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Monochromatic Light Interactions in the Early Hypocotyl Elongation of Sunflower (*Helianthus annuus* L.) Seedlings

Dragan Vinterhalter [1,*], Vaclav Motyka [2] and Branka Vinterhalter [1]

[1] Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, 11060 Belgrade, Serbia; horvat@ibiss.bg.ac.rs
[2] Institute of Experimental Botany of the Czech Academy of Sciences, Rozvojová 263, 165 02 Prague, Czech Republic; motyka@ueb.cas.cz
* Correspondence: vinter@ibiss.bg.ac.rs or dvinterhalter@yahoo.com; Tel.: +381-631995888

Abstract: Sunflower is a crop species well adapted for cultivation in open fields under full sunlight. Young plantlets can be grown in growth chambers under low irradiance, where different aspects of light can be easily tracked. Using time-lapse imaging, we have shown how monochromatic red, blue, and far-red light and their combinations interacted, affecting the rhythmicity and elongation of sunflower hypocotyls. Monochromatic light of any color, applied individually, canceled all manifestations of diurnal rhythmicity and anticipation of imminent light transitions present in diurnal photoperiods established by white LED light panels. Monochromatic light also significantly increased the rate of hypocotyl elongation, which became uniform (arrhythmic) and often triggered the appearance of guttation. The rate of hypocotyl elongation was highest with the blue light and lowest with red light. In double light combinations, red light suppressed the stimulative effect of blue light, but it promoted the elongation rate when used together with far-red light. A triple light combination of red, blue, and far-red light stimulated hypocotyl elongation to a high degree and increased the elongation rate more than twofold compared with red and fourfold compared with white LED light.

Keywords: *Helianthus annuus*; blue light; red light; far-red light; diurnal rhythmicity; hypocotyl elongation; guttation; root pressure

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1. Introduction

In this study, we will show that the effects of light on the progress and rhythmicity of hypocotyl elongation in sunflower seedlings are fundamentally different from the patterns and responses found in other plant species, including the model plant Arabidopsis. The study focuses on light requirements after germination and initial seedling development and explains the importance and advantages of sunflower as a model system for future studies in plant photobiology.

Light is considered an important external factor that regulates plant growth and development [1]. It provides environmental signals necessary for the control of plant photomorphogenesis [2] and the regulation of circadian clock functioning [3]. Light provides plants with the data [4,5] that they need in order to select a specific developmental strategy that is best suited for the current light conditions in their environment. Most importantly, light via photosynthesis provides plants with the energy needed to maintain their metabolic processes and support their growth and development.

Plants on Earth receive a complex mixture of solar radiation, composed mainly of wavebands from the visible part of the spectrum (400–750 nm) and some ultraviolet and

infrared light. The actual waveband composition can vary considerably depending on the light incident angle, the current conditions in the atmosphere [6], and the possible presence of neighboring plant leaves that obstruct the incident light [7,8]. Changes in the ratio of red to far-red light are known to induce shade avoidance response which leads to rapid shoot elongation. In sunflower, changes in the ratio of red to far-red light have been shown to strongly affect hypocotyl elongation [9].

A common feature of all plants living on Earth is their adaptation to diurnal photoperiods, in which periods of light (daytime) and dark (nighttime) alternate daily in a regular and predictable sequence. The rhythmicity dictated by the diurnal photoperiods enables proper interpretation of environmental stimuli, which leads to precise daily coordination of metabolic processes.

Extensive genetic studies have shown that a large proportion of genes in the plant genome are light dependent and change their daily activity in response to the presence of light [10–12]. Entire groups of related genes are rhythmically triggered to become active at the same time of day. In this way, plants can synchronize and start major metabolic processes at optimal time points of the day.

The rhythmic phenomena of plants and other living organisms are controlled by circadian oscillators, which are present in every living cell [13]. The concept of circadian oscillators assumes that each cell contains a small group of genes that are functionally organized as a circadian clock and rhythmically change their activity in a regular sequence throughout the day. The circadian clock enables plants to follow the daily timing and adjust to the duration of the light/dark periods of their photoperiods. Current models of the clock assume that the interactions between the genes that form the core of the circadian clock occur through negative feedback at the level of transcriptional/post-transcriptional regulation [14–16].

The adjustment of the clock, known as entrainment, can be triggered by light or by certain environmental stimuli such as daily temperature fluctuations. It has been shown that light entrainment is perceived by photoreceptors from the phytochrome and cryptochrome group and with some possible roles for S phototropins [17,18] and zeitlupe [19] photoreceptors. Multiple blue and red light-absorbing pigments involved in the entrainment allow white artificial light, same as that of solar radiation, to act as a light entrainment factor. Apart from entrainment, these photoreceptors participate in a number of other light-triggered plant responses such as de-etiolation.

