The relationship between internode elongation of soybean stems and spectral distribution of light in the canopy under different plant densities

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ABSTRACT

The present study carried out with a field plot experiment and light environment simulation experiment in 2017 to assess the effect of spectral distribution on internode elongation in soybean canopy. Henong 60 and Heinong 48 were used as the experimental materials. The spectral distribution in the soybean canopy was studied under four planting densities 200,000 (D20), 300,000 (D30), 400,000 (D40) and 500,000 (D50) plant ha⁻¹. Meanwhile, a pot experiment of light environment simulation with light-emitting diode (LED) was carried out to further analyze the effects of light quantity and quality on the elongation of soybean seedlings. The results showed that the intensity of PAR, blue light, red light and far-red light in soybean canopy decreased by varying degrees with density and canopy depth increasing, and the decrease of PAR and R/FR led to significant elongation of internodes and plant height in high density. The spectral intensity in the middle and base of the canopy was strongly reduced, resulting in the strongest internode elongation in the middle of soybean stem. The increase in soybean plant height was also mainly due to the internode elongation in the middle at high density. In addition, the mono-light of far-red light could promote the internode elongation, while the red light and blue light acted as inhibitors and the inhibitory effect of blue light was even greater.
Introduction

An increase in planting density raises competition for light among individual plants within a population. Dense planting increases leaf area index (LAI) and shading among individual leaves, which in turn changes the light environment that plants live in, including light intensity and light quality (Maddonni et al., 2001; Xue et al., 2016; Zhi et al., 2014). Shading generally reduces photosynthetically active radiation (PAR) and decreases of red light (R), blue light (B) and far-red light (FR) due to the absorption or reflection by the canopy vegetation. The decrease in red light is much greater than that of far-red light, leading to a decrease in the ratio of red light to far-red light (Franklin, 2008; Grant, 1997). These changes are also influenced by planting density and canopy depth, further influencing plant morphological and development (Carlos, 1999; Casal, 2012; Cosgrove, 2010). Studies by Rousseaux and Hall AJSanchez (2010) and Board (2000, 2001) have shown that under high densities, PAR and R/FR are significantly reduced in canopies of sunflower and soybean. Moreover, Board (2001) found that blue light was also an important signal in the light environment.

Low R/FR in the shade promoted the elongation of internodes and hypocotyl in sunflower and Chenopodium album L. and increased leaf area (Kurepin et al., 2007b; Morgan et al., 2010). Ballare et al. (1987), (1988) revealed that these changes were caused by a decrease in R, which the signal was perceived by photochromes. The decrease in R/FR can lead to the fewer roots and lower root biomass in Festuca rubra and wheat, what is more, the number of tillers was also significantly reduced in wheat (Kasperbauer & Karlen, 2010; Pecháčková, 1999). However, the effect of R/FR also varied among plant species. In shade-grown and shade-tolerant plants, a low R/FR could promote seed germination and plant development, whereas, for sun plants and shade-intolerant plants, low R/FR has an inhibitory effect (Kurepin, Walton, Pharis et al., 2010; Kurepin, Walton, Reid et al., 2010; Sánchez et al., 1996). The photosynthetic photon flux density (PPFD) could affect the physiological functions of leaves, plant relative growth rate and net assimilation rate (Caldwell et al., 1986). Gawronska et al. (1995) found that as the PPFD decreases, the height and the fresh weight of pea seedlings increase, whereas leaf expansion was inhibited. Meanwhile, low PPFD was also a signal for decreasing leaf surface area and leaf dry weight (Kurepin et al., 2007a). Stuefer and Huber (1998) found that a decrease in PAR mainly affected the growth of Potentilla, whereas a decrease in R/FR affected plant morphology. However, studies have shown that in the shade, a decrease in PAR and R/FR leads to an increase in soybean plant height and decrease in stem diameter, root length, branching and above-ground biomass (Board, 2001; Gong et al., 2015; Green et al., 2011; Liu et al., 2017; Yang et al., 2014).

Supplementing FR during plant growth can promote leaf expansion and stem elongation, on the contrary, with an increase in R, leaf expansion and stem elongation could be inhibited (Behringer & Davies, 1992; Morgan, O’Brien & Smith, 1980; Walton et al., 2010). When pepper was grown under a combination of R and B, the length of the stem, as well as dry weight, were greater than those of plants grown under-red light. Particularly, pepper plants are grown under a combination of red light and far-red light were the tallest. These results were agreed with the findings of Kim et al. (2004) and Park et al. (2015), which tested the effect of a combination of red light and blue light on the growth of chrysanthemum. Increasing the intensity of blue light could inhibit internode elongation due to low R/FR in Datura ferox L. and Sinapis alba L. It could also inhibit cell division in soybean stems and decrease internode length (Dougher & Bugbee, 2004). However, the low-intensity blue light can increase soybean leaf area and stem elongation (Board, 2001; Cope & Bugbee, 2013).

