

Article

Morphological Response of Soybean (*Glycine max* (L.) Merr.) Cultivars to Light Intensity and Red to Far-Red Ratio

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Abstract: In soybean production, the shade avoidance response can affect yield negatively in both mono- and inter-cropping systems due to increased heterogeneity of the crop and lodging. This is mainly regulated by photoreceptors responding to the ratio between red and far-red light (R:FR) and photosynthetic photon flux density (PPFD). In this study, three soybean cultivars were grown under different R:FR and PPFD in a light emitting diode (LED) climate chamber to disentangle the effect of each on morphology and dry matter. Results showed that plant organs were influenced differently and indicated an interaction with the increase in assimilates at high PPFD. Internode elongation was mainly influenced by low PPFD with an additive effect from low R:FR, whereas petiole elongation responded strongly under low R:FR. Hence, petiole elongation can be seen as the main response to the threat of shade (high PPFD and low R:FR) and both petiole and internode elongation as a response to true shade (low PPFD and low R:FR). Interactions between cultivar and light treatment were found for internode length and diameter and leaf mass ratio, which may be unique properties for specific cropping systems.

Keywords: photomorphogenesis; shade avoidance response; far-red radiation; photosynthetic photon flux density; red to far-red ratio; LED lighting

1. Introduction

Plants competing for light recognize neighboring vegetation through changes in the light quality, which leads to shade avoidance responses (SARs), with the aim to increase light interception and consequently competitiveness. Typical SARs of plant organs are thinner leaves, increased lamina:petiole length and elongation of hypocotyl, internode, petiole, and leaves [1,2]. The change in light quality is a result of different absorption and reflection of different wavelengths in leaves particularly due to the low absorption and high reflection and transmittance of far-red light (FR) (700–800 nm) compared to other wavelengths. A reduction in red to far-red ratio (R:FR) while photosynthetic photon flux density (PPFD) is still high, appears by the threat of future shade from surrounding short vegetation [3,4]. In this situation, FR is reflected from the surrounding vegetation, whereas red light (R) is absorbed and decreases the R:FR perceived by the taller crop. Under shading, both perceived PPFD and R:FR are reduced, due to the high absorption of other wavelengths when compared to FR by the tall vegetation. The light quality is recognized by plant photoreceptors, with phytochrome and cryptochrome being the main regulators of SAR to R:FR and PPFD, respectively [5]. The two forms of phytochrome (Pr and Pfr) regulate the response by a dynamic photo-equilibrium dependent on R:FR. By absorption of FR, Pfr is transformed to the inactive form Pr, which relieves gene suppression, and in turn, induces SARs [6]. Cryptochromes regulate, while interacting with multiple regulating proteins, the response to changed

PPFD by recognizing the change in blue light intensity (400–500 nm) associated with changed PPFD [7]. In *Arabidopsis thaliana* (L.) Heynh, several studies have shown that phytochrome and cryptochrome interact to fine-tune SAR [5,8–10]. Interacting of cryptochrome with Phytochrome Interacting Factors (PIFs) under low blue light enhanced the response of petioles [8] and hypocotyl [10] to low R:FR. Not only photoreceptors regulate the SAR, as increased leaf thickness under high PPFD has been found, independent of a blue light level [11] and cryptochrome mutations [12].

In crop production, the light environment can change with weed pressure or cropping system. Emerging weeds increase R:FR due to the increased reflection of FR while intercropping reduces both PPFD and R:FR perceived by the shorter crop. The SAR of the crop to the given environment can affect yield negatively e.g., SAR to emerging weeds reduced the yield of mono-cropped soybean (*Glycine max* (L.) Merr.), due to increased crop heterogeneity [4]. The intercropped soybean is often the shorter crop grown together with a taller crop like maize and a strong elongation response of soybean to the given light environments, which can lead to lodging [13]. Earlier studies showed that soybean shaded by maize had a reduced stem diameter [13,14], root length, aboveground biomass, total root biomass, and root–shoot ratio [14,15] and an increased seedling height [13,14,16]. Additionally, the shaded soybean has increased chlorophyll content and reduced the photosynthetic capacity and chlorophyll *a/b* ratio [16,17]. Plants are considered more shade tolerant if they have a high specific leaf area (SLA), leaf area ratio (LAR), and leaf mass ratio (LMR) [18]. Intercropped soybean leaves had reduced leaf area and mass per area [19] and increased SLA and LAR [16].

From field observations, it is difficult to determine the distinct effect from low PPFD and low R:FR. Studies on the distinct effect of decreased R:FR on soybean found elongated petioles, retarded rates of stem elongation [20], increased internode length only of epicotyl and hypocotyl [4], or increased length of the measured internode with no significant change of total stem length [21]. Regarding the effect of R:FR on leaf area, Green-Tracewicz et al. [4] found a decreased leaf area from the four-leaf stage to seed fill, while Pauch et al. [20] found an increase or insignificant decrease depending on other growth conditions. In addition, the response in LAR and LMR to R:FR depended on growth conditions, but could increase LAR and decrease LMR [20]. Only one study focused on the effect of PPFD and found increased plant height and decreased stem diameter and leaf thickness with decreased PPFD [22]. However, there are no studies on soybeans, which investigated the effect of a change only in PPFD, i.e., without any influence of FR, facilitating the disentanglement of the individual effects of PPFD and R:FR.

Breeding for cultivars with an optimal SAR for a specific cropping system could increase yield and is particularly important to improve productivity in intercropping systems of maize with a shorter crop like soybean [23,24], but can also be important for increasing weed tolerance [25]. For exploiting this potential, genetic variability in SAR has to be examined [16]. Intercropped soybean cultivars differed e.g., in their degree of vining, stem diameter [13], internode elongation [16], dry matter [15], yield [26], and photosynthetic capacity [17]. The underlying molecular mechanisms of the response in soybean are being explored, but are still unclear [25]. A disentanglement of the effects of PPFD and R:FR on morphology could help in understanding these mechanisms and to determine the most important molecular mechanisms for specific cropping systems.