The mode of action of individual photoreceptors (phytochromes, cryptochromes, phototropins)—together with factors involved downstream in the regulation of their light signaling, such as PIFs (phytochrome interacting factors), cop1, and other ligases, as well as the HY5 transcription factor—were elucidated in a relatively short period from the late 1990s to the early 2000s, as reviewed by [20]. This enormous breakthrough was achieved through the use of diverse experimental techniques and collaborative efforts of many researchers working on the model plant Arabidopsis, with its S ecotypes and mutants.

Two points of particular interest to us, both of which were presented in study [21], were that light inhibits hypocotyl elongation and that hypocotyl elongation is controlled by the circadian clock. These two simple, widely accepted and experimentally well-supported statements apply to the model plant Arabidopsis and many other plant species [22], but not to sunflower, in which blue light does not inhibit hypocotyl elongation but, rather, strongly stimulates it [23]. In fact, hypocotyl elongation in sunflower is low only under white light, whereas all monochromatic light wavebands applied individually support much higher hypocotyl elongation rates [24]. Thus, in sunflower seedlings, light is by no means an inhibitory factor for hypocotyl elongation, which is in complete contrast to the situation in Arabidopsis, where light arrests elongation and promotes the formation of leaf

rosettes [25]. However, hypocotyl elongation in Arabidopsis can also result from a balance of light components with promoting and inhibiting influences [22].

In a previous study [24], we showed that many factors of light can affect the hypocotyl elongation of sunflower seedlings, including spectral composition (light colors) and the periodicity (shifts in the presence and absence of light) and intensity of light irradiance. Here, we include a study on the effects of far-red light and its interactions with blue and red light in regulating hypocotyl elongation and rhythmic phenomena, including guttation.

2. Material and Methods

2.1. Seed Germination and Basic Growth Conditions

A number of sunflower genotypes were used for the study, including cultivars NK Sureli, NK Kondi, and NK Neoma (Syngenta, Basel, Switzerland); Achilles CLP (KWS, Einbeck, Germany); NS H7749 (Institute of Field and Vegetable Crops, Novi Sad, Serbia); P64 LL155 (Pioneer, Tokyo, Japan); and local lines AN1IMI, AN3IMI, CX1CON, and CX2TBM.

Seeds were rinsed and immersed in tap water for 3–4 h and then germinated for 36 h in closed plastic boxes under moist paper tissue. Germinated seeds, grouped in batches of plantlets with primary roots of similar length, were sown individually in a horizontal position in well-watered peat soil substrate dispensed in 50 mL PVP centrifuge tubes (diameter 31 mm) filled to the rim with substrate. To each tube filled with substrate, 8–9 mL of water was added, bringing all tubes to the same weight. The water added before sowing was sufficient to cover all the needs of the plantlets until the end of the treatments. After planting, the tubes were inserted in their 17.5 × 20 cm Styrofoam racks and placed in growth chambers that allowed easy access and rapid change or rearrangement of light sources during treatments. The baseline illumination of about 70 μmol m^{-2} s^{-1}, measured at the cotyledon level, was provided by two 30 × 30 cm Mitea 6500 K cool white LED panels. The emission spectrum of this light source, presented in reference ([24]), shows that wavebands of light above 700 nm, including far-red light, are mostly missing. The temperature in the growth chambers was maintained at 24–25 °C. The relative air humidity varied from 52–56% during the day to 60–68% at night.

Baseline cool white LED light illumination of growth chambers was provided in a 14/10 h light/dark diurnal photoperiod, applied for the first 120 h (5 full days) from the start of imbibition, which was done at dawn, corresponding to 7:00 local time, when illumination in the chambers was turned on. The duration of the treatment time (TT) was followed and expressed in hours from the onset of imbibition.

2.2. Monochromatic Light Sources

Monochromatic light was generally provided at irradiance levels of 10–50 μmol m^{-2} s^{-1} or less, with double and triple light combinations not exceeding the 70 μmol m^{-2} s^{-1} level of the cool white LED panels. Blue and red light illumination were provided by two modules, each with 9 high-intensity blue or red light LEDs, (assembled by Stratus, Printwork-Ledwork, Belgrade, Serbia). The peak spectral emission of the LEDs on the modules was at 470 nm for the blue and 660 nm for the red light LEDs. In the same way as the white LED panels, the colored light luminaires were placed directly above the plant racks. Far-red light (Solux, 7 W) was also provided from above, but usually at a small angle. LED luminaires (arrays, strips, lamps, panels) were supplied by Philips, V-TAC, and other manufacturers. Their irradiance was measured using a Li Cor 250 A light meter (Lincoln, NE, USA) equipped with a quantum sensor. Spectral light composition was measured by an Ocean NIR UV 2000 spectrophotometer (Ocean optics, Orlando, FL, USA), generously provided by the Institute of Physics, Zemun, Belgrade, Serbia.

