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Original Article

The Effect of Different Doses of Blue Light on the Biometric Traits and Photosynthesis of Dill Plants

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Abstract

The supplementation of blue light to red light enhanced plant growth compared with the use of red alone. The aim of the study was to determine the effect of different doses of blue light on the biometric traits and photosynthesis of dill plants. The plants were grown in pots in a growth chamber. They were grown in red light (100 μmol m^{-2} s^{-1}) and blue light (from 10 to 50 μmol m^{-2} s^{-1}) in five combinations. Light emitting diode modules were the source of light. The plants were evaluated every 7 days during vegetation, for the first time - seven days after germination and later on the 14th, 21st and 28th day after germination. The share of blue light in the spectrum significantly influenced the biometric traits of the dill plants. It significantly inhibited the elongation growth of the plants and negatively affected the increase in fresh weight. A small dose of blue light (20%) had positive effect on the plants' area. The research did not reveal a simple relationship between the amount of blue light and dry weight yield. The value of physiological indexes depended both on the combination and measurement time. The plants from the combination with 30% blue light were characterised by the greatest photosynthesis intensity. An effective share of blue light in the spectrum may range from 10 to 30% in relation to red light and depends on the plant's development phase and on the result we want to achieve in the cultivation of plants.

Keywords: *Anethum graveolens* L., light emitting diodes (LEDs), light quality, photomorphogenesis

Introduction

Spice plants grown and sold in containers tend to have excessively elongated hypocotyl at the initial period of cultivation due to their high density and usually insufficient amount of light. This situation results in worse quality of plants for sale (Callan *et al*., 2007). Dill is characterised by very high elongation of plants (Frąszczak *et al*., 2008). Decreasing light levels result in decreased numbers of dill leaves, lesser leaf area and plant height (Hälvä *et al.,* 1992a). Plant growth and morphogenesis are strongly influenced by the light spectrum and both yield and crop quality could be improved by controlling light quality (Whitelam and Halliday, 2007). Hanyu and Shoji (2002) suggested that yield and crop quality could be improved by controlling light quality. For example, blue light suppresses hypocotyl elongation and induces cotyledon expansion, whereas red light induces hypocotyl elongation and cotyledon expansion in *Arabidopsis* seedlings (Johkan *et al*., 2010). According to Hälvä *et al.* (1992b), red light increased dill plant growth and induced the elongation of internodes. By contrast, blue light-treated plants had shorter internodes and produced relatively high herb yields.

The combination of red and blue light is the most photosynthetically effective at the leaf level. The absence of one of the two light wavebands creates photosynthetic inefficiencies (Hogewoning *et al*., 2010). The percentage of absorption of blue or red light by plant leaves is about 90% (Terashima *et al*., 2009). The combination of red and blue light has proved to be an effective source of light for producing some vegetables (Hirai *et al*., 2006; Fan *et al*. 2013; Sabzalian *et al*. 2014). However, the optimal amount of blue light in the spectrum is still under discussion (Massa *et al*., 2008; Hogewoning *et al*., 2010). The optimal proportion of blue light in red-blue light is expected to range from 7 to 20% (Ptushenko *et al*., 2015). Many studies have demonstrated different combinations of red and blue light to be effective for the growth of some plants. 1:1 ratio was found to be more effective for the growth of cherry tomato plants (Fan *et al*., 2013), 0.9:0.1 would be an acceptable level for the growth of lettuce, spinach and radish (Yorio *et al.,* 2001; Wojciechowska *et al*., 2015). The abovementioned and other publications suggest that crops react to blue light in different ways (Massa *et al*. 2008) and their reaction depends on the species and the photosynthetic photon flux density (PPFD) (Hirai *et al*., 2006; Fan *et al*., 2013).

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Based on the data available to date, one can raise two questions. Firstly, how much blue light should be added to ensure normal growth and development of dill plants? Secondly, does the amount of blue light in the spectrum also depend on the amount of red light or should it be constant regardless of the quantity of red light? Therefore, in this study we tested how a constant amount of red light combined with blue light at different ratios (from 10 to 50%) affected the growth of dill.