We hypothesized that the morphological response of soybean to a change only in PPFD would differ from the response to a change only in R:FR due to the regulation by different photoreceptors and that the response would vary between cultivars. Therefore, a climate chamber experiment was conducted with different levels of PPFD combined with different ratios of R:FR and with no FR including three morphologically different soybean cultivars to investigate the effect of PPFD and R:FR on morphology of soybean and to assess the variability among cultivars.

2. Materials and Methods

2.1. Experimental Setup

Two experiments were performed in an LED climate chamber (poly klima[®]S-chamber, Poly klima GmbH, Freising, Germany) at the University of Hohenheim (Germany). The chamber was designed for research applications and had the following dimensions: 0.6 m high, 0.6 m wide, and 0.5 m deep. The chamber consisted of two compartments each with two LED modules comprised of several LED types adjustable in light intensity and spectrum (Figure 1). Each LED type corresponded to a different wavelength and was represented by several individual LEDs arranged across the LED module for homogenous illumination. Each set of LED type, which was called channel, could be turned on and adjusted independently in intensity, which allows for several spectral compositions from the same LED module. The photoperiod was 16 h with 28 °C day temperature, 22 °C night temperature, and a relative humidity of 45%. The three soybean cultivars of maturity group 000, namely Lissabon (I.G. Pflanzenzucht GmbH, Ismaning, Germany), Merlin (SAATBAU LINZ eGen, Leonding, Austria), and Sultana (R.A.G.T. Saaten Deutschland GmbH, Hiddenhausen, Germany) were selected for their known differences in plant height. According to the cultivar descriptions, among the three cultivars, cv. Lissabon is the shortest cultivar and cv. Merlin is the tallest. Additionally, Merlin has a fast youth development and an intermediate tendency for lodging, whereas cvs. Lissabon and Sultana have a low lodging tendency. Within each compartment of the LED chamber, nine soybean plants (three of each cultivar) were grown in pots (9.5 × 9.0 × 9.0 cm) containing peat substrate (Substrat 5+Perlite, Klasmann-Deilmann GmbH) and irrigated from below in a common tray. At the start of the experiment, four seeds were sown in each pot. During germination and early growth, PPFD was 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with very low photon flux density of far-red light (2 $\mu\text{mol m}^{-2} \text{s}^{-1}$). After one week, seedlings were thinned to one plant per pot, according to homogenous development, and the light treatments were applied. The plants were grown for another 13 days under the light treatments (in a total of 20 days of growth) until the first trifoliolate leaf was developed. During the experiment, the shelves with the plants were moved downward to maintain a distance of 10 cm to the LED modules ensuring a constant spectral photon flux density at the top of the canopy.



Figure 1. Soybean within the chamber (a) and the empty chamber showing the LED modules (b).

2.2. Light Treatments

To set the light treatments, quantitative measurements of the light spectrum ($\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$) were acquired with a FLAME-S-XR1-ES spectrometer (Ocean Optics Germany GmbH, Ostfildern, Germany). The spectrometer measured the light spectrum within the range from 200 to 1025 nm with a resolution of around 2 nm. It was equipped with a collimating lens (74-UV-MP) and a right-angle reflector with a cosine corrector (74-90-UV-CC3). The photon flux density was recorded for five ranges: 400–700 nm (photosynthetic active radiation), 400–500 nm (blue light), 500–600 nm (green light), 600–700 nm (R), and 700–800 nm (FR) and the R:FR was calculated. Setting of the light treatments

(Table 1) was done by adjusting the intensities of the channels of the LED modules by comparing them to measurements of the spectrometer. The setting was performed at a 10-cm distance from the LED modules equivalent to the distance to the soybean canopy during the experiments.

Different light treatments were applied in the two experiments. In the first experiment, the light treatments were low and high PPFD of $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ (PPFD¹⁰⁰) and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (PPFD⁴⁰⁰), respectively, in combination with a low or high R:FR or a very high R:FR further referred to as no FR. The low R:FR was 1.5 (rfr^{1.5}) and the high ratio was 5 (rfr⁵) (Table 1). In the second experiment, PPFD was $160 \mu\text{mol m}^{-2} \text{s}^{-1}$ (PPFD¹⁶⁰). This intensity was chosen because it enabled under the experimental maximum intensity of FR, which is the creation of a lower R:FR ratio of 0.6 (rfr^{0.6}). This can be considered comparable to natural shade by vegetation [14]. The R:FR ratios of 1.5 (rfr^{1.5}) and 5 (rfr⁵) were kept for comparison with the first experiment. All light treatments were comprised of a broad spectrum (shown for experiment one in Figure 2) with the same blue to R and blue to green ratios of 1 and 1.5, respectively.

Table 1. Photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$) within the four-color ranges, total photosynthetic photon flux density (PPFD), and the red to far-red ratio (R:FR, rfr) for each treatment within the first and second experiment.