2.3. Shoot Elongation and Tropic Bending Measurements

The relative hypocotyl length of the seedlings was measured as previously described [24], using the rim of the centrifuge tube as the lower hypocotyl position marker and the suture between the cotyledon bases as the upper hypocotyl position marker. Seedling development and hypocotyl elongation were monitored and documented by time-lapse imaging at 10 min intervals using Nikon P510 and P520 cameras. The relative hypocotyl length was measured from the images using ImageJ 1.54 d software and the diameter of the outer centrifuge tube (31 mm) as the scale for measurements. The images were taken in growth chambers, with minimal manipulation of the plants during the treatments. For measurements of hypocotyl bending magnitudes during phototropic stimulation, Gimp 2.10.22 was used.

2.4. Data Acquisition, Management, and Statistics

The numerical data of the measurements and the time-lapse images of all experimental treatments were stored in Origin8 (version 8E) data sheets and files (Originlab Corp. Northampton, MA, USA). Origin8 was also used to perform simple statistical analyses, ANOVA analysis, and graphing. Following the initial pilot test, specific light treatments of all investigated genotypes were replicated at least 2–3 times, with batches typically consisting of 16–24 plantlets.

3. Results

Monochromatic Light

Monochromatic light of any color, provided individually to the plants from the dawn of the sixth day from the onset of imbibition, canceled rhythmic hypocotyl elongation that had been established in the diurnal photoperiods, replacing it with a uniform (arrhythmic) elongation pattern (Figure 1). Illumination with monochromatic light also disabled previously established anticipation of light transitions and prevented plants from predicting daytime or nighttime duration, a fundamental advantage that circadian regulation offers plants. For all colors of monochromatic light, the elongation rates were higher than for white LED panel light. The effects of monochromatic light illumination on the hypocotyl elongation were studied here at rather low irradiances, usually about 10.0–15.0 μmol m^{-2} s^{-1} or less, since the effects of individually applied wavebands were mostly saturated, as shown for blue light in Figure 2. Thus, the light effects presented here can be considered as those related to signaling and not to acquisition of metabolic energy by photosynthesis.

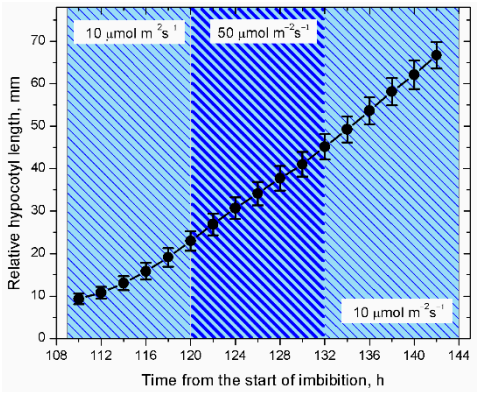


Figure 1. Hypocotyl elongation of cv. Kondi plantlets grown in a white light 14/10 h light to darkness diurnal photoperiod and then placed under green monochromatic light (9.0 μmol m^{-2} s^{-1}). n = 16.

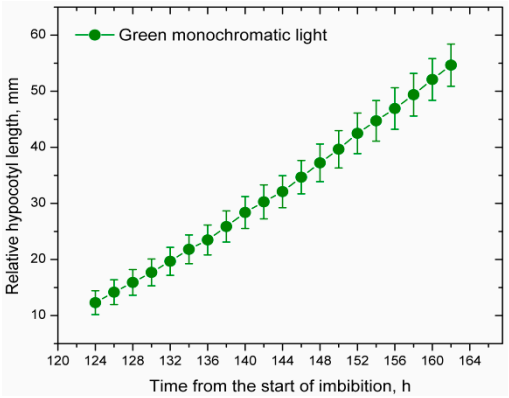


Figure 2. In blue and other monochromatic light treatments, hypocotyl elongation is saturated, showing little changes in the elongation rate if irradiance is increased or decreased. Cv. Kondi; irradiance levels 10 and 50 μmol m^{-2} s^{-1}; n = 16.

We have tested and found that the saturation effects of blue light are also present in phototropic bending. Even in plants illuminated with only 1.0–2.0 μmol m^{-2} s^{-1} blue light, strong bending effects were observed (data not presented).

In plants exposed to monochromatic light and then returned back to white light conditions (Figure 2), further hypocotyl elongation in white light commenced as uniform but at a rather low elongation rate (0.5 mm/h) that could erroneously be characterized as inhibitory. The actual, almost inhibitory effect of white light could only be observed in hypocotyl elongation of etiolated seedlings, where it induced de-etiolation.

