveniente del lado abierto (norte) y desde el sur, donde se ubicó el banco de luz desconectado utilizado como control. D. Irradiancia de luz alcanzada a nivel de las inflorescencias proveniente de la fuente de luz rojo lejana ubicada al sur de las plantas y del lado abierto opuesto (norte). Las unidades de irradiancia están en µmol de fotones por metro cuadrado por segundo*

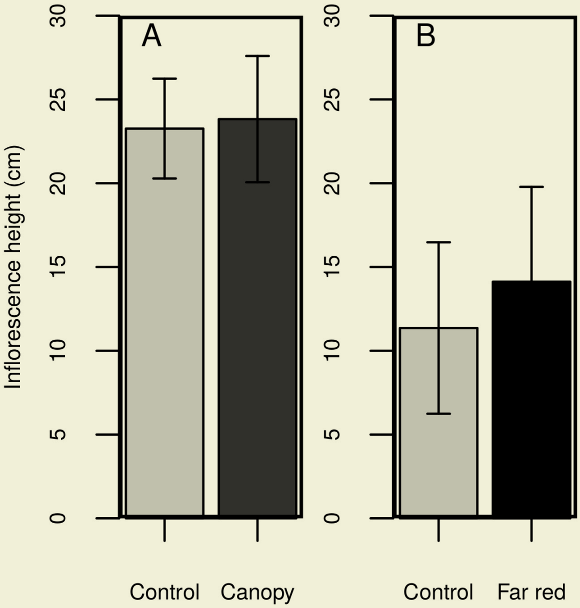

Figure 3. Mean height of inflorescence stems of *Arabidopsis* plants exposed to unilateral low R:FR conditions generated by the presence of a natural canopy (A) or an artificial far red light source (B) compared to their respective control treatments. Bars indicate standard error of 8 and 14 replicates (A and B respectively). Mann-Whitney test showed no significant differences between low R:FR treatments and the corresponding control

Figura 3. Altura media del tallo de la inflorescencia de plantas de Arabidopsis *expuestas a condiciones de baja relación R:RL generadas por la presencia de un canopeo natural (A) o una fuente de luz roja lejana artificial (B) comparada con sus tratamientos control respectivos. Las barras indican error estándar de 8 y 14 réplicas (A y B respectivamente). La prueba de Mann-Whitney muestra que no hay diferencias significativas entre los tratamientos de baja relación R:RL y el control correspondiente*

posure to the experimental conditions (**Figure 3A**). Similarly, there was no detectable increase in inflorescence height in far red-irradiated individuals compared to control plants (**Figure 3B**).

Escape in direction

In the WT, a higher inclination of the stem against the canopy (values significantly higher than 90°) was observed in plants subjected to the unilateral presence of neighbours, compared to control ones, which exhibited a random orientation around the vertical axis (values not different from 90°) (**Figure 4A**). This response was absent in the *phyB* mutants (i.e. random orientation around vertical axis regardless of the presence or absence of neighbours) (**Figure 4B**). Likewise, a bending reaction was also visible in plants irradiated unilaterally with artificial far red lamps and not in plants shad-