To study the effect of the spectrum in the canopy on soybean stem elongation, we selected two soybean cultivars with different plant type and lodging resistance according to the test results of Xu et al. (2017), Xu et al. (2020), Henong 60 (dwarf-stem lodging resistant variety with high lodging resistance coefficient) and Heinong 48 (tall stem, susceptible to lodging variety with low lodging resistance coefficient). They were used to measure the changes in spectral distribution in canopy under different densities and analyzed the effect of density on the light spectrum in a field experiment. At the same time, we further investigated the effects of light quantity and quality on stem elongation in two soybean cultivars through light environment simulation experiment. The natural light and shading with LED equipment (blue, red and far-red light) as light environment treatment. So, our study will provide evidence and reference for understanding the mechanism of spectral effect on internode elongation in soybean stems.

Materials and methods

Experiment design

The experiment was divided into field plot experiment and light environment simulation experiment. The field plot experiment was conducted at the experimental base of Northeast Agricultural University, Harbin (126° 45’ E and 45° 42’ N), Heilongjiang province, China in
2017. Based on the study of Xu et al. (2017), two soybean varieties of Heinong 48 (HN48, a tall variety) and Henong 60 (HN60, a dwarf variety) were selected as test materials. HN48 was susceptible to lodging and HN60 was a lodging resistant variety.

**Field plot experiment**

This experiment was designed as a field plot experiment. The plots were completely randomly arranged. The treatments of density were 200,000 plants ha\(^{-1}\) (D20), 300,000 plants ha\(^{-1}\) (D30), 400,000 plants ha\(^{-1}\) (D40) and 500,000 plants ha\(^{-1}\) (D50) with four replicates of each soybean variety. The soil of the field was the Phaeozem. The results of soil analysis were as follows: soil organic matter content was 32.1 g kg\(^{-1}\); available nitrogen content was 84.9 mg kg\(^{-1}\); available phosphorus content was 59.1 mg kg\(^{-1}\) and available potassium contents was 182.9 mg kg\(^{-1}\). The normal density in-field product of HN60 and HN48 was 400,000 plants ha\(^{-1}\) and 300,000 plants ha\(^{-1}\), respectively. Plants were planted as ridge culture and the ridge was 0.65 m wide. The plot was eight rows (including two border rows) by 10 m long. Seeds were sown by hands on 30 April 2017 and thinned to a design population density at V3. Crops were protected against weeds, insects and diseases as needed and were irrigated and fertilized to prevent water and nutritional stresses. The average amount of N, P, K fertilizer application rates was 51 kg ha\(^{-1}\), 62 kg ha\(^{-1}\) and 33 kg ha\(^{-1}\), respectively. The herbicide was the Enhalidine with a concentration of 12.5%, the application concentration was 10 ml/kg. The pesticide was the Lambda-cyhalothrin with spraying with 2.5% cream 1000 ~ 2000 times liquid.

**Simulation experiment of light environment**

Plants were grown with pots (30 cm length, 20 cm width, 20 cm depth) in outdoor. Soybean seeds were sown in a row along the middle of the pot. Six seedlings were saved in each pot, at an interval of 4 cm. Light environment simulation experiments were divided into Experiment I and Experiment II. In Experiment I, we set up six light conditions of natural light (N), shade (S), shade + red light (SR), shade + blue light (SB), shade + far-red light (SFR) and natural light + far-red light (NFR). In Experiment II, the four light conditions of natural light (N), shade (S), natural light + red light (NR) and natural light + blue light (NB) were set up. We arranged six pots per variety of each treatment and replicated this design in the three blocks, totally 144 pots for Experiment II. In each experiment, three pots were sampled at 2 times. Experiment II was conducted after the completion of Experiment I. Normal sunlight radiation was used as natural light. Shading nets with 35% transmittance were used to reduce solar radiation, thus creating a shaded environment. In addition, light-emitting diode (LED) equipment was 90 cm long and placed 1 cm away from soybean seedlings, at the height similar to that of soybean cotyledons with the vertical illumination on the stems. Light treatments began after the expansion of the true leaf on the soybean seedlings. The illumination time of the LED was consistent with local sunshine time. Experiments I and II were conducted on June 13–24 and July 19–30 in 2017, respectively. Daily average temperature and daily total radiation are shown in Figure 1.