In the treatment presented in Figure 3, the far-red light provided at dawn, instead of the expected white light, triggered fast and uniform (nearly linear) hypocotyl elongation. When the seedlings were placed back to white light after 12 h of far-red light illumination, hypocotyl elongation significantly declined; however, subsequent supplementation of far-red to white light fully restored the fast hypocotyl elongation.

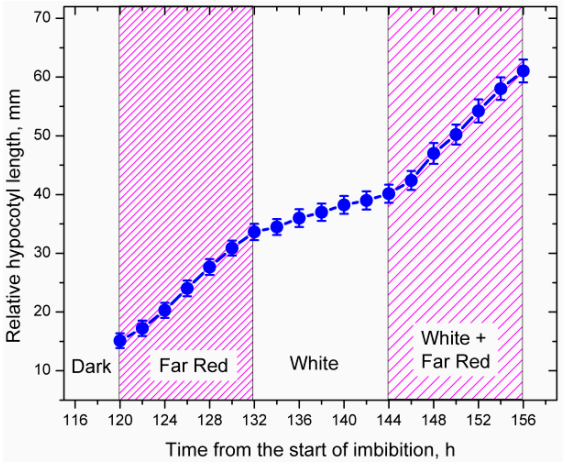


Figure 3. The hypocotyl elongation rate in 2.5 μmol m^{-2} s^{-1} of far-red light is significantly higher than in 70.0 μmol m^{-2} s^{-1} of white LED light, even if it is supplemented with white light. The light of white LEDs either prevents high hypocotyl elongation or requires more of the stimulatory far-red light or both. Cv. H7749; n = 21.

Six hours from the start of far-red illumination in the treatment presented in Figure 3, guttation droplets were observed to appear on cotyledon tips and edges. Guttation was later shown to be triggered by fast light-induced hypocotyl elongation, but only at certain times of the day—namely at midday at about six hours after dawn and again at nighttime several hours before dawn.

In the dark periods that followed illumination with monochromatic light, hypocotyl elongation also became uniform, with an elongation rate of about 0.46 mm/h, similar to the uniform elongation rate of 0.53 mm/h measured for white light following monochromatic light illumination.

The effects of genotypes and light colors on hypocotyl elongation were surveyed using a number of cultivars and breeding lines, illuminated with blue, red, or far-red light for 12–24 h. Four cultivars (Achilles, Sureli, P64LL155, and H7749) were exposed to six different light treatments (Figure 4). Significant differences were observed not only between light color treatments but also between the cultivars. But, the differences between the cultivars were apparent mostly in the time at which seedlings appeared above the soil surface (Figure 5). The fastest emergence from the soil was always in cvs. H7749 and Sureli, and the slowest was in cv. P64LL155. In the graphs showing hypocotyl elongation in cultivars grown under the same light color (Figure 5), the lines representing elongation patterns of each cultivar were parallel.

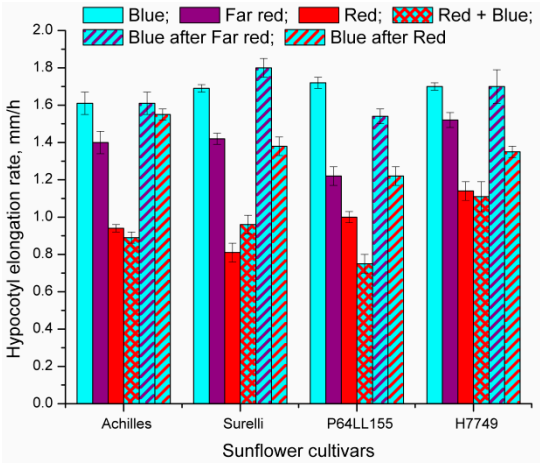


Figure 4. Genotype and monochromatic light treatments as factors affecting the relative rate of hypocotyl elongation mm/h. Average values ± SE of the mean; n = 15–20.

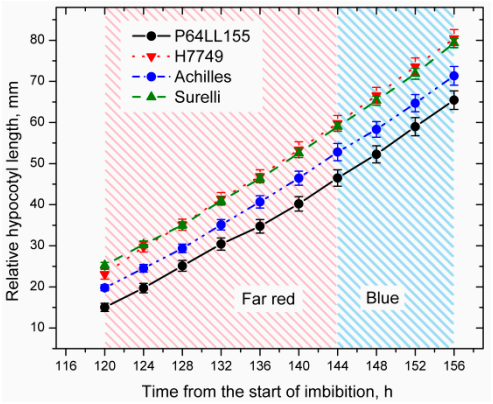
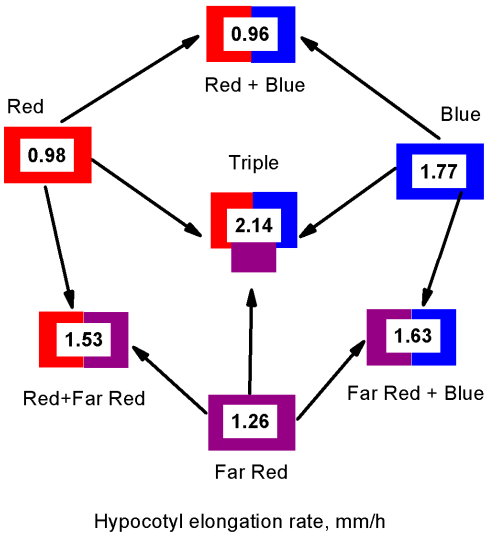