Materials and Methods

Plant material and growth conditions

The experiments were carried out in 2014 in growth chambers of the Marcelin experimental station of the Poznań University of Life Sciences, Poland. Dill (*Anethum graveolens* L., cv. 'Ambrozja') was used as a plant material. The 'Ambrozja' cultivar was chosen after another research conducted by the author of this study (Frąszczak, 2009). It is characterised by the highest growth dynamics in comparison with other dill cultivars. The plants were grown in 280 cm^3 pots, in a cultivation area of 49 cm^2, filled with peat substrate for vegetable transplanting production (Klassmann-Deilman, Germany). The number of plants grown in a pot was identical and amounted to 40 (±5 plants). A 16-h photoperiod and a day/night temperature of 23/18 °C were maintained. The relative air humidity was 65-70%.

After germination the dill plants were cultivated for 28 days (four weeks). Red and blue diode modules (type SMD, Seoul Semiconductor, South Korea) were the source of light (Fig. 1). The red light photosynthetic photon flux density (PPFD) was about 100 μmol m^{-2} s^{-1}. The blue light PPFD varied (10-50 μmol m^{-2} s^{-1}) depending on the combination (Table 1). The PPFD was measured with a quantum sensor (PAR-10; Sanopan, Białystok, Poland). The spectral distribution of light treatments was measured with a spectroradiometer BLACK-Comet CXR, 280-900 nm (UV-VIS by StellarNet Inc., Tampa, USA). The measurements were made 15 cm under the lamps, more or less at the height of the tops of the plants.

Growth and morphology analysis

The plants were evaluated every 7 days during vegetation, for the first time – seven days after germination and later on the 14th, 21st and 28th day after germination. Harvesting involved hand-cutting of the plants close to the surface of the substrate. After harvest, the weight of the fresh matter of the plants from eight pots was determined. In addition, measurements of the plants' height, hypocotyl length and leaf area were taken (ten plants per pot, eight pots per treatment). A scanner (Mustek 1200 UB) and the *Skwer* program (*IksmodaR*, Poland) were used to calculate the area of leaves. Dry mass was determined by drying the material to constant weight at 105 °C for 24 h (PN-90/A-75101/03 1990). Photosynthesis was measured using a gas exchange measurement system (LCpro+, ADC BioScientific). Gas exchange was measured using a custom-made leaf chamber - 6.25 cm^2. After steady-state rates of A had been recorded (approx. 1 h), leaves were removed from the chamber and the leaf area was measured. Photosynthesis was measured under the conditions in which plants were grown, in the fourth week of cultivation.

Physiological indices

The relative growth rate (RGR), net assimilation rate (NAR), specific leaf area (SLA), and leaf area index (LAI) were calculated as described by Hunt (1982). The RGR was calculated according to the following formula: $RGR = (\ln W_2 - \ln W_1)(t_2 - t_1)$, where: W_2 and W_1 are plant dry mass (g), at times t_2 and t_1, respectively. The NAR is the increment of biomass per unit of time and per unit of any measure of magnitude of the assimilation organs: $NAR = dW/(A \cdot dt)$, where: A - area of assimilation organs (dm^2), dW- dry mass increment (g), dt - time of cultivation (day).

The SLA is defined as the ratio between the leaf area and the dry mass of leaves. $SLA = L_A/L_W$, where: L_A- leaf area (dm^2), L_W - dry mass of leaves (g). The LAI refers to the area of the leaf surface in relation to the pot area taken up by all plants. It was calculated according to the following formula: LAI =A/P, where: A - plant assimilation area (dm^2), P - pot area (dm^2).