Treatment	PPFD (400–700 nm)	Blue (400–500 nm)	Green (500–600 nm)	Red (600–700 nm)	Far-Red (700–800 nm)	R:FR
First Experiment						
PPFD ¹⁰⁰	100	37.5	25	37.5	1	37.5
PPFD ¹⁰⁰ rfr ⁵	100	37.5	25	37.5	7.5	5
PPFD ¹⁰⁰ rfr ^{1.5}	100	37.5	25	37.5	25	1.5
PPFD ⁴⁰⁰	400	150	100	150	4	37.5
PPFD ⁴⁰⁰ rfr ⁵	400	150	100	150	30	5
PPFD ⁴⁰⁰ rfr ^{1.5}	400	150	100	150	100	1.5
Second Experiment						
PPFD ¹⁶⁰ rfr ⁵	160	60	40	60	12	5
PPFD ¹⁶⁰ rfr ^{1.5}	160	60	40	60	40	1.5
PPFD ¹⁶⁰ rfr ^{0.6}	160	60	40	60	100	0.6

2.3. Plant Measurements

In both experiments, measurements were performed 20 days after sowing. The morphological measurements comprised length and diameter of the third internode and the petiole of the first trifoliate leaf (located on the third node) and length of rachis bearing the center leaf of the first trifoliate leaf. The third phytomer was chosen because it included the first and fully developed trifoliate leaf. Measurements of leaf area and dry mass were made separately for leaves and stem (internodes and petioles) of the entire above ground plant and were used to calculate the specific leaf area (SLA = leaf area/leaf dry mass), leaf area ratio (LAR = leaf area/total above ground dry mass), and leaf mass ratio (LMR = leaf dry mass/total above ground dry mass). Diameters were measured with a caliper and lengths with a ruler. Leaf area was measured with a leaf area meter (LI-3100 Area Meter, LI-COR, Lincoln, NE, USA) and dry mass was recorded after drying for at least 48 h at 60 °C until it reached a constant weight.

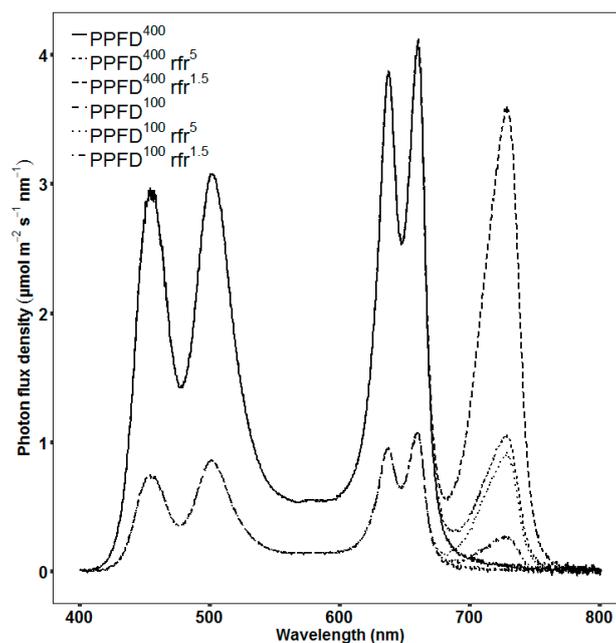


Figure 2. Spectral photon flux density of the six light treatments in the first experiment at high (PPFD⁴⁰⁰) and low (PPFD¹⁰⁰) photosynthetic photon flux density (PPFD) and high (rfr⁵) and low (rfr^{1.5}) red to far-red ratio (R:FR).

2.4. Statistical Design and Analysis

In both experiments, the effect of light treatments and cultivars on SAR were tested. In the first experiment, six light treatments were tested. In the second experiment, there were three light treatments (Table 1). Both experiments were performed with two replicates within the single chamber with two compartments. This resulted in six runs for the first experiment and three runs for the second experiment. In each run, two out of six or three light treatments, respectively, could be tested. An α -design with two replicates and a block size of two was used in the first experiments. An incomplete non-resolvable block design with two replicates was used in the second experiment. Within a compartment, each cultivar was tested in triplicates. The nine pots were arranged according to a 3×3 latin square to block out effects in two dimensions. In total, each treatment was measured on six plants including two runs with three plants each.

To verify the number of replicates to be statistically sufficient, the statistical power for the trait length of the third internode was estimated after performing the first experiment. Error variances were heterogeneous. Therefore, we calculated an average variance using a generalized linear model, by assuming a gamma distribution. The light treatment specific variances varied between 0.07 cm^2 and 3.61 cm^2 and the estimated average was 1.075 cm^2 . With this error variance, we calculated the power for 1 to 5 cm and two or three replicates using the approach of Stroup [27]. The power satisfied two replicates, as shown in Table 2.

Table 2. Power for the trait length of the third internode.

Difference in cm	Two Replicates	Three Replicates
1	0.362	0.509
2	0.894	0.977
3	0.998	>0.999
4	>0.999	>0.999
5	>0.999	>0.999

According to the design, the following mixed model was used to analyze the data in the Statistical Analysis System (SAS):

$$y_{ijklmno} = \mu + b_k + i_{kl} + p_{lm} + r_{lmn} + c_{lmo} + \tau_i + \varphi_j + (\tau\varphi)_{ij} + e_{ijklmno}, \quad (1)$$

where μ is the intercept, b_k is the fixed effect of the k th complete replicate, i_{kl} is the random effect of the l th incomplete block (=run) within the k th replicate, p_{klm} is the random effect of the m th compartment within the l th run, and r_{lmn} and c_{lmo} are random row and column effects within the m th compartment of the l th run. τ_i , φ_j , and $(\tau\varphi)_{ij}$ are the main effects of the i th light treatment, the j th cultivar, and their interactions, respectively. $e_{ijklmno}$ is the error effect of observation $y_{ijklmno}$ with either homogeneous variance, PPFD-specific variance, or R:FR-specific and PPFD-specific variance. All three error structures were fitted and the model with the best model fit measured via the Akaike information criterion (AIC) [28] was used. Studentized residuals were checked graphically for normal distribution and homogeneous variances. After finding significant effects via the F-test, a multiple t -test was used to create a letter display [29]. Note that, for the second experiment, b_k was dropped from the model and i_{kl} was replaced by i_l since there are no complete replicates. Further note that ratios of means were calculated from least square means for solely a presentation purpose.