Figure 5. Hypocotyl elongation in seedlings of sunflower cvs. Sureli, Achilles, H7749, and P64LL155 grown under far-red light (2.5 μmol m^{-2} s^{-1}) for 24 h with a transfer to blue light (10.0 μmol m^{-2} s^{-1}). n = 20–24.

Numerous interactions between different light colors were observed in the regulation of hypocotyl elongation. The first interaction observed was between red and blue light when they were applied together (red + blue light doublet), as shown in Figure 6. In this doublet, the rapid elongation promoted by blue light when provided individually was prevented, and the elongation rate of the doublet as a whole was suppressed. It was as high as when the red light was administered individually (Figure 6). However, the suppression

of hypocotyl elongation induced by red light was also visible when the illumination with blue light followed the previous illumination with red light. This interaction was not present when red light was replaced with far-red light, showing that only red light suppressed the promoting effect of blue light (Figure 6). Taken together, these results indicate a strong negative interaction between red and blue light.



(**A**)

Group	Number of plants	Elongation rate mm/h ± SE of the treatment	Statistical significance	Light color of the treatment
1	120	0.910 ± 0.046	a	Red + Blue
5	106	0.979 ± 0.046	a	Red
4	95	1.268 ± 0.05	b	Far Red
3	107	1.532 ± 0.048	c	Red + Far Red
2	107	1.632 ± 0.048	c	Blue + Far Red
6	102	1.777 ± 0.050	d	Blue
7	116	2.142 ± 0.048	e	Red + Blue + Far Red

(**B**)

Figure 6. (**A**) Hypocotyl elongation rates (mm/h) in doublets and the triple light combination compared with elongation rates in treatments with individually provided light colors. Average rate of relative hypocotyl elongation ± SE of the mean. Treatments consisted of batches with 15–20 plants from 4–6 different cultivars and lines (Achilles, Sureli, P64LL166, H7749, Kondi, CX1CON, AN3IMI) providing n = 95–120 plants per light treatment. (**B**) One-way ANOVA average values labeled with the same letter are not significantly statistically different according to Duncan's multiple range test at $p \leq 0.05$.

Thus, there are two types of light color interactions. A major interaction appears when lights of two different colors are applied simultaneously as a doublet, while the second, somewhat less pronounced interaction comprises post illumination interactions that occur when two different colors of light are applied in succession. For this reason, we tested the other two doublet light combinations (far-red + blue and far-red + red) and their post illumination combinations with shorter illumination times and additional genotypes.

Both (far-red + blue) and (far-red + red) doublets supported similar, fairly high hypocotyl elongation rates. In the first doublet, the presence of far-red only slightly reduced rapid elongation by blue light. In the other doublet (far-red + red), red light did not suppress but rather promoted hypocotyl elongation, acting synergistically with far-red light.

A triple light combination consisting of red + far-red + blue light (10 + 2.5 + 20 µmol m^{-2} s^{-1} irradiance, respectively) was also tested; surprisingly, it supported the highest elongation rate recorded among the different treatments with monochromatic light. In the triple light combination, the hypocotyl elongation rate was four times higher than in the light of the white LED panels, which provided a uniform elongation rate of 0.53 mm/h. With an elongation rate exceeding 2 mm/h, the triple light combination matched the elongation rate of the etiolated seedlings grown in the dark. To test whether the plants simply interpreted the triple light combination as darkness, it was applied to rapidly elongating etiolated shoots, where it immediately stopped any further elongation and triggered de-etiolation. The hypocotyl elongation rates of the individual blue, red and

far red light treatments, their doublets and the triple light combnation are shown together in Figure 6.

When various monochromatic light treatments were provided in a sequence of shorter (up to 8 h long) illumination periods, interactions after illumination were more easily detected. They consisted mostly of initial lags or of carry-over effects in which changes of elongation rates were delayed.

Far-red light was the most constant light color, showing no lags or decay. Blue light had an important directional effect (phototropism) and showed a clear initial lag when applied after the red light or after a period of darkness (Figure 7). Hypocotyl elongation under red light was unstable, showing carry-over effects from previous light treatments that stimulated fast elongation, as in the case where red light followed elongation in a triple color combination that showed postponed decay (Figure 7).