Statistical analysis

The experiment was performed in eight replicates with three pots being treated as one replicate. The investigations were

Table 1. The content of red and blue light in the spectrum

Treatment	Red light (640 nm)	Blue light (455 nm)	Total (PPFD*)
	μmol m^{-2} s^{-1}		
10% BL*	107.8	9.82	117.6
20% BL	107.8	23.2	131.0
30% BL	107.8	33.6	141.4
40% BL	107.8	39.7	147.5
50% BL	107.8	52.3	160.1

* PPFD - photosynthetic photon flux density, BL – blue light

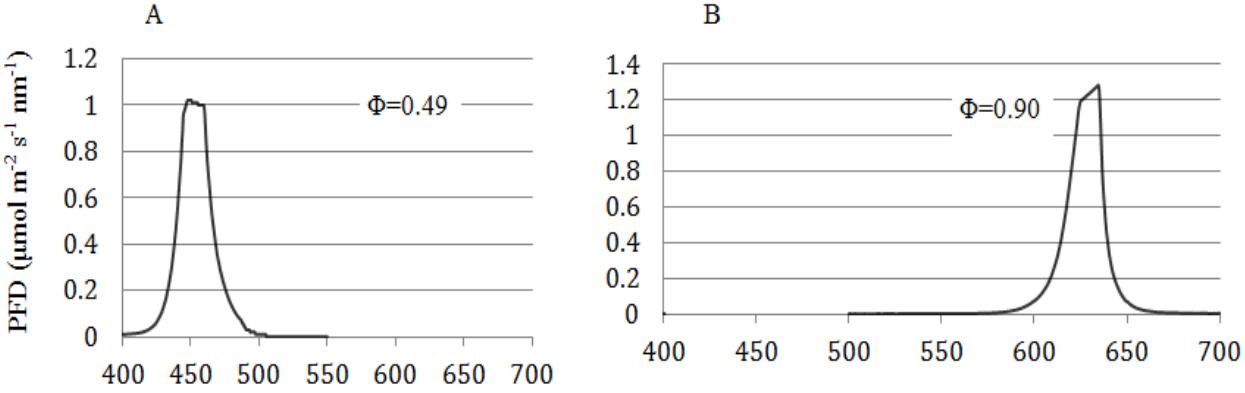

Fig. 1. The spectral photon distribution of LED light sources: blue light (A) and red light (B)

conducted in two consecutive cycles. The results show the mean values of the two series. Measurements of morphology, dry matter, photosynthetic rate and physiological indices were analysed statistically with one-way analysis of variance (ANOVA). Differences between the means were estimated with the Newman-Keuls test at a significance level of $\alpha = 0.05$. All statistical analyses were carried out with the *Statistica* program (StatSoft, Poland).

Results

Morphological parameters

The amount of blue light in the spectrum had significant influence on the hypocotyl length (Table 2). During the first two weeks of growth the shortest hypocotyl was observed in the plants growing under 50% blue light. In the consecutive weeks the hypocotyl was also the shortest in the plants growing under 40% blue light. Blue light had similar effect on the height of the plants. During the whole growth period the tallest plants were observed with the smallest amount of blue light (10%). In the consecutive weeks of cultivation, when the share of blue light was as low as 20-30%, it significantly inhibited the elongation growth of the plants. During the first three weeks of cultivation the plants growing under 20% blue light were characterised by greater area. During the last week of cultivation the area of the plants in all combinations was similar, except the plants growing under 50% blue light.

Dry mass content

It is difficult to prove the direct dependence between the content of dry weight in the herbage and the share of blue light in the spectrum (Table 3) upon analysis of the research findings.

Table 2. The effect of different doses of blue light on the morphological parameters of dill

