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Original Article

The Effects of Various LED Light Wavelengths to the Physiological and Morphological Parameters of Stevia (*Stevia rebaudiana*) Bertoni

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Abstract

In this study, it was investigated the growth of stevia (*Stevia rebaudiana* Bertoni) under various wavelengths of LED lamp (Light Emitting Diodes), which can emit daylight (cool white; 400–700 nm), red (620–630 nm) and blue (465–485 nm) wavelengths of the light in the electromagnetic spectrum. In all applications, quantity of PAR (photosynthetically active radiation) was adjusted as 150 $\mu mol.m^{-2} s^{-1}$. Study had maintained in plant breeding cabin 16 hours light and 8 hours dark environment. Results demonstrated that while the highest plant height was determined in the "30% blue light+ 70% red light" application, the highest stem length was found in the "50% blue light + 50% red light" application. In addition, the number of the stems reached the highest value in the "70% blue light + 30% red light" application. Consequently, a correlation was observed between negative "*a*" value and the amount of chlorophyll. Because of the hereby obtained results, comparing to other applications, the "50% blue light + 50% red light" was found as the best light application to obtain optimum yield values of stevia.

Keywords: blue light, stevia, light-emitting diodes, red light, PAR

Introduction

Stevia (*Stevia rebaudiana* Bertoni) is a perennial plant from *Asteraceae* family. Stevia has nearly 230 species as herbaceous, bush and half bush (Cimpeanu *et al*., 2006; Yadav *et al*., 2011). Because this plant includes steviol glycosides, it is used commercially in many countries with the purpose of sweeting and flavorant instead of synthetic sweetings. The leaves of stevia include glycosides of dulcoside-A, rebaudiosides A, B, C, D, E, stevioside that are sweeter than sucrose (Carneiro *et al*., 1997). The dry leaves of this plant are 15-20 times sweeter than sugar and also powder extracts of this plant are 300 times sweeter than sugar and it is zero calories (Singh and Rao, 2005). So, diabetics and dieters (Fronza and Folegatti, 2003) can conveniently consume stevia. Stevia has diuretic, analgesic and blood pressure-lowering characteristics and it can be used for stomach ache with the purpose of herbal treatment (Argueta and Cano, 1993). Moreover, it has important biological activities such as antimicrobial and antifungal properties (Cerda-García-Rojas and Pereda-Miranda, 2002) and it has antioxidant effects (Okawa *et al*., 2001). The research on rats has been shown that stevia has no side effect and its usage has been recommended to diabetics (Megeji *et al*., 2005). Because of these prominent properties, the production and trading of stevia are desired in many countries. Therefore, stevia is produced commonly in Paraguay, Brazil, Mexico, Uruguay, Guatemala, Peru, Japan, South Korea (Inanç and Cınar, 2009). In terms of cultivation, this plant has been grown better in the regions, which have sufficient moisture and well drainage clayey, alluvial or red soils and 1500-1800 mm rainfall per year with 25 °C average temperature (Shock 1982; Singh and Rao, 2005). With greenhouse farming, small areas are made useful in order to take high efficiency from unit area without depending on climatic conditions (Sevgican *et al*., 2000). However, light is critically important, in order to get a good efficiency from plants, because light energy is needed for photosynthesis and producing organic matter via using inorganic matters (water, carbon dioxide) and chlorophyll (Anonym, 2015). The quality of light has important effect on morphological characteristics of plants such as lengthening of plants, stem forms, leaf anatomies and leaf sizes (Yağcıoğlu, 1996). Especially in greenhouse farming, the amount of light may not be enough for sufficiently growth and flourishing of plants, therefore, most times it is necessary to make photoperiodic illumination and provide the lack photosynthetic active radiation (PAR) energy with synthetic light sources. Photoperiodic illumination provides artificially organizing of illuminated time in order to control the plant growth (Çağlayan and Ertekin, 2011). Red lights is major important for plant growth because it induces transformations in photochrome system of plants (Furuya, 1993). Blue light is necessary for plants because it is effective on consisting of chlorophyll, opening of stomas and photomorphogenesis (Urbonaviciute *et al*., 2007).