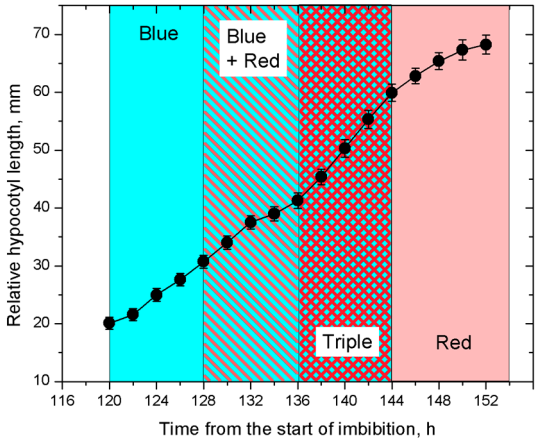


Figure 7. Carry-over and decay of hypocotyl elongation in red light. Fast elongation, in blue light TT 120–128 h, continues initially in the blue + red doublets (carry-over) but later declines in TT 128–136 h. In the triple blue + red + far-red light combination, elongation is strongly promoted (TT 136–144 h), but it gradually declines in the following red light treatment (TT144–152 h). Cv. Sureli; n = 20.

In the red + blue doublets, the hypocotyl elongation rate depended on the ratio of red to blue light irradiance. Higher blue light irradiance favored an increase in the hypocotyl elongation rate, which was suppressed in the doublets with higher red than blue irradiance (Figure 8).

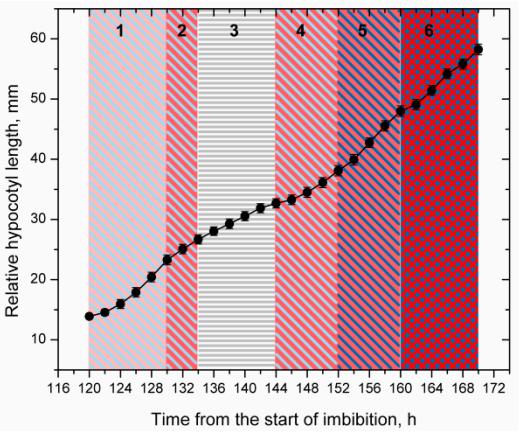


Figure 8. Hypocotyl elongation in red + blue color doublets with components at different irradiance levels. Treatments include: 1. white LEDs; 2. red + blue; 3. dark; 4. strong red + blue; 5. red + strong blue; and 6. strong blue + far-red light. Light irradiance (μmol m^{-2} s^{-1}): red (10.0), strong red (20.0), blue (20.0), strong blue (40.0), and far-red light (7.0). Cv. P64LL155; n = 17.

Circumnutations are a fundamental feature of sunflower hypocotyls, visible in young plantlets as soon as they break and appear above the soil surface. With increasing hypocotyl length, the circumnutations became more conspicuous. By irradiating plantlets with blue light, the circumnutational movements could be suppressed. Blue light was equally effective when applied alone (individually) or in combination with other light colors (Figure 9A).