Treatment	Hypocotyl length (cm)	Height (cm)	Fresh mass (g pot^{-1})	Plant area (dm^2 pot^{-1})
		7th day		
10% BL	3.0 a*	5.7 a	1.58 ab	0.46 b
20% BL	3.1 a	5.3 a	1.76 a	0.63 a
30% BL	3.2 a	5.7 a	1.26 bc	0.43 b
40% BL	2.9 a	5.4 a	1.46 b	0.40 b
50% BL	2.4 b	4.5 b	1.50 b	0.49 b
		14th day		
10% BL	3.7 a	9.4 a	3.96 b	0.61 ab
20% BL	3.4 b	7.6 b	5.52 a	0.91 a
30% BL	3.4 b	6.6 c	2.96 c	0.52 b
40% BL	3.1 b	5.9 d	3.04 c	0.53 b
50% BL	2.4 c	5.8 d	2.96 c	0.59 ab
		21st day		
10% BL	3.8 a	9.6 a	5.32 ab	0.82 b
20% BL	3.7 a	8.9 ab	6.80 a	1.10 a
30% BL	3.8 a	6.8 c	3.28 b	0.55 c
40% BL	3.1 b	8.5 b	3.98 b	0.85 b
50% BL	2.8 b	8.4 b	5.50 ab	0.91 b
		28th day		
10% BL	3.9 a	11.2 a	8.72 ab	1.46 a
20% BL	4.0 a	9.0 b	9.42 a	1.56 a
30% BL	3.9 a	8.8 b	7.24 b	1.54 a
40% BL	3.7 ab	8.8 b	7.60 b	1.34 ab
50% BL	3.4 b	8.7 b	7.04 b	1.10 b

*Values followed by the same letters for individual dates do not differ significantly at α=0.05,

BL-blue light, the percentage relative to 100 μmol m^{-2} s^{-1} red light, see Table 1.

During the whole vegetation period the dry weight yield was significantly lower in the combination with 30% blue light. On the other hand, the highest dry weight yield in the last week of measurements was observed in the combination with 40% blue light.

Net photosynthetic rate

The highest intensity of photosynthesis was observed in the plants with smaller shares of blue light (10-30%) (Fig. 2). The lowest intensity of photosynthesis was observed in the plants from the combination with 40% BL.

Physiological indices

Both the relative growth rate (RGR) and net assimilation rate (NAR) values varied depending on the measurement time and combination (Fig. 3).

At the initial growth period the greatest values of both indices were observed for the plants grown under 20% BL. However, at later periods both the RGR and NAR values were significantly smaller in this combination. The combination 50% BL was characterised by very high variability of these indices, depending on the measurement time. During the whole growth period there was a great value of the specific leaf area (SLA) in the combination grown under 30% BL. The combination was also characterised by a significantly smaller LAI value during the first three measurements. The greatest LAI value was observed in the combination 20% BL. By contrast, this combination was characterised by a significantly smaller SLA value than in the other combinations.

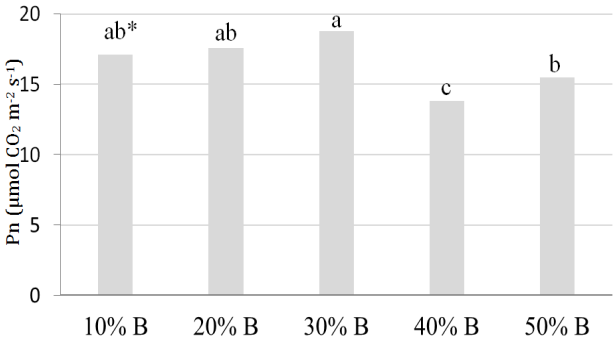

Fig. 2. The effect of different doses of blue light on the net photosynthetic rate (Pn); *values followed by the same letters for individual days do not differ significantly at $\alpha = 0.05$, B-blue light, the percentage relative to 100 μmol m^{-2} s^{-1} red light, see Table 1.

Table 3. The effect of different doses of blue light on the content of dry mass in dill herbage

Treatment	Dry mass							
	7th day		14th day		21st day		28th day	
	(gpot^{-1})	(%)	(gpot^{-1})	(%)	(gpot^{-1})	(%)	(gpot^{-1})	(%)
10% BL	0.11 a*	6.85	0.27 b	6.85	0.45 b	8.40	1.01 b	11.59
20% BL	0.13 a	7.33	0.43 a	7.80	0.84 a	12.40	0.95 b	12.50
30% BL	0.07 b	5.95	0.17 c	5.85	0.36 b	10.95	0.49 c	6.80
40% BL	0.11 a	7.26	0.21 bc	6.87	0.46 b	11.80	1.43 a	15.30
50% BL	0.13 a	8.61	0.20 bc	6.80	0.73 a	13.35	0.74 bc	10.50

*values followed by the same letters for individual days do not differ significantly at $\alpha = 0.05$, ; BL – blue light, the percentage relative to 100 μmol m^{-2} s^{-1} red light, see Table 1.