**Sampling and measurements**

**Field plot experiment**

The canopy spectrum of soybean plants was measured in the R1(initial flowering), R3(initial pod), R5(beginning of seed filling) stage. We chose sunny days to perform every measurement from 11:00 to 14:00. Five soybean plants were selected from an area in each plot where plants grew uniformly. The probe of the fibre optic spectrometer (manufactured by Aventes, Apeldoorn, Gelderland, Netherlands, AvaSpec-2048-USB2) was with the cosine corrector and the FOV was 2m. The spectrometer was placed at three positions as adjacent to the stem base, the middle and the growing point of the soybean stem. The base position was at the 5 cm height from the surface of the soil and the growing point was the top position, the middle position was at the half-height between the base and top position. Thus, the middle position was changing with the growth of soybean at different development stages. At each location, measurements were taken horizontally along four directions (east, west, south, north) and vertical upwards along the stem. The spectral intensity of PAR (\(\lambda = 400–700\) nm), blue light (B, \(\lambda = 455–465\) nm), red light (R, \(\lambda = 645–655\) nm) and far-red light (FR, \(\lambda = 730–740\) nm) in each location was calculated as the sum of those from five directions. Soybean plants were cut along the soil surface and the length of every internode above the scar of the cotyledons was measured at maturity stage.

**Light simulation experiment**

Samples were collected on Day 6 and 12 after the light simulation experiment started. The above-ground portion of the plants was cut from the scars of the
cotyledons. The length from the scar of the cotyledons to the growing point (plant height) and every internode length was measured.

Statistical analyses

There were four and three replicates of each treatment for each variety of field plot experiment and light environment simulation experiment, respectively, and the mean value of replicates was used for ANOVA to detect a significant effect of treatment only and the effect of genotypes between two soybean varieties was not analyzed here. Analysis of variance was performed with IBM SPSS Software version 17.0.

Results

The effect of density on soybean plant height, internode length and spectral distribution in the canopy

The precipitation, average temperature and average daily radiation during the growth period of soybean field plot experiment (from May to September) are
shown in Table 1. In Table 2, HN60 and HN48 exhibited a significant increase of plant height and internode length as plant density increasing in maturity. In terms of plant height, the sequence of plant height was D50 > D40 > D30 > D20 for both two cultivars. HN60 did not show any significant difference in plant height between D20 and D30, whereas HN48 had no significant difference between D40 and D50. The differences among all other treatments were significant. For the internode length, in HN60, except for I4 and I11 that had no significant difference, all other internodes exhibited significantly different lengths. For I1, there was a significant difference only between D20 and D40, D50 and all other treatments did not show significant differences. For I2 and I3, there was no significant difference among D20, D30 and D40, whereas all others were significantly different when compared with D50. From I5 to I10, except for I7 and I9, all other internodes showed significant differences when comparing D40, D50 with D20 and D30. There was no significant difference in I7 and I9 between D30 and D40. For the internodes in HN48, the I1-I4 of D20 was significantly shorter than that of D50. I5-I7 of D20 and D30 was significantly different from D50. From I8 to I13, except for I10, the internode length of D20 was significantly shorter than those of all other treatments. There was no significant difference observed for I14-I17 among all treatments. Overall, for both two soybean cultivars tested, the length of the internodes located in the middle of the plant increased as density increased. The internode length between D20 and D50 was significantly different. Due to the fact that higher density induced the elongation of internodes in the middle of the stem, plant height then increased.

Figure 3 shows similar changing patterns of the spectral distribution in the canopy of HN60 and HN48 with the developmental stages, density and canopy depth changed. The spectral intensity decreased as soybean development progressed and increasing of density and canopy depth. From the top to the middle of the canopy, the spectral intensity had the greatest decrease, whereas from the middle to the lower part of the canopy, those showed a relatively smaller decrease. Comparing with two cultivars, we found that photon flux densities of PAR, red light, far-red light, blue light and the ratio of red light to far-red light (R/FR) in the HN48 canopy were slightly higher than those in HN60 and the percentage of decrease under high density in HN48 was higher than that in HN60.