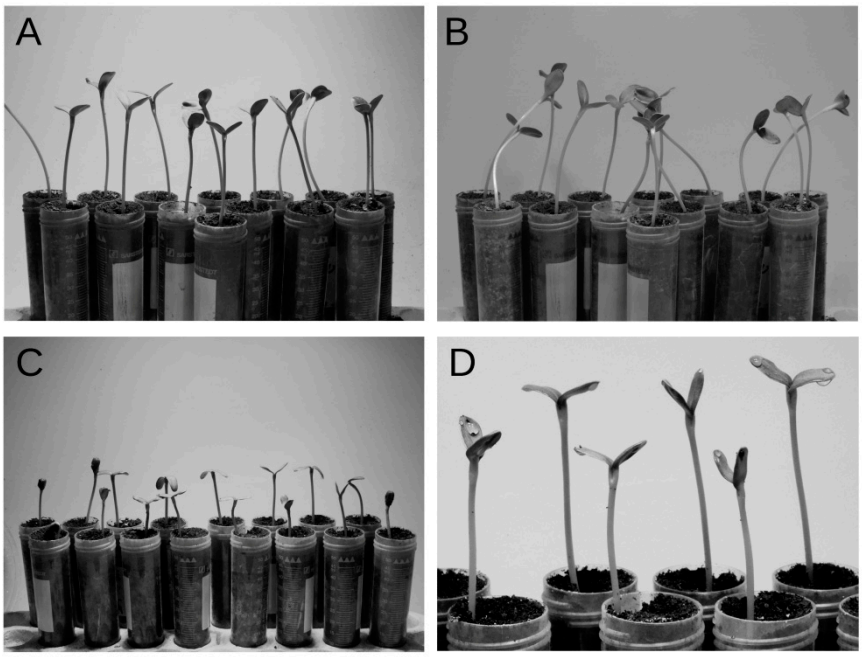


Figure 9. Circumnutations (**A**,**B**) and guttation (**C**,**D**). (**A**) At TT 146 h, which was at the end of a 6 h treatment with a blue + far-red light doublet, the average hypocotyl length exceeded 60 mm. (**B**) Blue light was then replaced by red light, while far-red light was maintained. The replacement of blue light with red light was sufficient to trigger circumnutations, which became intense after 150 min. (**C**) Guttation in a batch of plants exposed to blue light for 8 h at TT 120 h became visible at TT 126 h and continued with the doublet application of blue + far-red light for the next 8 h, reaching the highest expression at TT 136 h (**D**), before it gradually declined in the triple combination of blue + far-red + red light. Treatments were set with AN3IMI plants.

Treatments with monochromatic light usually had to be discontinued after about 55–60 h of illumination. At this point, the exceptionally long hypocotyls showed a tendency to enter vigorous circumnutations or simply sag under their own weight and lean to one side (Figure 9B).

Guttation droplets were usually observed at the cotyledon tips and edges of seedlings illuminated with monochromatic light. Although guttation was first observed in response to far-red light, as in the treatment shown in Figure 3, it is actually not triggered by any particular light color or light combination. Guttation occurs only when rapid light-driven hypocotyl elongation (any color or combination of colors) is going on at specific times of day, six hours from dawn (TT 126 h) or four hours before the dawn (TT 140 h). Guttation is therefore the result of a coincidence or rather cooperation between light signaling and circadian regulation.

Guttation (Figure 9C,D) was generally absent during the daytime in plants cultured in growth chambers with white LED light in which the air humidity varied between 52 and 58% during the day. However, in treatments with monochromatic light, guttation occurred even without high air humidity—a factor that triggers guttation in the late night and early morning hours in many plant species [26]. Guttation is only one of the many manifestations

of positive root pressure [27] whose function in sunflower has been known for more than a century [28].

Attempts to create diurnal photoperiods using periods of monochromatic illumination alternating with periods of darkness were not successful. In this case, hypocotyl elongation in darkness simply continued at the rate characteristic of the last monochromatic light before darkness. Thus, when monochromatic light replaces white light, darkness actually becomes redundant.

4. Discussion

At dawn of the sixth day, sunflower plantlets that had emerged above the soil substrate the previous day were well entrained to the 14/10 h light to dark photoperiod in which they had grown, anticipating the approach of dusk and the light transitions in the following days. This is actually a very fast entrainment, as the plants were hardly exposed to the white light of daytime for more than a day. The real question here is how long the plants need to be exposed to the light of a photoperiod to become successfully entrained? If this time is just a single day or slightly more, as we have shown here, then, we can hypothesize that their entrainment reflects the simple expectation that the next daytime and nighttime duration will be the same as the previous day. The circadian clock has no problem adopting to and overcoming small changes in light period duration, similar to those that occur in nature; however, large changes can apparently disrupt the fragile circadian rhythm freshly established the previous day.

An extended daytime duration in the diurnal photoperiod, resembling the start of constant light conditions, can induce drastic changes in the production and accumulation of important phytohormones in sunflower seedlings [29]. These changes are transient, as in treatments with continuous light, they are absent at subjective dawn. However, the abolition of circadian regulation upon illumination with monochromatic light is a different case and somewhat more difficult to explain.

A more interesting explanation for the occurrence of rapid entrainment is that plants can adjust and utilize some basic circadian rhythms that started earlier in the plantlets. In order for such an adjustment to take place, the two processes sharing the same circadian rhythm need to be functionally connected. And, with positive root pressure, we have a good candidate for a process to which hypocotyl elongation timing can adjust. Positive root pressure in sunflower [30] is responsible for upward water translocation, allowing water and minerals to move from the roots into the shoot system [31].

Root pressure is a basic process that begins early in the individual life of sunflower plants. It does not initially require light for its functioning, which can be easily demonstrated as the decapitation of young etiolated seedlings leads to the exudation of liquid droplets from damaged hypocotyls. Under diurnal photoperiods, root pressure is just as rhythmic [32] as under continuous light conditions [33].

The circadian rhythmicity of root pressure in sunflower plants grown under diurnal photoperiods is well documented and shows a daily maximum at midday [32]. The rhythm of root pressure can be altered by applying light at different times during the nighttime, which induces phase shifts. Phase shifts are also susceptible to the quality of light, as they respond strongly to the color of the used monochromatic light. It has also been shown [32] that the light signal that regulates the timing of root pressure is perceived by the apical shoot segments at the level of cotyledons and is transduced downwards from there, taking about half an hour to reach the effectors located in the root region.