In photoperiodic illumination, if high dose red light and blue light is given together, it incites blooming and seed

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production. Blue light is responsible for vegetative growth and it supplies the morphologically healthful of the plant. Chlorophyll that exists in the green leaves of the plant affects the photosynthesis ability and speed. In artificial illuminations for agricultural purpose, LED lamps are more preferred because they can emit a specific wavelength of light. The AllGaP based and powerful LEDs are able to emit 660 nm wavelength of light and this wavelength is important because it is near to 640 nm wavelength, which is important for plants. So, the PAR energy, which is necessary for plants, is utilized more efficiently (Koç *et al*., 2009; Çağlayan and Ertekin, 2011). In this research, the physiological and morphological differences of stevia, which was grown under various wavelengths of LED light, were examined. Therefore, this study goal is to determine the most appropriate wavelengths of light, in order to increase the stevia yield.

Materials and Methods

Experiments have been performed in 252-Liter capacity climatic controlled plant cultivation cabin (Nuve, TK252) located in Akdeniz University Faculty of Agricultural, Agricultural Machinery and Technologies artificial illumination laboratory in 2013 March-November (Fig. 1). LED lamp is designed specially for cabin and it consists of Power LED belongs to CREE firm XLamp XP-C high flux (1W, 350 mA). The lamp can emit daylight (cold white; 400-700 nm; 6500 K), red (620-630 nm) and blue (465-485 nm) wavelengths of the light (Fig. 2).

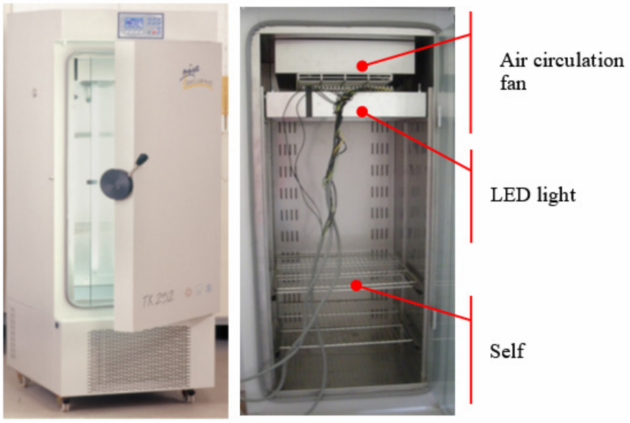

Fig. 1. Plant cultivation cabinet (Nüve, TK252)