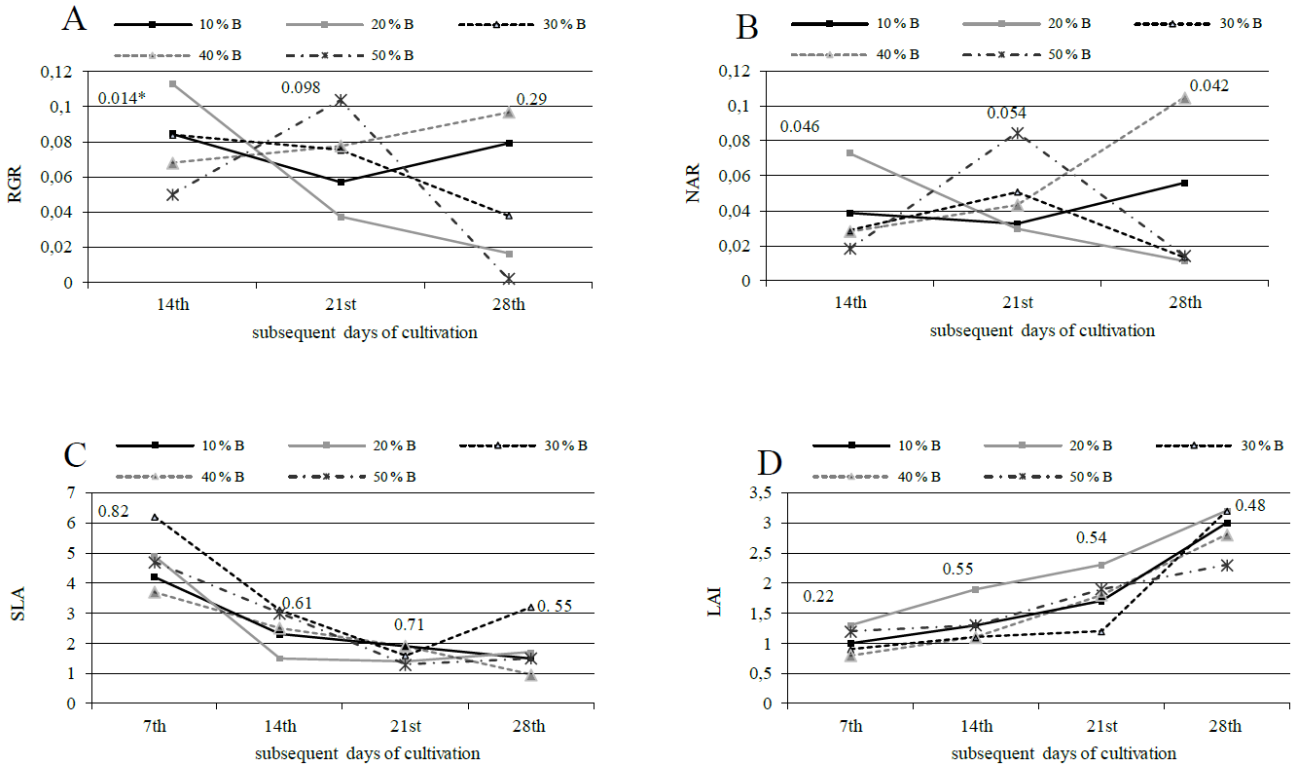

Fig. 3. The effect of different doses of blue light on A: relative growth rate - RGR (g g^{-1} d^{-1}). B: net assimilation rate - NAR (g dm^{-2} d^{-1}). C: specific leaf area - SLA (dm^2 g^{-1}). D: leaf area index LAI (dm^2 dm^{-2});* LSD - least significant differences, n.s. - not significant at $\alpha = 0.05$, B-blue light, the percentage relative to 100 µmol m^{-2} s^{-1} red light, see Table 1.