3. Results

All soybean cultivars responded significantly to both factors, PPFD and R:FR, but the quantitative response to each of the two factors differed between plant organs. Cultivars differed significantly in their response of internode length and diameter, petiole length LAR, SLA, and LMR. Regarding the response on plant level, plant height increased under both low PPFD and low R:FR in the first experiment. Furthermore, the experiment had few significant differences that were present. Plant weight decreased under low PPFD and increased under low R:FR (Table 3).

Table 3. The least square means of plant height and weight for the three cultivars in the first (top) and second (bottom) experiment. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

Treatment	Plant Height (cm)			Plant Weight (g)		
	Lissabon	Merlin	Sultana	Lissabon	Merlin	Sultana
First experiment						
PPFD ⁴⁰⁰	12.31 ^{eA}	11.88 ^{fA}	8.89 ^{eB}	0.71 ^{bA}	0.64 ^{bAB}	0.52 ^{bB}
PPFD ⁴⁰⁰ rfr ⁵	13.97 ^{eA}	15.07 ^{eA}	10.48 ^{eB}	0.69 ^{bA}	0.80 ^{abA}	0.61 ^{bA}
PPFD ⁴⁰⁰ rfr ^{1.5}	17.16 ^{dB}	18.65 ^{dA}	16.50 ^{dB}	0.92 ^{aA}	0.98 ^{aA}	0.86 ^{aA}
PPFD ¹⁰⁰	39.74 ^{cA}	29.87 ^{cB}	25.54 ^{cC}	0.32 ^{cA}	0.29 ^{cAB}	0.23 ^{cB}
PPFD ¹⁰⁰ rfr ⁵	44.34 ^{bA}	36.73 ^{bB}	32.83 ^{bC}	0.37 ^{cA}	0.29 ^{cA}	0.31 ^{cA}
PPFD ¹⁰⁰ rfr ^{1.5}	52.01 ^{aA}	48.73 ^{aAB}	46.63 ^{aB}	0.37 ^{cA}	0.41 ^{cA}	0.33 ^{cA}
Second Experiment						
PPFD ¹⁶⁰ rfr ⁵	45.15 ^{aA}	36.01 ^{aB}	33.35 ^{bB}	0.50 ^{bB}	0.61 ^{aA}	0.49 ^{bB}
PPFD ¹⁶⁰ rfr ^{1.5}	45.47 ^{aA}	44.61 ^{aA}	44.22 ^{abA}	0.77 ^{aA}	0.52 ^{aB}	0.59 ^{bB}
PPFD ¹⁶⁰ rfr ^{0.6}	40.16 ^{aB}	42.03 ^{aB}	44.26 ^{bA}	0.72 ^{abA}	0.70 ^{aA}	0.87 ^{aA}

3.1. Internodes

The length of the third internode was influenced significantly by PPFD and R:FR with the maximum length reached by the combination of low PPFD and low R:FR, but with the strongest effect of decreasing PPFD (Figure 3a). Comparing high PPFD with low PPFD, the internode length of, for instance, cv. Merlin increased substantially from 1.48 to 7.02 cm, which corresponds to 373%, whereas a reduction in R:FR increased length to 3.68 cm at high PPFD and 14.47 cm at low PPFD, which corresponds to an increase by 148% and 106%, respectively (Figure 3a). The cv. Lissabon responded lesser to low R:FR than the other two cultivars with a 63% increased length at high PPFD and a 53% increase at low PPFD in the first experiment and no significant response in the second experiment. The response of cv. Sultana was stronger to a decrease in R:FR at high PPFD than the other two cultivars with an increased length by 192%. Except from cv. Lissabon, the tendency of the responses to reduced R:FR in the second experiment were similar to the first experiment and showed an additional elongation under R:FR of 0.6 compared to 1.5 (Figure 3b).

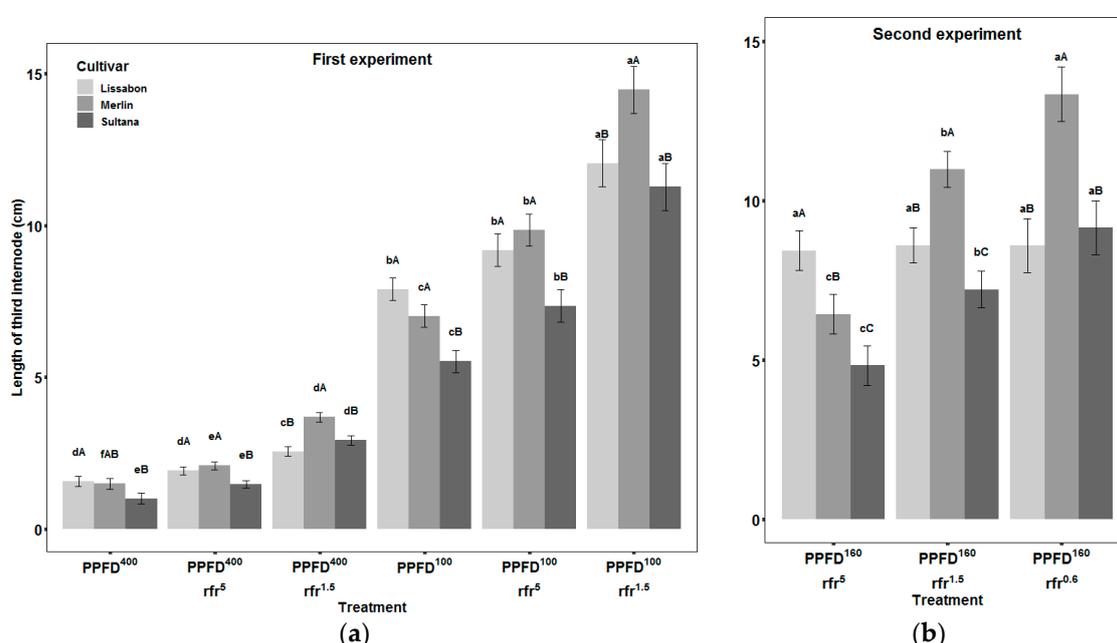


Figure 3. The least square means of third internode length for the three cultivars in first (a) and second (b) experiment. Error bars indicate standard error of the LS-mean. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