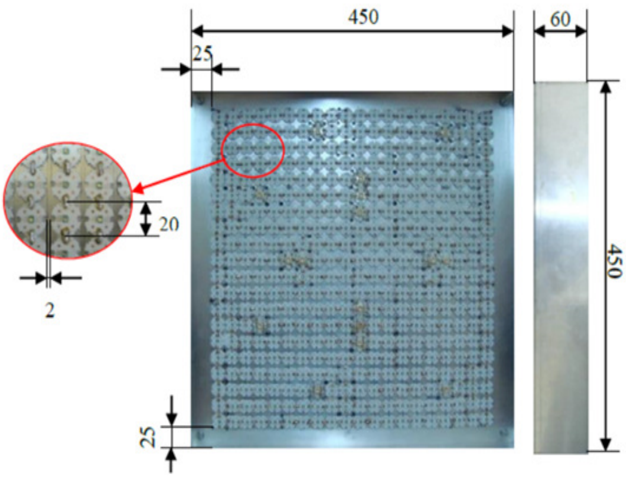

Fig. 2. LED lamp

In the research, four light types were used as "50% blue + 50% red light"; "70% blue + 30% red light"; "30% blue + 70% red light" and daylight and lighting experiments, which each one lasts 60 days were performed. In each day, 16 hours light and 8 hours dark applications were carried out. The total PAR quantity of red light and blue light was kept constant at 150 $\mu mol.m^{-2}.s^{-1}$. Cabin interior environment, air temperature and relative humidity values were arranged as 22 °C, 70% during illuminated time and 18 °C, 75% during dark time respectively. PAR quantum sensor (LI–190, LI-COR Inc.), has been used for measurements of PAR values and a data logger (DL2e, Delta-T Devices) has been used for recording PAR temperature and humidity values.

At the beginning of the study, seeds were taken from stevia seedlings cultivated in open field and these were germinated in viols. After one week, the seeds were germinated and they were transported to climate-controlled growth cabin. Experiments were conducted as a randomized complete design method with three replicates and five plants were used for each application, according to experimental design. Seeds were sowed to viols as for that totally fifteen plants for each viol.

Each application lasted 60 days and after the application and for each plant, the amounts of chlorophyll L, *a*, *b* values, stem and root lengths, the number of leaf and the thickness of plant were measured. The obtained data were analyzed by appropriate analysis of variance and Duncan grouping method was performed whether there was a relationship between the different light applications. MSTAT-C software was used for performing of analysis (Freed, 1988).

For determining the chlorophyll amount, measurements were performed from each leaf with a chlorophyll meter (SPAD 502) for three times and average values were calculated (Zhu *et al*., 2012). In determining color changes in leaves of plant, leaf samples were taken randomly from all plants and these samples were measured by using a chroma meter (Minolta CR-200) as CIE L, *a*, *b*. The value of L expresses the brightness and takes different values between 0 and 100. Upon the value of L is 0, which means black color, there is not any reverberation and when it takes 100, it means white color that has perfect reverberation (Fig. 3). On the other hand, positive "*a*" value represents red color and negative "*a*" represents green color. While positive "*b*" values present yellow color, the negative "*b*" values represent blue color. At the point of zero-cut (*a*=0 and *b*=0), it becomes colorless or grey (Fig. 4). Chroma (C*) and hue angle (h*) values have been calculated by using equations below (McGuire 1992):

$$C^* = \sqrt{(a^*)^2 + (b^*)^2} \quad (1)$$

$$h° = \tan^{-1} (a^*/b^*)] \quad (2)$$

Results and Discussion

Chlorophyll amounts of leaves

The chlorophyll quantity existing in leaf is one of the factors that affect the photosynthesis speed and it is a significant indicator used for measuring the growth of chloroplast, photosynthetic capacity, content of leaf nitrogen and general vitality of plant (Yol and Uzun, 2011). During light reaction, light is absorbed by chlorophyll pigment and induced an electron transport (Jao and Fang, 2003). In this research, a chlorophyll meter (SPAD 502, Spectrum Technologies, Inc.) was used for determining the chlorophyll quantity in plant samples.