Discussion

Morphological parameters

Blue light is known to play the main role in photomorhogenesis - therefore, it is necessary for the growth and development of higher plants (Terfa *et al.,* 2012; Woźny 2011). In this study blue light significantly affected the length of hypocotyl and the height of plants. The hypocotyl was the shortest in the combination grown under 50% BL. Kwack *et al.* (2015) also found that blue light suppressed hypocotyl elongation in several vegetable sprouts. However, the response of stem elongation under blue or red light is apparently dependent on plant species (Hirai *et al.*, 2006). Eggplants grown under blue light were characterised by much longer stems than the plants grown under other colours of light. Lettuce exhibited a completely opposite reaction. Plants' sensitivity to blue light and its influence on growth inhibition could be an individual quality of each species.

It is noteworthy that especially at the initial period of vegetation only the greatest dose of blue light (50%) significantly influenced the inhibition of hypocotyl elongation. It is not only the existence of blue light in the spectrum but also the amount of blue light that matters. In the study by Ouzounis *et al.* (2014) an increased blue light ratio resulted in lower heights of roses and chrysanthemums. Also, in the study by Samuolienė *et al.* (2011) the hypocotyl was much shorter when plants were grown under red light with 10% blue light than in the combination with 5% blue light.

At a later period of dill growth blue light did not have such strong influence on the hypocotyl elongation, because the plants' growth was much less intensive.

It was observed other dependences about the height of plants. At the initial period only 50% blue light inhibited the hypocotyl elongation. At later growth periods the hypocotyl elongation was also inhibited by 20-50% blue light. This situation may have been caused by more intensive growth of dill during that period and the plants' greater sensitivity to blue light. Fan *et al.* (2013) observed that the height of young tomato plants decreased as light intensity increased; a similar dependence was observed in this study.

The study proved a noticeable dependence between the share of blue light in the spectrum and the fresh weight of dill. The greatest amount of fresh weight was produced under 20% BL and it did not differ significantly from 10% BL (except the second measurement). Thus, when there was more than 20% of blue light in the spectrum, the growth of fresh weight of dill was inhibited. Ouzounis *et al.* (2015) revealed that the total fresh weight was not affected by additional blue light. Blue LEDs in combination with high light intensities are more efficient for biomass production in plants than red light (Muneer *et al.*, 2014). In the study by Johkan *et al.* (2010) fresh weight was higher in lettuce plants treated with blue light after 45 days from sowing. But after 17 days from sowing there was higher fresh weight in plants treated with red light than in those treated with blue light. Red light may have caused a greater increase in biomass production at the initial period of growth than blue light.

In general, blue light is thought to stimulate the growth of leaf area in plants (Johkan *et al.*, 2010; Samuolienė *et al.,* 2014). In this study at the initial period of measurements the greatest area and LAI were observed in the plants treated with 20% BL. Only the fourth measurement did not show differences in the plants' area or LAI under 10-40% blue light. Hogewoning *et al.* (2010) also reported that in cucumber plants grown under HPS (5% B) the area was twice as large as in plants grown under fluorescent tubes with 23% B proportion. Terfa *et al.* (2013) also observed in their study that the area of rose leaves grown under white light with 20% BL was much smaller than the area of rose leaves grown under 5% BL. There were similar findings in the study by Ouzounis *et al.* (2014), where the largest leaf area was observed in the rose plants in the combinations 20%B/80%R and 100%R, as compared with the combination 40%B/60%R. Dougher and Bugbee (2001) defined long-term B light dose-response curves for leaf area in soybean and lettuce. They showed that this parameter in soybean decreased with increment of the B light proportion while the leaf area in lettuce increased with an increasing B light fraction. In their experiment with soybean, they showed a 23% decrease in the leaf area when the B light fraction increased from 6% to 26%. This was associated with a 15% decrease in the cell area (expansion) and 11% decrease in the cell number. According to these authors, the much lower average leaf area with increasing blue light fraction may be associated with a B light-mediated inhibition of cell expansion (Dougher and Bugbee, 2004).