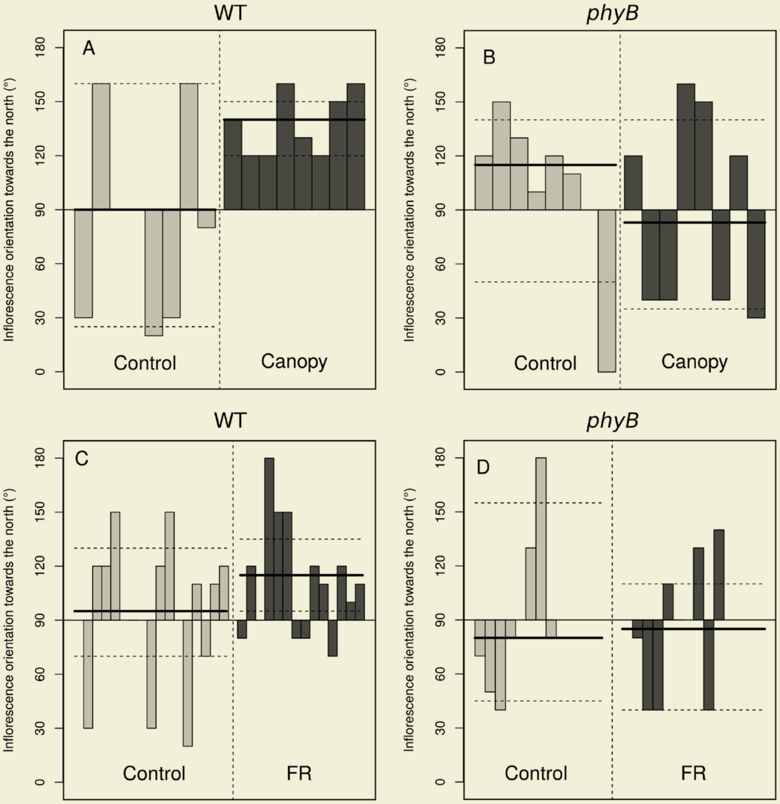

Figure 4. Inflorescence orientation of WT and *phyB* mutant plants placed at the north of a grass column (Canopy) or in an isolated site (Control) (A and B). Inflorescence orientation of WT and *phyB* mutant plants placed at the north of the far red light source turned on (FR) or off (Control) (C and D). In *y* axis, 90° represents a vertically oriented stem, while 180° and 0° are north- and south-oriented stems respectively. Each bar represent the orientation of one individual to show the data distribution (absent bars are plants which orientation was exactly 90°). Broad black horizontal lines are the median, and dashed lines are th 95% confidence interval (c.i.). A 95% c.i. excluding the value 90° is indicative of a putative lateral shade avoidance response, according to one-sample Wilcoxon tests

Figura 4. Orientación de inflorescencias de plantas WT y mutantes phyB *ubicadas al norte de una columna de pasto (Canopeo) o en un sitio abierto (Control) (A y B). Orientación de inflorescencias de plantas WT y mutantes* phyB *ubicadas al norte de la fuente de luz roja lejana encendida (FR) o apagada (Control) (C y D). En el eje y el valor de 90° representa una inflorescencia en posición vertical, mientras que 180° y 0° corresponden a inflorescencias orientadas hacia el norte y hacia el sur respectivamente. Cada barra indica la orientación de un individuo, para mostrar la distribución de los datos (barras ausentes corresponden a plantas con una orientación de exactamente 90°). La línea negra horizontal indica la mediana y las líneas punteadas, el intervalo de confianza (i.c.) del 95%. Un i.c. 95% que excluye al valor de 90° indica que verdaderamente existe una respuesta de escape al sombreado lateral, de acuerdo a pruebas de Wilcoxon para una muestra* ed by the turned off device used as a control, conspicuously mimicking the effect generated by the canopy (**Figure 4C**). As in the previous case, *phyB* mutants showed no directional movement against the far red light source (**Figure 4D**), revealing that the inability to perceive the light gradient in this particular region of the spectrum abolishes the unidirectional bending response.

Similarly to what was found in stem, a bending response was also observed at the apical level in WT plants subjected to the unilateral presence of neighbours and those exposed to unilateral far red light and it was completely absent in control plants and in *phyB* mutants (**Figure 5**).

Lateral branches

In addition, we evaluated whether lateral branches were asymmetrically distributed in plants exposed to the unilateral signal of neighbours but we did not found significant differences in this variable between light conditions (**Figure 6**).

Discussion

In this study we show that some of the responses triggered by a low R:FR ratio directed to avoid the actual or potential shade of surrounding vegetation (usually linked to photosynthetic advantages in vegetative organs) are also evoked by non-photosynthetic structures like inflorescences.

We found no differences in height between plants beside neighbours or the far red source and isolated plants, which contradicts one of the most widespread shade avoidance response evoked by plants, which is the enhancement of growth throughout successive developmental stages, resulting in taller individuals (Casal, 2012). This result particularly disagrees with a previous study, which showed that *A. thaliana* plants grown under low R:FR conditions throughout the entire life cycle developed taller inflorescences than plants grown under high R:FR conditions (Finlayson et al., 2010). The discrepancies between these findings and our results can be explained because in our experiments the far red or neighbour stimulus was applied after flowering was triggered, and not from seedling stage. We chose not to apply the far red or neighbour signal before flowering initiation because plants flower earlier under shade conditions (Halliday et al., 1994), and it would cause far red-irradiated stems to start growing earlier than control ones. In addition, our experimental conditions had two more differences with those used by Finlayson et al., (2010): i) our experiments were carried out inside a greenhouse rather than in chamber conditions, and ii) we applied the low R:FR signal from a side instead of applying it from above. Both differences implied, in the present study, a greater ambient irradiation, which could cause an overall repression of stem elongation, minimizing the effect of shade in inflorescence height.