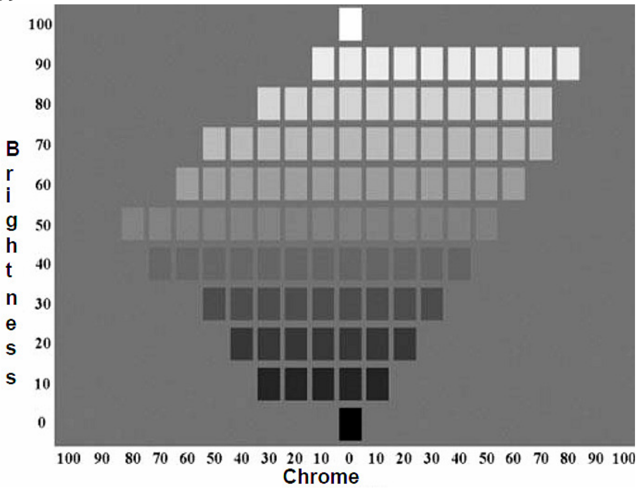

Fig. 3. Brightness - chrome diagram

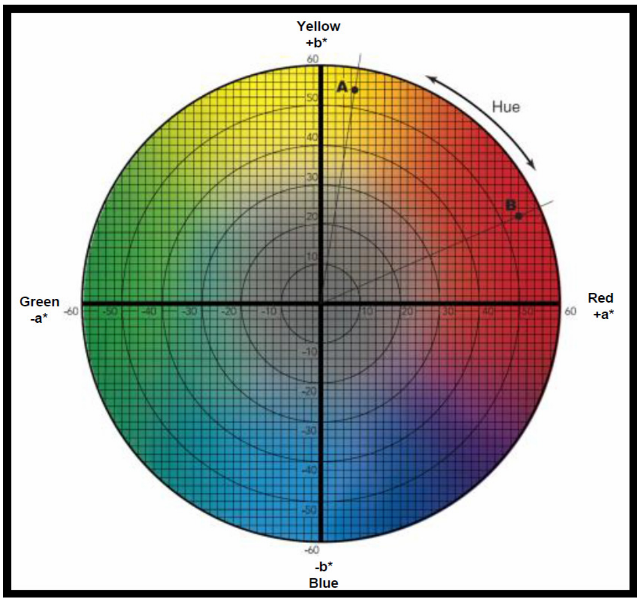

Fig. 4. Colour diagram

According to obtained results, the plants applied the blue light, red light and daylight have statistically significant (P ≤ 0.05) effects on chlorophyll amounts (Table 1). Hence, the maximum chlorophyll amount was acquired in "50% blue light + 50% red light" application (39.08) and "70% blue + 30% red light" and "30% blue + 70% red light" applications respectively follow it. Chlorophyll molecules absorb the blue (460-480 nm) and red (650-700 nm) radiations better and these wavelengths are more effective on plant growth (Yeh and Chung, 2009). Therefore, the minimum chlorophyll amount (32.44) was determined in plants cultivated under daylight. In a study on tomatoes (Çağlayan and Ertekin, 2011), while the maximum chlorophyll amount was obtained in seedlings under MHL (Metallic Halide Lamp) light, the nearest measurement to this value was determined in the "10% UV-A + 10% red light + 70% blue light" light application. In another study, the effect of red LED light on chlorophyll synthesis was emphasized (Tripathy and Brown, 1995). While in a study carried out by Brazaityte *et al*. (2010), decreasing was observed in chlorophyll amount with the orange (622 nm), yellow (595 nm), green (520 nm) and UV (380 nm) light applications on tomatoes, in an another study in which was used the same lights and same plant, the highest chlorophyll content was determined under yellow light (595 nm) (Brazaityte *et al*., 2010). In addition, when Jao *et al*. (2005) compared LED applications, the blue light mainly took the chlorophyll growth control mechanism task. According to Wu *et al*. (2011), the highest SPAD value was obtained with "70% red light + 30% blue light" application on strawberry plant.

L, a, b values of leaves

When the PAR that is active radiation area for photosynthesis is considered, it is observed that light is a factor that affects the photosynthesis speed of plants. Upon the blue and red lights are removed from environment, life of plants stops and death occurs. Furthermore, the amounts of red light and blue light change depending on the incidence angle of solar lights to the earth and seasons. According to Seeman (1952), when solar lights come to the earth with 10-16 degree angles, the amount of red light is twice more than the amount of blue light. Because incidence angle of solar lights increases in summer months, the amount of blue light increases. On the other hand, owing to decreasing of incidence angle in the winter, the amount of red light increases. Sometimes, insufficient blue light can causes to yellowed leaves (Mc Cree, 1972; Çağlayan and Ertekin, 2011). In addition, the color differences are observed in plant leaves indifferent periods depending on the seasonal or nutrient content changes. In our study, on behalf of these different wavelengths of the light, the color parameters of plant leaf were measured with color spectrophotometer and the effects of light applications on negative "*a*" and positive "*b*" values (respectively P ≥ 0.01 and P ≥ 0.05) were regarded as statistically significant.