It is remarkable that, even though sunflower has been used for decades as a model system for shoot elongation and similar studies, the outstanding property of its root pressure feature has never been considered or analyzed in connection to hypocotyl elongation.

But, why is hypocotyl elongation suppressed in white light compared with monochromatic light? First, it is important to note that after illumination with monochromatic light, subsequent treatments with white light or with darkness only result in uniform hypocotyl elongation, with no trace of the earlier diurnal rhythmicity. Second, the light from white LEDs, which corresponds to natural solar radiation, is also a mixture of different wavebands in which all colors except blue and far-red support fairly low hypocotyl elongation. We have shown for blue and red doublets that the ratio of blue (high support) to red light (low support) determines the resulting rate of hypocotyl elongation (Figure 8). As most of the radiation in the spectrum of solar radiation or white LEDs are wavebands that are less supportive of hypocotyl elongation, such as red light [24], it is not surprising that white light mixtures generally do not support hypocotyl elongation.

It is difficult to estimate how many different light colors must be provided to the plants before they can accept the light mixture offered as the white light of daytime that follows diurnal rhythmicity. It is definitely not a triple combination of blue, red, and far-red light—which provided very fast elongation—or a combination of red, green, and blue light from RGB LED strips—which also supported high hypocotyl elongation [24].

Recently, multiple pigment systems and large-scale gene expression involved in phototropism, heliotropism, and autostraightening were studied in tissues of mature sunflower plants at anthesis [34]. The results suggest that transcriptional regulation of heliotropism is distinct from phototropism and that multiple light signaling pathways are likely involved, with photoreceptors for red light potentially playing a role.

The effects of individual monochromatic light colors that disrupt the rhythmicity of hypocotyl elongation are of particular interest because these effects are saturated, i.e., the light responses do not depend on the light irradiance applied. Light intensity interactions are temporarily suspended under monochromatic light illumination; darkness becomes redundant and plants lose the ability to anticipate upcoming changes in the light regime. This also explains why nothing special happens in the dark after illumination with monochromatic light, as the hypocotyls continue to elongate at the rate characteristic of the last light color with which the plants were irradiated before the start of darkness.

We previously observed that the diurnal rhythmicity of hypocotyl elongation does not function under certain light conditions such as prolonged extended daytime duration [24], indicating a possible malfunction of the circadian clock. However, it was recently found that this is not the case, as the major genes of the circadian clock function and maintain their rhythmicity even under prolonged daytime conditions in which the rhythmicity of hypocotyl elongation was immediately abolished [29]. Thus, the occurrence of a monochromatic light-triggered arrhythmia in hypocotyl elongation only means that this output can be rapidly uncoupled from the circadian clock, which otherwise continues to run with some synchronization in all cells and tissues, ticking as usual. This may have some adaptive value for plants, as a rapid response of any kind seems to be a much better solution than simply waiting for the circadian clock to reset at late night.

We have shown here a strong positive interaction between red and far-red light and a negative interaction between red and blue light. The positive interaction of red + far-red light, which is visible in doublets, falls into the category of shade avoidance responses that have already been demonstrated and studied in sunflower [9]. Shade avoidance has limited effects in field-grown sunflower, as crop plant density is planned in advance at the time of sowing. Shade avoidance is considered an undesirable trait in all major crops [35], but it still receives considerable attention in experimental studies.

A low proportion of far-red light in the spectrum of white LED panels [21], resulting in the establishment of a high ratio of red to far-red light, simply prevents increased shoot elongation caused by shade avoidance [36]. For in vitro propagation, however, the lack

of shade avoidance in growth chambers with white LED panels can be considered as an advantage, as these light panels allow for a longer subculture duration and uniform growth of rooted plantlets during acclimation.

In the diurnal photoperiod, dark (nighttime) seems to be of great importance for the diurnal rhythmicity of phototropic bending [37]. The presence of pivotal points in treatments starting at night, visible as trend changes of phototropic bending magnitudes and as changes in the duration of phototropic bending lag phases, suggests that darkness in the diurnal photoperiods is a time of significant metabolic activity. Uniform rates of hypocotyl elongation both during illumination with monochromatic light and in the subsequent dark periods indicate rather simple metabolic support. Such a situation has been described for plants that utilize starch for metabolic activities during darkness [38,39].

However, it should be noted that the main metabolic reserves of sunflower seedlings are stored lipids, which account for more than 40% of the dry weight of seeds and were readily available to the plants at the time these experiments were conducted. The high rate of hypocotyl elongation under monochromatic light remained unchanged for more than 48 h, indicating that the internal acquisition and provision of resources required for hypocotyl elongation under monochromatic light or in the darkness is not a problem for young sunflower seedlings.

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