For both HN60 and HN48, the PPFD of the top, middle and bottom canopy all decreased in the R1, R3, R5 stage as the density increased. However, significant levels of variation at different depths and stages. For HN60 at the R1 stage, the PPFD of the top, middle and bottom canopy at D20 was significantly greater than those at D40 and D50. The PPFD of the top and middle canopy at D30 was also significantly different from those at D40 and D50. At the R3 stage, the PPFD at the top and middle canopy at D20 was significantly different from those at D30 and D40, the difference of PPFD at D40 and D50

| Internode number | D20  | D30  | D40  | D50  |
|------------------|------|------|------|------|
| I1               | 2.75 ± 0.12b | 3.14 ± 0.14ab | 3.33 ± 0.17a | 3.48 ± 0.15a |
| I2               | 2.29 ± 0.11b | 2.59 ± 0.13b  | 2.40 ± 0.11b  | 3.15 ± 0.21a |
| I3               | 2.86 ± 0.13b | 2.69 ± 0.15b  | 2.76 ± 0.17b  | 2.97 ± 0.13b |
| I4               | 3.28 ± 0.17a | 3.07 ± 0.14a  | 3.42 ± 0.27a  | 3.42 ± 0.37a |
| I5               | 3.43 ± 0.18b | 3.50 ± 0.21b  | 5.19 ± 0.42a  | 5.76 ± 0.50a |
| I6               | 4.51 ± 0.28b | 4.39 ± 0.22b  | 6.05 ± 0.44a  | 5.91 ± 0.36a |
| I7               | 4.68 ± 0.25c | 5.36 ± 0.31bc | 5.96 ± 0.42ab | 6.56 ± 0.25a |
| I8               | 5.11 ± 0.20b | 5.48 ± 0.29b  | 6.31 ± 0.23a  | 6.71 ± 0.20a |
| I9               | 4.88 ± 0.37b | 4.93 ± 0.30b  | 5.77 ± 0.27ab | 6.26 ± 0.32a |
| I10              | 3.84 ± 0.42b | 4.41 ± 0.35ab | 5.16 ± 0.38a  | 5.28 ± 0.34a |
| I11              | 3.35 ± 0.43a | 3.93 ± 0.39a  | 4.32 ± 0.46a  | 4.01 ± 0.39a |
| I12              |               |               |                | 5.70 ± 0.25c |
| I13              |               |               |                | 7.05 ± 0.24b |
| I14              |               |               |                | 7.26 ± 0.32a |
| I15              |               |               |                | 8.04 ± 0.23a |
| I16              |               |               |                | 8.21 ± 0.37a |
| I17              |               |               |                | 7.71 ± 0.37a |

Table 2. The plant height and internode length in different densities.

Note: Population means for an internode and plant height at maturity followed by different letters (a,b,c) were significantly (P > 0.05) different according to Duncan’s multiple range test.
were significant at the top canopy, the PPFD at the bottom canopy did not significantly differ among treatments. At the R5 stage, the PPFD of the top canopy at D20 and D30 was significantly different from that of D50, the middle and bottom canopy’s PPFD at D20 was significantly different from the other treatments, whereas the other of the treatments did not show significant differences. For HN48, at the stages of R1 and R3, the PPFD of the top canopy was not significantly different between D40 and D50, but there was a significant difference among other treatments. The PPFD of the middle canopy at D20 was significantly greater than those of D40 and D50, whereas the PPFD of the bottom canopy at D20 and D30 was significantly different from that at D50. At the R5 stage, the PPFD of the top canopy of D20 and D30 were significantly different from those at D40 and D50. The PPFD of the middle and bottom canopy at D20 was significantly greater than those of the other treatments. There was no significant difference among other treatments.

The changes in photon flux density of R, FR and B were similar to those in PPFD between two soybean cultivars. This spectral difference accumulated as density increased and exhibited a significant difference between D20 and D50. Table 3 shows that among the three stages and three canopy depths measured, there was a significant decrease in photon flux density in the D50 canopy compared to D20 in both cultivars. The percentage decrease in PPFD in the canopies of both cultivars reached peaked at the R1 stage. The average percentage decrease of PPFD, R and B at the three depths of HN60 and HN48 were 53.7% and 63.3%, 52.4% and 60.0%, 57.4% and 67.5%, respectively. The average percentage decrease in FR at the three depths was 19.25% and 28.72%. The percentage decrease declined in R3 and R5, where at the R3 stage, the average percentage decrease in PPFD, R, B and FR was 37.6% and 57.9%, 37.6% and 54.7%, 42.4% and 61.1% and 11.7% and 23.6%, respectively. At the R5 stage, the average percentage decrease in PPFD, R, B and FR were 38.7% and 40.8%, 36.9% and 45.0%, 41.2% and 46.4% and 20.9% and 22.7%, respectively. Because the decrease percentage in R was far greater than that of FR in the canopies of both cultivars, R/FR also significantly decreased as density increased. The significant differences between treatments are shown in Figure 2, where D20 and D50 showed significant differences in R/FR. Other treatments exhibited significant differences in R/FR among various developmental stages and canopy depth.