Net photosynthetic rate and dry weight

According to literature data, plants can have a full life cycle only under red light, but the addition of blue light increases photosynthetic production in some plants (Lee *et al.*, 2007; Wang *et al.* 2015). It is so because cryptochromes (CRYs) and phototropins are specifically sensitive to blue light and phytochromes are specifically sensitive to red light (Whitelam and Halliday, 2007). The greatest Pn value was observed in 30% BL, which did not differ significantly from 10% and 20% BL. On the other hand, the lowest intensity of photosynthesis was observed in the plants grown under 40% BL. Thus, in this study the intensity of photosynthesis increased along with increasing BL only to a level of 30 μmol m^{-2} s^{-1} BL. The plants grown under 30% BL were also characterised by the lowest yield of dry weight. The study by Liu *et al.* (2011) proved that the leaf thickness, the length of palisade tissue cells and the number of stomata in the epidermis of the leaves of cherry tomato seedlings increased significantly under the spectra containing blue light, as compared with the plants grown under other light treatments. According to Sun (2008), the abovementioned factors greatly influence photosynthesis in the leaves. However, it is intriguing that the intensity of photosynthesis decreased in 40% and 50% BL. In the study on Westar plantlets conducted by Li *et al.* (2013) the best photosynthetic parameters were observed in the combination B:R = 3:1, where the share of blue light was much greater than the share of red light. On the other hand, Hogewoning *et al.* (2010) observed in their study that the photosynthetic capacity (A_{max}) increased with increasing blue light percentage during growth measured up from 7% to 50% blue light (the remaining percentage was red light). According to Wang *et al.* (2015), blue light optimised photosynthetic performance by improving the photosynthetic rate under low irradiance.

Greater photosynthetic intensity is usually reflected by an increase in the dry weight in herbage (Darko *et al.*, 2014). When plants are grown in a mixture of red and blue light, the yield of dry weight is also greater than when plants are grown in red light only (Wang *et al.*, 2009). On the other hand, according to Ouzounis *et al.* (2015) lettuce grown under blue LED lighting did not enhance FW and DW, but rather partitioned assimilates for other processes. In our study the smallest content of dry weight was observed in the combination 30% BL, where the photosynthetic intensity was the greatest. According to Yorio *et al.* (2001), a possible explanation for the discrepancy between Pn and dry weight accumulation could lie in the single point Pn measurement in our study.

Physiological indices

The diversified share of blue light had significant influence on assimilative indices. High SLA values were observed in the plants from the 30% BL combination. It was correlated with the greatest Pn value in this combination. Sirtautas *et al.* (2014) conducted a study on lettuce, whereas Samuolienė *et al.* (2010) experimented on strawberries and they proved that the addition of blue light in the spectrum had positive effect on the SLA value. In the studies of Johkan *et al.* (2010) the SLA in lettuce under FL and red light was higher than under blue-containing LED lights. There was a 54% decrease in the SLA between 0 and 2% blue light.

The growth of plants is the result of interaction between environmental factors and biomass allocation parameters determining the potential RGR (Galmés *et al.*, 2005). The RGR was correlated with its physiological (NAR) component. However, studies have not proved a simple dependence between the amount of blue light and the RGR and NAR values. In a study on baby leaf lettuce different supplemental blue light wavelengths had different effects on the growth rate: 455 nm supplemental blue light resulted in an increased NAR value, whereas 470 nm light had no significant effect (Sirtautas *et al.*, 2014).

Conclusion

Blue light significantly affected the biometric traits and Pn value in dill plants. At the initial period of growth only a considerable share of blue light in the spectrum inhibited the elongation growth of the plants. At later periods smaller doses of blue light also affected the elongation of the plants. Thus, we can suppose that the reaction of the dill plants to blue light depended not only on the dose but also on the stage of development of the plants. In general, blue light inhibited the elongation growth, increase in dry weight and the area of dill plants. A high share of blue light (40-50%) also inhibited the net photosynthetic intensity.

The research showed that blue light modified the growth of dill plants and could be used as a tool for controlling plant elongation, especially at the initial growth period.

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