In addition to inflorescence height, low R:FR conditions applied from above repressed branching and lateral bud development in *A. thaliana* (Finlayson et al., 2010; Holalu & Finlayson, 2017). Therefore it was tempting to hypothesize that such a repression of buds development could also happen asymmetrically in plants exposed to a unilateral stimulus. However, in our study, unilateral exposure to low R:FR conditions did not caused any differential development of

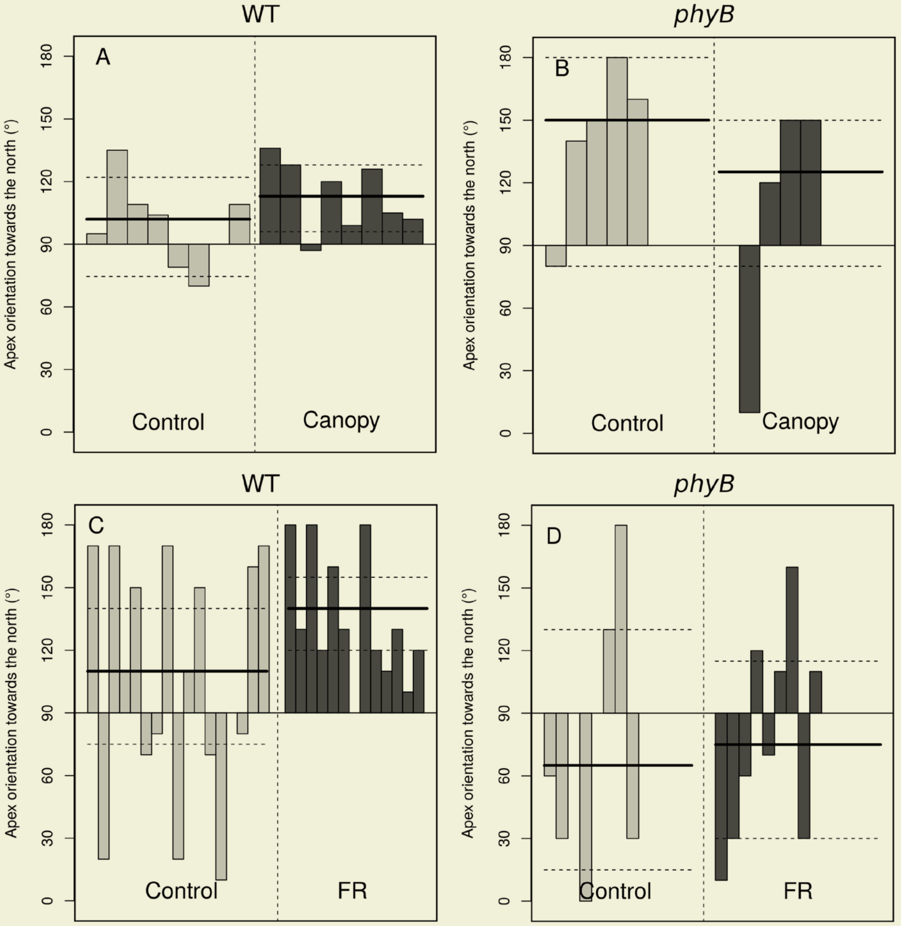

Figure 5. Apex orientation of WT and *phyB* mutant plants placed at the north of a grass column (Canopy) or in an isolated site (Control) (A and B). Apex orientation of WT and *phyB* mutant plants placed at the north of the far red light source turned on (FR) or off (Control) (C and D). All graphical parameters are the same as in **Figure 4**
Figura 5. Orientación del ápice de inflorescencias de plantas WT y mutantes phyB *ubicadas al norte de una columna de pasto (Canopeo) o en un sitio abierto (Control) (A y B). Orientación del ápice de inflorescencias de plantas WT y mutantes* phyB *ubicadas al norte de la fuente de luz roja lejana encendida (FR) o apagada (Control) (C y D). Todos los parámetros gráficos son los mismos que en la Figura 4*

branches or buds between opposite sides of the inflorescence (**Figure 6**). Conversely, our results show that the main effect of the unilateral presence of neighbours is an inclination of the whole inflorescence stem as well as the inflorescence apex against the stimulus and controlled by phytochrome B (**Figures 4** and **5**). It is