The effect of simulated light environment on plant height and internode length at the soybean-seedling stage

During the experiment, spectral photon irradiance from 5 cm above seedlings in natural light and shade environment was measured at noon of sunny days and shown in Figure 3. The intensity of PAR, R, FR, B and the ratio of R/FR in natural light or shade environment are shown in Table 4. The intensity of LED light was measured at 1 cm directly facing the LED bulbs. The intensity of red light was 171.0 ± 4.5 μmol·m⁻²·s⁻¹, FR light was 102.1 ± 4 μmol·m⁻²·s⁻¹ and blue light was 194.0 ± 6.8 μmol·m⁻²·s⁻¹.

In Table 5, the plant height and internode length of HN60 and HN48 significantly increased in the S compared with N treatment in Experiment I and II, indicating that shading could significantly induce the internode elongation of soybean, therefore increasing the plant height.

When comparing treatment N with NFR, we found that on Day 6, the plant height and the length of the 1st internode of HN60 under natural light + FR were significantly greater than those under N treatment. The plant height and internode length of HN48 did not significantly differ between these two treatments. On Day 12, both HN60 and HN48 showed significantly greater plant height and internode length under NFR treatment than those under N. When comparing SFR with S treatment, on Days 6 and 12, FR resulted in a significant increase in plant height and the length of the 1st and 2nd internodes in both HN60 and HN48. This suggested that supplementing FR in natural light and shading both led to an increase in the elongation of internode and plant height and this effect was more obvious when FR was supplemented in the shade. Moreover, comparing with two cultivars, it showed that
HN60 was more sensitive to FR and the increase in internode length was more extensive.

By comparing SR and SB with S treatment, respectively, we found that the plant height and internode elongation was significantly inhibited in both cultivars. For HN60, the difference was significant on both Days 6 and 12. HN40 was slightly different than HN60, which exhibited significant differences in the 2nd internode length on Day 12 between SR and S. When comparing with SR and SB, we found that on Day 12, plant height and 2nd internode length in HN60 and HN48 under SB were significantly shorter than those under SR, indicating that when under shading, red and blue light can both inhibit internode elongation, decrease the plant height and the inhibitory effect of blue light on internode elongation was stronger than that of red light (Figure 4).

In Experiment II, under natural light, red and blue light also imparted an inhibitory effect on the plant height and internode length. When comparing NR and
Figure 3. Spectral photon irradiance in natural light and shade environment.

Table 4. The percentage of spectral decrease (%) in the soybean canopy from D20 to D50 treatments.

| Treatment      | PAR (μmol·m⁻²·s⁻¹) | R (μmol·m⁻²·s⁻¹) | FR (μmol·m⁻²·s⁻¹) | B (μmol·m⁻²·s⁻¹) | R/FR |
|----------------|---------------------|-------------------|-------------------|------------------|------|
| Natural light  | 1508.15 ± 7.49      | 570.87 ± 2.49     | 508.90 ± 1.75     | 399.36 ± 2.45    | 1.12 ± 0.01 |
| Shade          | 540.05 ± 5.23       | 211.92 ± 3.54     | 207.75 ± 2.40     | 134.77 ± 1.48    | 1.02 ± 0.01 |

Table 5. Changes in soybean seedling height and internode length under different-simulated light environments.