In obtained findings, while the "*a*" value representing green color decreases, green color of leaves increases and the highest "*a*" values were obtained in "50% blue + 50% red light" application (18.51) and the lowest "*a*" values were obtained in daylight application (15.46). According to Öztürk (2008), blue light caused the darker green leaves. Furthermore, because the plants grown under red LED had less chlorophyll and photosynthesis ratio, yellowing was observed in their leaves. Nevertheless, according to some reports, a normal plant growth can be observed even just under red light without blue light depending on plant type (Nhut and Nam, 2010). As for our study, very low chlorophyll amount was determined in intense red light application and the negative "*a*" value representing the chlorophyll quantity in leaf and green color of leaf was obtained in same application. Therefore, there is also a strong relationship between negative "*a*" values and chlorophyll content of stevia. Hence, it was determined that the leaf chlorophyll content and color changings of stevia showed a parallel alteration depending on the amount of light that applied to plant. In another study, there is a strong relationship between leaves "*a*" color values and chlorophyll contents (Demirtaş and Kırnak, 2009). Furthermore, upon positive "*b*" values representing yellow color were evaluated, while the highest "*b*" value was obtained in white light application (24.77), it was not observed a significant difference between white light and "50% blue light + 50% red light" application (23.88). Additionally the lowest "*b*" value was determined in "70% blue + 30% red light" application (21.40). Therefore, according to these obtained data, light applications in different wavelengths had a significant effect on leaf color of stevia plant.

The "L" value represents brightness of plant leaf for L, a, b measurements. According to our results, the different light applications had a statistically significance ($P \le 0.01$) on "L" values (Table 1). Hence, the highest "L" value was obtained from "70% blue light + 30% red light" application (44.50) and "50% blue + 50% red light" and daylight applications followed this respectively. On the other hand, the lowest "L" value was determined in "30% blue + 70% red light" application (40.34). In other words, at the end of the 60 daily lighting experiment, it is determined that "70% blue + 30% red light" application, which has intense blue light, generates more bright leaves than other applications.

Growth parameters

Growth, yield and quality of plants are not directed just by genetic factors. Light is also one of the environment factors that necessary for photosynthesis and growing of plant just like plant nutrition elements, quality of water and temperature. These factors are among the delimiting or enhancing factors of plant growth. Light is the elementary energy source for photosynthesis (Yağcıoğlu, 1996) and plants need to sufficient light for growth. Increase in light intensity, which is in certain limits and ratios, speeds up growing in plants. In addition, it has a significant impact on determining of some morphological characteristics such as lengthening of plants, root shape and leaf anatomy (Vardar, 1975; Heuvelink, 1989; Uzun, 1996; Kevseroğlu, 1999; Aybak, 2002). In addition, photosynthesis reaches the highest speed in the blue and red regions of the electromagnetic spectrum (Öztürk, 2008).

According to statistically analysis of obtained data, in various wavelengths of light applications have statistically importance on the plant height, the number of root and the root lengths. While the longest plant height was obtained in "30% blue +70% red light" application (13.6), the shortest plant height was determined in "50% blue + 50% red light" application (8.04). Generally, while the cold colors (e.g. blue, 460-480 nm) cause to short plants, the hot colors (e.g. red, 650-700 nm) incite the elongation of plants (Çağlayan and Ertekin, 2011). Red light is effective on stem elongation of plant, leaf growth and chlorophyll synthesis (Tripathy and Brown, 1995) but it can cause to decrease in chlorophyll content of leaf (Nhut and Nam, 2010). When the plants that include extremely high chlorophyll are compared, the plants that include less chlorophyll use chlorophyll more efficiently (Saebo *et al*., 1995). As for our study, less chlorophyll ratio (35.92) was determined in "30% blue + 70% red light" application, which had more intense red light compared to applications that had more intense blue light. In this application, the longest plant height was also obtained.