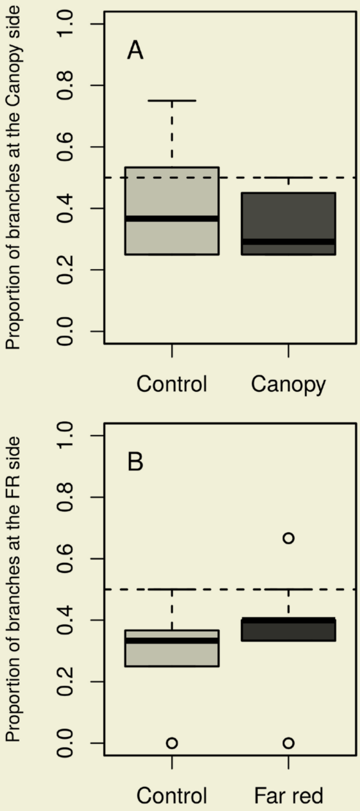

Figure 6. Lateral distribution of branches developed at both sides of the inflorescence stem of WT plants. A. Proportion of branches developed at the side facing to the canopy in WT plants, and at the equivalent side in isolated plants used as control. B. Proportion of branches developed at the side facing the far red light source and the equivalent side, facing the turned-OFF light source. For each box the broad line in the middle represents the median, upper and lower box limits are the first and third quantiles of the data distribution respectively, the error bars indicate the observations within 1.5 times the distance between the median and the quantiles and the circles represent extreme cases. A Chi-squared test showed no statistical differences between treatments

Figura 6. Distribución lateral de ramas desarrolladas a ambos lados del tallo de la inflorescencia de plantas WT. A. Proporción de ramas desarrolladas en el lado enfrentado al canopeo y en el lado equivalente (sur) en las plantas aisladas usadas como control. B. Proporción de ramas desarrolladas del lado enfrentado a la fuente de rojo lejano y el lado equivalente (sur), frente al banco de luces desconectado. Para cada caja la línea gruesa en el medio representa la mediana, las líneas de los límites superior e inferior corresponden al primer y tercer cuartil de la distribución de los datos respectivamente, las barras de error indican las observaciones dentro de 1.5 veces la distancia entre la mediana y los cuartiles; y los círculos representan casos extremos. Una prueba de Chi cuadrado mostró que no hay diferencias estadísticas entre tratamientos possible that a lateral escape at the apical level (like that shown in **Figure 5**), might have an important effect on flower display and visibility, and consequently on pollination, even though *A. thaliana* is a self-compatible species, because there are strong evidences that demonstrate that *A. thaliana* flowers existing in wild populations are visited by a great variety of insects, causing high cross pollination rates (Hoffmann et al., 2003). In turn, cross pollination is well known to rise seed production even in self-compatible species (Groom, 1998; Ghazoul, 2005). Therefore, it would be interesting to carry on future experiments in the presence of pollinators to test this hypothesis and assess if it could represent an adaptive advantage for *A. thaliana*.

In addition, the lateral escape observed at the whole inflorescence level might be relevant for reproductive success because it is reasonable to suppose that plants evoking this response may leave their seeds far from the site where they have detected the presence of neighbours, thus favouring post-dispersal processes such as germination, establishment and growth of the progeny in an open ambient, and reducing the potential competition with neighbouring plants at seedling stage. Seeds that perceive a high R:FR ratio (i.e. those present in an open ambient), have a high proportion of phytochromes in their active Pfr state and this condition stimulates germination (Vázquez Yañez & Smith, 1982; Franklin & Quail, 2009).

The aforementioned variability in the responses of inflorescences to a uniform (Finlayson et al., 2010) or a unilateral reduction of R:FR light, provide a complementary view of the floral distribution of *A. thaliana* plants growing at the centre of a patch or at the edge, respectively. For example, in the case of a plant growing in the centre of a dense population it is expected that it will allocate more resources to overgrow its neighbours, placing its flowers in a higher position where they have more probabilities to be visited by pollinators (Hainsworth et al., 1984, Larson & Larson, 1990; Peakall & Handel, 1993; Lortie & Aarssen, 1999). However, this resource allocation might be detrimental for seed production, like it happens in dense sunflower fields (Liebenson et al., 2002). Because of this, it is reasonable that plants that are at the edge of a patch, and less shaded, evolve different and less expensive strategies, like the bending responses shown in this study.

Our study presents, however, some unexpected results. For example, since the low R:FR conditions were deeper in the artificial light set up than in the natural canopy treatment, we would have expected the former condition to induce a stronger response than the latter. Contrarily, the far red artificial light source produced a weaker effect in inflorescence orientation. This might be due to the fact that plants do not only rely on light cues to detect and interact with neighbours, but also on chemical signals such as the release of volatile organic compounds, which are known to drive plant to plant communication (Pichersky & Gershenzon, 2002). This could provide an additional signal, absent in artificial conditions that might enhance the avoidance response.

CONCLUSIONS

In conclusion, *A. thaliana* inflorescences growing beside neighbouring plants (like those growing at the edge of a dense population) are able to detect a gradient of R:FR light established by the shade of neighbours. Consequently, they undergo a change in stem orientation, rather than an escape in height, which is triggered by phytochrome B. In a population of flowering plants, the variation in R:FR light may play an essential role in the achievement of an optimal arrange of flowers and inflorescence stems, which probably have an important impact in pollination biology and reproductive success.

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