| Experiment | Day | Treatment | Plant height (cm) | 1st internode length (cm) | 2nd internode length (cm) | Plant height (cm) | 1st internode length (cm) | 2nd internode length (cm) |
|------------|-----|-----------|-------------------|---------------------------|--------------------------|-------------------|---------------------------|--------------------------|
| I          | 6   | N         | 2.37 ± 0.07d      | 2.37 ± 0.07d              | 5.18 ± 0.29e             | 3.98 ± 0.24d      | 1.15 ± 0.06de             |
|            |     | S         | 6.95 ± 0.18b      | 5.7 ± 0.22b               | 11.03 ± 0.27b            | 8.43 ± 0.34b      | 2.17 ± 0.17b              |
|            |     | NFR       | 4.26 ± 0.21 c     | 3.46 ± 0.19 c             | 5.8 ± 0.06e              | 4.57 ± 0.3d       | 0.9 ± 0.15e               |
|            |     | SR        | 4.58 ± 0.14 c     | 3.47 ± 0.17 c             | 8.63 ± 0.51 c            | 6.73 ± 0.33 c     | 1.9 ± 0.20c               |
|            |     | SB        | 4.2 ± 0.12 c      | 3.44 ± 0.09 c             | 7.55 ± 0.29d             | 6 ± 0.39 c        | 1.5 ± 0.17 cd             |
|            |     | SFR       | 10.3 ± 0.18a      | 8.25 ± 0.25a             | 12.9 ± 0.26a             | 10.47 ± 0.03a     | 2.5 ± 0.29a               |
| 12         | N   | 7.38 ± 0.1e | 2.98 ± 0.03e      | 3.05 ± 0.14d             | 7.53 ± 0.34e             | 3.72 ± 0.26e      | 3.03 ± 0.19e              |
|            |     | S         | 15.85 ± 0.24b     | 6.53 ± 0.37b             | 18.95 ± 0.55b            | 8.77 ± 0.66b      | 7.95 ± 0.23b              |
|            |     | NFR       | 8.95 ± 0.05d      | 4.08 ± 0.05 c             | 10.43 ± 0.19d            | 5.4 ± 0.21d       | 4.03 ± 0.23d              |
|            |     | SR        | 10.03 ± 0.64 c    | 3.7 ± 0.24 c              | 15.2 ± 0.43 c            | 6.83 ± 0.47 c     | 5.93 ± 0.45 c             |
|            |     | SB        | 7.93 ± 0.46d      | 3.7 ± 0.25 d              | 11.18 ± 0.65d            | 5.95 ± 0.38 cd    | 4.23 ± 0.36d              |
|            |     | SFR       | 18.15 ± 0.36a     | 8.35 ± 0.46a             | 24.03 ± 0.84a            | 10.75 ± 0.22a     | 10 ± 0.2a                 |
| II         | 6   | N         | 6.7 ± 0.17b       | 5.27 ± 0.18b             | 7.68 ± 0.4b              | 5.35 ± 0.14b      | 2.18 ± 0.09b              |
|            |     | Se        | 10.3 ± 0.21a      | 8.03 ± 0.27a             | 12.9 ± 0.33a             | 8.64 ± 0.11a      | 4.3 ± 0.24a               |
|            |     | NR        | 5.93 ± 0.13b      | 4.63 ± 0.09bc            | 6.5 ± 0 c                | 4.9 ± 0.1 c       | 1.65 ± 0.09b              |
|            |     | NB        | 5.43 ± 0.41b      | 4.28 ± 0.3 c             | 7.47 ± 0.09bc            | 5.57 ± 0.03b      | 1.87 ± 0.07b              |
| 12         | N   | 12.6 ± 0.46b | 6.38 ± 0.19b     | 4.02 ± 0.02b             | 11.34 ± 0.26b            | 4.9 ± 0.1b       | 2.73 ± 0.15b              |
|            |     | S         | 19.6 ± 0.30a      | 8.45 ± 0.21a             | 24.38 ± 0.44a            | 9 ± 0.29a        | 5.85 ± 0.29a              |
|            |     | SR        | 10.15 ± 0.25c     | 5.78 ± 0.3b              | 11.41 ± 0.4b             | 4.63 ± 0.23bc     | 2.88 ± 0.13b              |
|            |     | NB        | 9.21 ± 0.13c      | 5.1 ± 0.15 c             | 11.26 ± 0.31b            | 4.13 ± 0.13 c     | 2.55 ± 0.05b              |

Note: the means for every treatment in Experiment I and II followed by different letters (a,b,c) were significantly (P > 0.05) different, according to Duncan’s multiple range test in 6D and 12D.

S: shade, N: natural light, SR: shade+ red, SB: shade+ blue, SFR: shade+ FR, NFR: natural light +FR, NR: natural light + red, NB: natural light + B.

NB with N only, we found that on Day 6, the difference in plant height of HN60 was not significant, but the internode length was significantly different. HN48 exhibited a significant difference in plant height and the 1st internode length. On Day 12, HN60 showed a significantly different plant height. The 1st and 2nd internode lengths were significantly different in NB treatment, whereas only the 2nd internode length was significantly different in NR. NH48 did not show any significant difference in plant height; only the 1st internode length was significantly different in NB. This suggests that under natural light, red and blue light can also inhibit internode elongation, but this inhibitory effect is more obvious when under shading. Moreover, blue light exhibited a stronger...
inhibitory effect than red light under both natural light and shading (Figure 4).