The diameter of the third internode was mainly influenced by low PPFD with a reduction of 37% to 42% across cultivars (Table 4). On the contrary, the internode diameter tended to increase under low R:FR. For cv. Merlin, there was a significant increase by 23% under low R:FR at a low PPFD and 34% to 38% at an R:FR of 0.6 in the second experiment for the cvs. Lissabon and Sultana (Table 4). Still, internode length to the diameter ratio increased under both low PPFD and low R:FR.

Both internode length, diameter, and their ratio showed, for the same R:FR ratios in the second experiment, an intermediate response between high and low PPFD of the first experiment (Figure 3, Table 4).

Table 4. The least square means of internode diameter for the three cultivars in the first (top) and second (bottom) experiment. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

Treatment	Third Internode Diameter (mm)			Third Internode Length:Diameter		
	Lissabon	Merlin	Sultana	Lissabon	Merlin	Sultana
First Experiment						
PPFD ⁴⁰⁰	2.47 abB	2.88 aA	2.73 aAB	6.48 cA	5.33 dAB	4.06 eB
PPFD ⁴⁰⁰ rfr ⁵	2.41 bB	2.95 aA	2.90 aA	8.10 cA	7.12 dA	5.08 eB
PPFD ⁴⁰⁰ rfr ^{1.5}	2.86 aA	2.79 aA	3.03 aA	8.72 cB	12.93 cA	9.44 dB
PPFD ¹⁰⁰	1.47 cA	1.66 cA	1.72 bA	47.99 bA	42.98 bAB	31.66 cB
PPFD ¹⁰⁰ rfr ⁵	1.65 cA	1.73 bcA	1.59 bA	58.91 bA	57.10 aA	49.58 bA
PPFD ¹⁰⁰ rfr ^{1.5}	1.65 cB	2.05 bA	1.68 bB	76.33 aA	73.55 aA	68.67 aA
Second Experiment						
PPFD ¹⁶⁰ rfr ⁵	1.84 bB	2.25 aA	2.19 bA	27.71 bA	27.66 cA	28.14 bA
PPFD ¹⁶⁰ rfr ^{1.5}	2.40 aA	2.15 aA	2.33 bA	44.84 aA	37.38 bA	35.62 bA
PPFD ¹⁶⁰ rfr ^{0.6}	2.47 aB	2.29 aB	3.03 aA	47.81 aA	50.04 aA	48.09 aA

3.2. Petioles

Contrary to internode length, the length of the third petiole increased less under low PPFD and showed the strongest increase under low R:FR (Figure 4a). In cv. Merlin, the response to low PPFD was an increased length from 2.05 to 3.07 cm corresponding to 50% and the response to low R:FR was an increased length to from 2.05 to 6.33 cm at high PPFD and an increase from 3.07 to 6.14 cm at low PPFD, which corresponds to 209% and 100%, respectively. In cv. Merlin, this led to no difference in petiole length between high and low PPFD at low R:FR and in cv. Lissabon to significantly longer petioles under low R:FR at high PPFD than at low PPFD. In the second experiment, the elongation responses to R:FR were further increased in the treatment with an R:FR of 0.6 compared to 1.5 (Figure 4b). For instance, the length of the third petiole of cv. Merlin increased from 6.14 cm to 8.49 cm under a R:FR of 1.5 and to 11.73 cm under a R:FR of 0.6, which corresponds to 38% and 91%, respectively. The cv. Lissabon had the strongest response to a R:FR of 0.6 with an increase of 147% when compared to a R:FR of 5. This led to the petioles of the cv. Lissabon no longer being significantly different from the other cultivars under a R:FR of 0.6, even though they were significantly shorter than the other cultivars under an R:FR of 5.

Similar tendencies of a strong response to low R:FR were present for the lengths of the third center rachis. Only rachis length of the cv. Sultana responded significantly at low PPFD, but all cultivars responded significantly under low R:FR at high PPFD (Table 5). Comparing PPFD levels of the two experiments, both petiole and rachis had a tendency of increased length in the second experiment compared to both high and low PPFD in the first experiment (Figure 4, Table 5).