There are indirect or direct effects of light intensity, light type and light influence time on plant growth. Both red light and blue light incite the cell elongation in plant stem and leaves. Moreover, photosynthesis ratio and dry matter production increase (Matsuda *et al*., 2004; Spalding and Folta, 2005). According to Jao and Fang (2004), irradiation of the plants with red and blue lights at the same time had positively effects on plant growth. The responses of plants to blue and red LED ratios change depending on their species (Nhut and Nam, 2010). Çağlayan and Ertekin (2011) reported that under more intense blue light applications, they obtained short seedlings but had thicker stems. According to Brazaityte *et al*. (2010), while the red, blue and UV lights positively affected the growth of tomatoes. Wu et al. (2011) has reported the maximum plant dry weight in strawberry under "70% red and 30% blue light" application. While Piszczek and Głowacka (2008) observed the maximum height in cucumber under the blue light application, Jao *et al*. (2005) determined the longest plants under the red LED light for black magic.

In our study, the different wavelengths of light showed significant effects on root lengths of stevia. Therefore, while the maximum root length was observed under "50% blue + 50% red light" application as 16.44 cm, the minimum root length was determined under daylight application as 13.20 cm. It is most probably, the low red light ratio obstructs growth of roots of stevia, because red light positively affects the root growth of plant (McCree, 1972; Çağlayan and Ertekin, 2011). On the other hand, while maximum number of root was obtained under "70% blue and 30% red light" application (7.8), minimum number of root was observed under red light and "30% blue light and 70% red light" applications (4.6). As a result, wavelengths of light have significant effects on plant heights, root lengths and the number of root of stevia.

Conclusions

Electrical artificial light sources are generally used for irradiation of plants in order to provide PAR (effective radiation in photosynthesis) energy when the natural light is

Table 1. The effects of lighting applications in wavelength on morphological and physiological properties of stevia plant

Applications	Test index						
	L**	a**	b*	Chlorophyll content*	Plant height*	Root height*	Root number**
T1	44.22a	-18.51a	23.88ab	39.08a	8.04b	16.44a	4.6b
T2	44.50a	-15.48b	21.40b	36.05ba	9.04b	15.56ba	7.8a
T3	40.341b	-16.26b	23.23ab	35.92ba	13.6a	14.00bc	4.6b
T4	43.5a	-15.46b	24.77a	32.44b	9.20b	13.20c	6.4ba

In lines and columns, according to Duncan Test at the level of $P \le 0.05$ different environments were denoted with different letter. (According to *0.05, according to **0.01 statistically significant) ("50% blue+50% red", "70% blue+ 30% red", "30% blue+70% red", daylight) not sufficient. One color wavelength of light application could be performed by using LED lamps on herbal production and plants can be irradiated with wavelengths of light in blue and red regions. Thusly, artificial irradiation possibilities are provided in wavelengths (colors) that are suitable for plants need for photosynthesis. The growth ratios of plants under various wavelengths of light show differences depending on the species of plants, therefore the yield of a plant can be increased under various wavelengths of light. As a result of study, the chlorophyll amounts of leaf, the leaf color values (L, *a*, *b*), the plant heights, the root lengths and the number of root of stevia demonstrate the differences depending on the applications of different wavelengths of light. So, while the highest plant height was determined in the "30% blue light + 70% red light" application, the highest stem length was found in the "50% blue light + 50% red light" application. The number of the stem reached the highest value in the "70% blue light + 30% red light" application. Consequently, a correlation was observed between negative "*a*" value and amount of chlorophyll. Finally, comparing to other applications, the "50% blue light + 50% red light" was found as the best light application to obtain optimum yield values of stevia.

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