Discussion

Light environment in the soybean canopy

Light is an abiotic factor that plays important roles in regulating plant growth. In the agricultural production, after solar radiation enters the canopy of crops, light with different wavelengths is absorbed and reflected by the leaves or transmitted through the leaves. Meanwhile, such changes are tightly correlated with planting density and canopy depth (Board, 2000; Grant, 1997). In this study, the PPFD of the spectrum with different wavelengths significantly decreased as the plant density and canopy depth increased (Figure 2). The PPFD (400–700 nm) at the middle and bottom canopy was considerably lower than that at the top canopy and significantly lower at high density. These results were due to that fact that the spectrum between 400 nm and 700 nm wavelength was absorbed and utilized by plants, among which blue light (455–465 nm) and red light (645–655 nm) changed in a similar pattern as that of PPFD. A proportion of FR (730–740 nm) in the canopy is absorbed by plants, while the rest is reflected; therefore, FR exhibited a decreasing trend as canopy depth and density increased. However, the extent of decrease of FR was far less than that of red light, causing R/FR to significantly decrease in the canopy. The R/FR in solar radiation is between 1.0 and 1.2 (Board, 2000). In this study, the R/FR in the canopy of the two soybean cultivars were between 0.15 and 0.45, significantly lower than that of solar radiation. The low

Figure 4. Comparison between Henong 60 and Heinong 48 in different light environments of Experiment I and II for 12 days, respectively. S: shade, N: natural light, SR: shade+ red, SB: shade+ blue, SFR: shade+ FR, NFR: natural light +FR, NR: natural light+ red, NB: natural light+ B.
PAR and R/FR in the soybean canopy formed a typical shade environment, which is an important factor that affects soybean morphogenesis during growth. Also, the spectrum with different wavelengths in the canopy plays different roles in regulating soybean morphology.

Soybean morphology at the seedling stage

Light intensity and quality in the spectrum can induce a series of responses in plant morphology (Kurepin & Pharis, 2014). A decrease in PAR can promote the elongation of hypocotyls and stems, as well as inhibit leaf expansion (Carlos L Ballaré, 1999; Board, 2000; Casal, 2012; Franklin, 2008). Other studies have also shown that a decrease in R/FR could cause different effects on the root system, internode length, branching and tillering (Kasperbauer & Karlen, 2010; Kurepin et al., 2007b; Pecháčková, 1999). The low blue light intensity can also lead to stem elongation (Board, 2001; Cope & Bugbee, 2013). The purpose of these morphological changes in plants is to be better adapted to the shade environment in the canopy and to be more competitive for essential resources for plant growth among individuals.

This study utilized the outdoor natural environment, supplemented with different monochromatic light for the simulated light environment. The R/FR in natural light and shade treatment was 1.12 and 1.02, respectively, both within the range of R/FR in solar radiation. Thus, the feature of two simulated light environments was normal PAR + normal R/FR and low PAR + normal R/FR. The internode length and plant height in the shade treatment was greater than those in natural light treatment for HN60 and HN48, indicating that a decrease in light intensity can promote soybean internode elongation. This coincides with the observed changes in soybean plant height and internode length in a maize-soybean intercropping system (Gong et al., 2015; Liu et al., 2017). However, in Experiment II, plant height in natural light and shade was higher than those in the same treatments in Experiment I. This was due to the natural conditions in Experiment II, the intensity of solar radiation was close to that in Experiment I, but the air temperature was significantly higher than that in Experiment I. Higher temperature led to a rapid growth in soybean plants, thereby causing the difference in plant height in the same treatments between Experiments I and II. In Experiment I, the treatments of NFR and SFR created a light environment with normal PAR + low R/FR and low PAR + low R/FR, respectively, when compared to natural light and shade separately, plant height and internode length increased significantly in both soybean cultivars. This suggests that low R/FR can also cause internodes to elongate and this elongation was most significant under low PAR + low F/FR, consistent with the results from a study involving sunflowers (Kurepin et al., 2007b).

In this study, after supplementing blue light for 12 days under shade conditions, the plant height of HN60 and HN48 decreased by 49.96% and 41.00%, respectively. The same pattern was also observed in HN60 under treatments of SR, NB and NR. HN48 did not show a significant decrease in plant height under NB treatment. Supplementing blue light and red light can decrease internode length by inhibiting cell division in soybean (Dougher & Bugbee, 2004). This indicated that HN60 was more sensitive to changes in the light environment, while blue and red light had an inhibitory effect on internode elongation and the blue light inhibitory effect was greater. The inhibition of blue light and the red light was more obvious under shading than under natural light. Kurepin et al. (2007b) showed that high R/FR under both low or normal PAR can inhibit stem elongation. In our study, adding red light to natural light and shade increased F/FR under different PAR conditions, which inhibited the elongation of stem internodes and plant height at the soybean-seedling stage. Compared to red light, blue light has a more significant effect in inhibiting stem internode elongation and plant height in soybean.