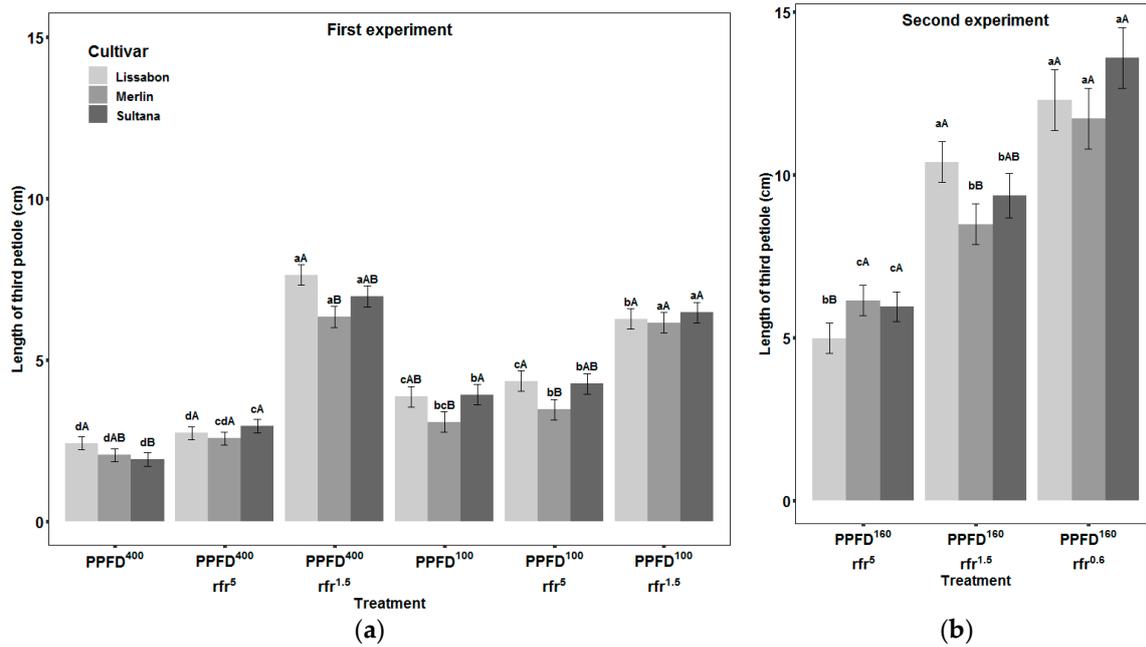


Figure 4. The least square means of third petiole length for the three cultivars in first (a) and second (b) experiment. Error bars indicate standard error of the LS-mean. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

Table 5. The least square means of rachis length for the three cultivars in the first (top) and second (bottom) experiment. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

Treatment	Third Center Rachis Length (cm)		
	Lissabon	Merlin	Sultana
First Experiment			
PPFD ⁴⁰⁰	0.92 ^{bA}	0.80 ^{cAB}	0.72 ^{dB}
PPFD ⁴⁰⁰ rfr ⁵	1.07 ^{bA}	0.98 ^{bAB}	0.87 ^{cdB}
PPFD ⁴⁰⁰ rfr ^{1.5}	1.62 ^{aA}	1.67 ^{aA}	1.30 ^{aA}
PPFD ¹⁰⁰	0.95 ^{bA}	0.85 ^{bcA}	0.98 ^{bcA}
PPFD ¹⁰⁰ rfr ⁵	0.97 ^{bA}	0.83 ^{bcA}	0.88 ^{cdA}
PPFD ¹⁰⁰ rfr ^{1.5}	1.13 ^{bA}	1.03 ^{bcA}	1.18 ^{abA}
Second Experiment			
PPFD ¹⁶⁰ rfr ⁵	0.96 ^{bA}	1.06 ^{bA}	1.03 ^{bA}
PPFD ¹⁶⁰ rfr ^{1.5}	1.38 ^{aA}	1.24 ^{bA}	1.42 ^{aA}
PPFD ¹⁶⁰ rfr ^{0.6}	1.61 ^{aA}	1.78 ^{aA}	1.65 ^{aA}

3.3. Leaves

Leaf length was generally less influenced by PPFD and R:FR compared to internodes and petioles. Low PPFD had no significant influence on leaf length of the cv. Merlin, but increased the leaf length of the cvs. Lissabon and Sultana with 11% to 13% (Figure 5). R:FR did not influence the leaf length at low PPFD or in the second experiment, but at high PPFD, low R:FR increased leaf length with 18% to 26% across cultivars.

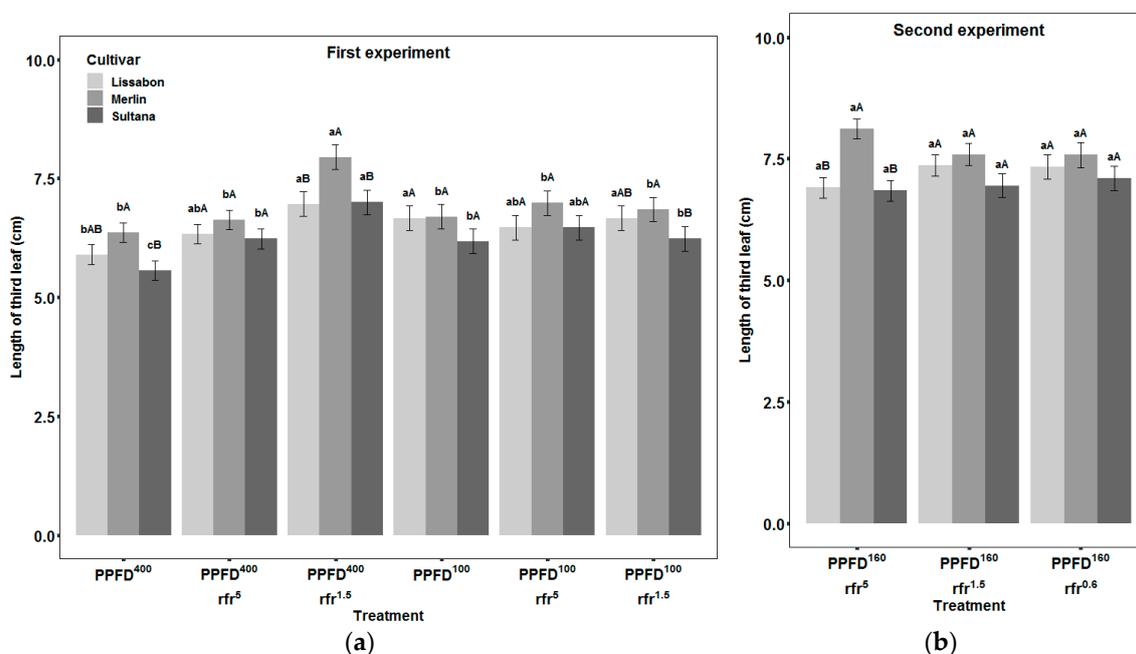


Figure 5. The least square means of leaf length of the third leaf for the three cultivars in the first (a) and second (b) experiment. Error bars indicate standard error of the LS-mean. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

The SLA and LAR responded mainly to low PPFD with an increase of 178% to 200% and 142% to 181%, respectively (Table 6). In the first experiment, R:FR had an additive influence on SLA with a maximum increase in cv. Lissabon at low PPFD of 23%, while R:FR decreased LAR with a maximum decrease in cv. Sultana of 18%. In the second experiment, leaf length, SLA, and LAR were not influenced significantly by R:FR. This was in accordance with the first experiment at either low or high PPFD, where they were also not influenced significantly by R:FR, which compared an R:FR of 5 and 1.5. Similar to internodes, SLA and LAR were intermediate in the second experiment compared to low and high PPFD. Under low PPFD, LMR significantly decreased by 9% to 14% in cvs. Merlin and Lissabon and, under low R:FR LMR, decreased by 10% to 24%. In the second experiment, LMR decreased by 15% to 23% from R:FR of 5 to 0.6. Comparing the cultivars, cv. Sultana maintained the highest LMR at low PPFD and low R:FR.

Table 6. The least square means of specific leaf area, the leaf area ratio, and the leaf mass ratio for the three cultivars in the first (top) and second (bottom) experiment. For each experiment, lower case letters indicate significant differences between treatments for each cultivar, and upper case letters indicate significant differences between cultivars for each treatment ($p < 0.05$).

Treatment	Specific Leaf Area (cm ² g ⁻¹)			Leaf Area Ratio			Leaf Mass Ratio		
	Lissabon	Merlin	Sultana	Lissabon	Merlin	Sultana	Lissabon	Merlin	Sultana
First experiment									
PPFD ⁴⁰⁰	247.31 cdB	242.19 cB	280.22 cA	170.72 bB	170.86 cB	209.30 dA	0.69 aB	0.71 aB	0.75 abA
PPFD ⁴⁰⁰ rfr ⁵	245.21 dB	243.60 cB	326.17 bA	168.32 bB	167.59 cB	248.35 cA	0.69 aB	0.69 aB	0.76 aA
PPFD ⁴⁰⁰ rfr ^{1.5}	278.16 cB	277.83 bB	319.13 bA	172.66 bB	169.71 cB	209.63 dA	0.62 bB	0.61 bcB	0.66 cA
PPFD ¹⁰⁰	687.99 bB	726.19 aAB	816.74 aA	412.36 aB	466.52 abB	588.27 aA	0.59 bcC	0.65 bB	0.72 bA
PPFD ¹⁰⁰ rfr ⁵	695.94 bB	817.27 aA	791.52 aA	395.18 aB	485.04 aA	526.43 bA	0.57 cB	0.60 cB	0.67 cA
PPFD ¹⁰⁰ rfr ^{1.5}	843.77 aA	833.10 aA	850.56 aA	405.19 aB	409.10 bB	481.52 bA	0.49 dB	0.49 dB	0.57 dA
Second Experiment									
PPFD ¹⁶⁰ rfr ⁵	592.98 aB	614.07 aAB	701.66 abA	330.74 aB	357.98 aB	457.75 aA	0.55 aC	0.59 aB	0.65 aA
PPFD ¹⁶⁰ rfr ^{1.5}	599.19 aB	703.47 aA	793.77 aA	298.79 aB	365.25 aA	416.26 aA	0.50 bA	0.51 bA	0.53 bA
PPFD ¹⁶⁰ rfr ^{0.6}	658.65 aA	672.45 aA	604.34 bA	306.23 aA	309.16 aA	299.78 bA	0.47 bB	0.47 cAB	0.50 bA

4. Discussion

4.1. Response to PPFD and R:FR

The results showed that the response of soybean to low PPFD and low R:FR can be characterized as an interactive response with differences between organs. Internodes elongated the most in response to low PPFD with an additive effect from the addition of low R:FR. Yet, the increase of petiole elongation was more stimulated by R:FR than PPFD. The main elongation response of petioles to low R:FR and of internodes to low PPFD are consistent with a study by Pauch et al. [20]. However, in their study, the internode length was not influenced or even reduced under low R:FR. On the contrary, Yang et al. [14] showed that the increase in seedling height of soybean intercropped with maize correlated more with R:FR than with PPFD. This might be explained by a greater effect of R:FR on the hypocotyl and epicotyl than the following internodes [4], which would not have been fully captured in our study, because the light treatments were applied after the development of the epicotyl had already started. The internode diameter responded mainly to decreased PPFD with a tendency toward an increased diameter under low R:FR. Only a few studies have examined the internode diameter response to low R:FR under high PPFD. Hussain et al. [21] also found the internode diameter of soybean to decrease mainly as a response to decreased PPFD, but with a tendency toward a decreased diameter under low R:FR and similar results were found for the sunflower [30]. However, as expected, an increase of the length to the diameter ratio under low R:FR was present and the tendency of increased diameter might be a necessary response to stabilize the plant due to the more extreme elongation of the internodes, than in the study of Hussain et al. [21].

The results indicated that, in addition to the expected elongation response to low PPFD, an increased PPFD might increase the elongation response to R:FR. The response to low PPFD is considered a response to decreased blue light mediated by cryptochrome, whereas a response to increased PPFD could be a result of increased availability of assimilates. This effect could be observed in petiole length under low R:FR, since the length was not increased under low PPFD compared to high PPFD and a tendency of an increased response at $160 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to the low PPFD treatments at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$. These results should be examined further with more levels of PPFD between 100 and $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ and measurements of photosynthesis for a better understanding. Earlier studies on soybean found changes in the photosynthetic system [17] and stem carbohydrate concentration as well as composition under low PPFD [21], and changes in carbon export from tomato leaves depending on PPFD and light quality [31].