The relationship between soybean plant height, internode elongation and light environment

In this study, the internode length and plant height in HN60 and HN48 increased significantly as density increased. Although internode numbers and plant height of two soybean cultivars tested were different, when comparing to the internode elongation in D20 and D50, which had the most significant difference, we found that in HN60 and HN48, the internodes in the middle of the plant showed the most extensive elongation. This was followed by the internodes from the bottom canopy, whereas the internodes from the top canopy had the lowest level of elongation. A previous study also showed that 85% of the difference in plant height among various densities came from the 7th to 13th internode (Board, 2001). One of the environmental factors that induces internode elongation is the light intensity and quality in the canopy, both of which affect the photomorphogenesis in plants (Franklin, 2008; Gommers et al., 2013).

Plants use the decrease in R/FR as an early signal to detect adjacent plants in the canopy (C. L. Ballare et al., 1987; Ballaré et al., 1988; Ballare et al., 1990). Thus, in this study, from germination to shading each other, the elongation of base internode and plant height in HN60.
and HN48 was the signal of decreasing R/FR. However, as PAR and blue light were sufficient in the canopy, the elongation of base internode was relatively small. As the soybean grew, shading began to occur, which led to a drastic decrease in PAR and B in the middle and bottom canopy as the density increased. R/FR also significantly decreased at this point. Studies have shown that a decrease in PAR and R/FR in the canopy leads to a significant increase in soybean internode length (Gong et al., 2015; Liu et al., 2017; Yang et al., 2014), while the relatively low blue light in the canopy is the main cause for internode elongation (Board, 2001; Cope & Bugbee, 2013).

The middle and bottom parts of the canopies on HN60 and HN48 had severe shading. The internodes in the middle of the stem were under shading for a long period of time and therefore had the greatest level of elongation. The PAR and the intensity of blue light were higher in the top canopy than those in the middle and bottom canopy, but the R/FR ratio remained relatively low. Thus, internode elongation in the top canopy was relatively small and did not exhibit significant differences among densities. For the two cultivars, we found that the plant height of HN60 at D20 showed a 46.4% increase compared to D50 and HN48 exhibited a 25.7% increase. The plant height of HN60 showed a greater increase, which is related to the low PAR, B and R/FR in the canopy, as well as a higher sensitivity of HN60 to changes in the light quality in the canopy.

The different response to the change of light quantity and quality between HN60 and HN48 were probably due to the sensitivity of photoreceptors and regulation of endogenous hormones. The phytochromes of plants can perceive the changes in R/FR, a family of photoreceptors which can detect R and FR quanta (Smith, 2000). The phytochrome gene family consists of PHYA, PHYB, PHYC, PHYD and PHYE (Clack et al., 1994). Phytochrome B-deficient Arabidopsis plants display increased elongation, decreased leaf expansion, increased apical dominance and early flowering responding to the changes in R/F ratio (Devlin et al., 1997). Multiple mutant analyses have also revealed roles for PHYD and PHYE in the PHYB-mediated response to changes in R/FR (Devlin et al., 1998, 1999). In S.longipes, the phytochrome gene family consists of PHYA, PHYB and PHYC and all have shown to alter their gene expression in response to changes in R/F ratio (Li et al., 2011). The changes in the growth of plants were regulated by at least four groups of plant hormones: gibberellins (GAs), cytokinins (Cks), auxin and ethylene (Kurepin et al., 2007a, 2007b) abscisic acid (ABA) (Kurepin et al., 2007b) and salicylic acid (Kurepin, Walton, Pharis et al., 2010) may be involved.

At present, there are few studies on the differences in gene expression levels and endogenous hormone content of phytochromes under different light conditions between different genotype soybeans, so that will be our goals of the further researches.

Our research found that when the density increased, the intensity reduction of PAR, blue light and R/FR ratio in the canopy spectrum was the main reason to promote internode elongation and plant height of soybean. The blue light and red light can inhibit the internode elongation of soybean and the inhibition effect of blue light was stronger. The HN60, was more sensitive to spectral changes, resulting in an increase in plant height at higher density than that of HN48.

**Disclosure statement**

The authors declare that there is no conflict of interest.

**Funding**

This work was supported by the National Key Research and Development Programme, Physiology and Regulation of High Quality Soybean Production under Grant number 2018YFD1000903.

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