In general, the response of the leaf length was minor when compared to internodes and petioles, but length increased under low PPFD and low R:FR at high PPFD. Soybean shaded by maize in an intercropping system (low R:FR at low PPFD) had a decreased leaf area [19]. Pauch et al. [20] found an increase in leaf area with decreased R:FR in the greenhouse, but smaller leaf area under climate chamber conditions, which had a lower average light intensity. This indicated a response of leaf area similar to petioles, which results in opposite responses to R:FR depending on the PPFD level.

The response of leaves was mainly a considerable increased LAR and SLA, i.e., decreased thickness of the leaf under low PPFD, and no effect of R:FR on SLA. An increase in SLA has also been observed in an earlier study on the PPFD level [22], in intercropped soybean [19], and is generally consistent with observations from other species [11]. In *Arabidopsis thaliana* (L.) Heynh, it was shown that leaf thickness is regulated mainly by light intensity through redox-signaling or sugar signaling and not through photoreceptors [12,32]. In accordance with earlier studies in soybean [16,20], elongation responses in this study resulted in a decreased LMR.

The second experiment with a lower R:FR treatment, which is comparable to field conditions, underlined the results from the first experiment and showed that the internode diameter, the elongation of the internode, and petiole were further increased under an R:FR of 0.6. A comparable study with sunflower also showed that the elongation response of internodes to decreasing R:FR were similar when comparing unnaturally high to high R:FR or high to low R:FR [30]. Regarding field conditions,

the strong response of petioles to low R:FR in this study, indicated that petiole elongation would be the main response in soybeans in response to the threat of shade recognized by a decreased R:FR due to reflected FR from neighboring vegetation [3], e.g., emerging weeds. While the response to true shade, for instance, by taller neighboring plants in intercropping decreases both PPFD and R:FR, the response would lead to an elongation of both internodes and petioles.

4.2. Cultivar Differences and Aspects for Crop Management

Assuming that the data sheet information given on the three cultivars are recorded in a mono-cropping system, it is the most relevant to compare these data with the treatments under high PPFD including R:FR. The cultivar Merlin was, as expected, the tallest cultivar, whereas cv. Sultana, not in line with the cultivar description, was shorter than cv. Lissabon. This is likely to be due to the early termination of growth or problems with comparing information on relative height from different companies. Assuming the ratio of the internode length to diameter as an expression for the lodging tendency, cv. Merlin had the highest ratio as expected from the cultivar description.

Relevant for mono-cropping systems is the response to R:FR, since this determines the response to the threat of shade at high light intensity, which can affect the yield negatively [4,25]. In this context, cultivars differed in their response to R:FR under high PPFD with a preferable low internode elongation of the cv. Lissabon and low petiole elongation of cv. Merlin. The response of petioles to a change in R:FR indicated that petiole elongation could be one reason for the weed induced upregulation of PIF-like genes in soybean [25]. In the context of intercropping, knowledge on gene regulation under low R:FR should be accompanied by further knowledge on gene regulations under low PPFD, which influences e.g., internode length, which was not influenced in the study of Horvath et al. [25]. A shade tolerant plant is expected to have a high SLA, LAR, and LMR, according to the hypothesis of maximizing net carbon gain [18]. Considering the shade tolerance of the three cultivars, cv. Sultana had a higher LMR and LAR and smaller decrease in these by decreased PPFD or R:FR than the other cultivars, which indicates potential cultivar differences in the carbon investment in the SAR. Intercropping of the soybean can be further divided depending on the duration of the overlapping growth period. If the overlap with e.g., maize is only during the early soybean growth, a stable crop is still important to avoid lodging, but shade tolerant traits e.g., a high LAR might be of less importance when shade is no longer present. In this context, it would be interesting to further disentangle the effect and underlying mechanisms of internode elongation regulated by photoreceptors and leaf morphology, which is regulated by other mechanisms [12,30]. In addition, interactions between morphological and photosynthetic adaptations to shade [17] could be interesting for further studies and cultivar improvements. For instance, it would be intriguing to determine whether an increased photosynthetic shade adaptation results in a stronger elongation response due to an increase in assimilate availability. The effect of fluctuating light occurring diurnally or for certain time periods during the co-growing period should also be taken into account in future studies for better understanding the influence of acclimation to different levels of PPFD and R:FR on carbon assimilation and translocation.

The interactions between the cultivar and the light environment and the different morphological responses e.g., between internodes and petioles, substantiate the need for a genetic selection within intercropping trials [23,24]. Based on this, a selection for the optimal response in e.g., internode elongation, length to diameter ratio, and allocation of assimilates between organs e.g., for an optimal LAR would be possible.

5. Conclusions

The shade avoidance response of the soybean to low PPFD and R:FR differed between organs. This supported a differentiated response to the threat of shade (low R:FR) with strong petiole elongation and to the true shade (low R:FR and PPFD) with considerable elongation of both internodes and petioles. Regarding petiole elongation, the results indicated an additional interaction with the availability of assimilates. For a deeper understanding, this interaction should be further studied together with a

disentanglement of the responses of leaf morphology, which is dependent on photoreceptors and those dependent on e.g., sugar signaling.

The different responses of organs to the light environment typical for different crop practices and the interactions between the cultivar and the light environment showed interesting possibilities for future breeding. Consideration of these differentiations and interactions could help in understanding molecular responses of soybean to shade and those important for developing cultivars with an optimized shade avoidance response for specific crop management (e.g., mono-cropping and intercropping). Further studies should examine the effect of PPFD and R:FR on carbon assimilation and translocation, include a larger and more diverse genetic panel, and align experiments under controlled and field conditions to transfer knowledge into practice.

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