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ผลของไดโอดเปล่งแสงต่อความงอกของเมล็ด และการสะสมปริมาณฟีนอลิกในต้นอ่อนพืช

Effects of Light-Emitting Diodes on Seed Germination and the Accumulation of Phenolic Content in Sprouts

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บทคัดย่อ

ไดโอดเปล่งแสง (LED) เป็นแหล่งให้แสงที่ประหยัดพลังงาน และเป็นเทคโนโลยีการให้แสงที่มีศักยภาพที่สามารถนำมาปรับใช้สำหรับการผลิตพืช แสงจาก LED มีผลทั้งกระตุ้นความงอกของเมล็ดพืช และการสังเคราะห์สารออกฤทธิ์ทางชีวภาพ ซึ่งช่วยเพิ่มคุณค่าทางโภชนาการและสารต้านอนุมูลอิสระในต้นอ่อนพืช การศึกษานี้มีวัตถุประสงค์เพื่อทดสอบผลของ LED ต่อความงอกของเมล็ด และการสะสมสารต้านอนุมูลอิสระในต้นอ่อนพืช โดยใช้แสงผสมระหว่างสีแดง (630 nm) และสีน้ำเงิน (470 nm) ที่ความเข้มแสงต่างกัน ซึ่งได้ทำการเพาะเมล็ดข้าว ถั่วเขียว ทานตะวัน และงา ภายใต้ LED แสงสีแดงร่วมกับสีน้ำเงินอัตราส่วน 7:3 ที่ความเข้มแสง 3 ระดับ (400, 200 และ 145 μmol/m^2/s) เปรียบเทียบกับการไม่ให้แสง ผลการทดลองพบว่าการใช้ LED สีแดงร่วมกับสีน้ำเงินที่ความเข้มแสง 145 μmol/m^2/s แก่เมล็ดพืช ส่งผลให้มีกิจกรรมของเอนไซม์แอลฟาอะไมเลสในเมล็ด และปริมาณฟีนอลิกในต้นอ่อนข้าวสูงที่สุด และยังพบว่าความเข้มแสง 200 และ 145 μmol/m^2/s ส่งผลให้เมล็ดพืชทุกชนิดมีเปอร์เซ็นต์ความงอกสูง ดังนั้นการให้แสง LED สีแดงร่วมกับสีน้ำเงินที่ความเข้มแสง 145–200 μmol/m^2/s สามารถเพิ่มกิจกรรมเอนไซม์แอลฟาอะไมเลส และความงอกของเมล็ดพืช รวมถึงเพิ่มปริมาณฟีนอลิกในต้นอ่อนพืชได้

ABSTRACT

Light–emitting diodes (LEDs) are a new type of energy-saving light source with the potential to revolutionize horticultural lighting technology for crop production. They can induce the germination and synthesis of bioactive compounds, which may improve the nutritional values and antioxidant properties of sprouts. This study aimed to investigate the combination of red (630 nm) and blue light (470 nm) from LEDs, with different light intensities on seed germination and accumulation of antioxidants in sprouts. Rice, mungbean, sunflower and sesame seeds were germinated under a combination of red and blue LED lights (7:3) at three light intensities (420, 200 and 145 μmol/m^2/s) using dark condition as control. The results revealed that germinated rice and sunflower seeds cultivated under the combination of red and blue LEDs at a light intensity of 145 μmol/m^2/s showed the highest α–amylase activity and total phenolic content. In addition, a high germination percentage of these crops was found at light intensities of 200 and 145 μmol/m^2/s. Therefore, the combination of LED light at an intensity of 145–200 μmol/m^2/s could be used to improve the α–amylase activity and germination of seeds and total phenolic content in sprouts.

คำสำคัญ: ความเข้มแสง ต้นอ่อนงา สารต้านอนุมูลอิสระ ความเร็วในการงอก

Keywords: Light intensity, Sesame sprout, Antioxidants, Speed of germination

INTRODUCTION

Currently, there is increasing attention focused on a healthy diet. One popular healthy diet trend involves highly nutritional sprouts. The most common sprouts include broccoli, watercress, peas, mungbean, wheatgrass and alfalfa, which can be added into salads, soups, sandwiches, stir fries, pasta and even smoothies. The cultivation of sprouts in a greenhouse requires controlled temperature, humidity, light, water, and nutrients (Butsabasri, 2012). Among these conditions, the lack of natural sunlight is a major problem for sprout production in a greenhouse, so artificial lighting is typically used as a light source, such as incandescent, halogen, neon and fluorescent lamps. However, these types of light sources often have limitations. For example, incandescent, halogen and neon lamps have short lifetimes and emit high energy, resulting in higher power waste, while the fluorescent lamp is not suitable for cultivating sprouts because of low energy level and expense. In addition, the lighting used to cultivate sprouts requires corresponding wavelengths and intensities. Light intensity, wavelength and duration have different effects on plant growth. The duration can be easily regulated by the lamps mentioned above, but wavelength and light intensity cannot be controlled using these lamps. Additionally, each plant responds differently to various wavelengths and light intensities. Therefore, an artificial light source with adjustable wavelength and intensity properties is needed for the cultivation of sprouts. Light-emitting diodes (LEDs) comprise a semiconductor light source that emits light when current flows through them. The LEDs can produce highly photosynthetic active radiation (PAR), which is used for photosynthesis by green plants, including blue, green, yellow, orange, red and far-red light spectrums. It is also possible to combine several LEDs to provide a higher light intensity or mix different LEDs to produce a specific light wavelength. Moreover, these LEDs are high in energy-saving efficiency, have low operating-temperature and long lifetime. They are deemed to have often replaced fluorescent and high-intensity discharge lamps in greenhouse systems to revolutionize controlled growth environments (Darko et al., 2014).

Sprouts are qualified as a functional food due to being good sources of phytochemicals such as ascorbic acid and phenolic compounds as well as antioxidants such as thiols and ascorbic acid (vitamin C). They include compounds that inhibit oxidation, which is reported to benefit human health. The cultivation of sprouts in a greenhouse is feasible due to the use of LEDs, which solves the problem of insufficient illumination from sunlight (Boonyakiat, 2011). However, the optimal light conditions for sprout production of each crop in closed environment growing systems are required. Vaštakaitė et al. (2015) studied the effect of LED light on red pak choi (*Brassica rapa* var. chinensis ‘Rubi’ F1), tatsoi (*Brassica rapa* var. rosularis) and basil (*Ocimum basilicum* L. ‘Sweet Genovese’). They reported that the absence (0 %) and the highest (33 %)

blue light intensity (447 nm total photosynthetic photon flux density (PPFD) ~ 302.5 μmol/m^2/s; 16 h photoperiod) led to higher 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH radical scavenging activity) in tatsoi and basil. In pak choi, however, the value was significantly lower. Johkan et al. (2010) studied the effects of raising seedlings with different light spectra such as blue, red, and blue with red LED lights on the seedling quality and yield of red leaf lettuce plants. They found that the polyphenol contents and total antioxidant status (TAS) were greater in lettuce seedlings treated with blue containing LED light than in those treated with fluorescence light source at 17 days after sprouting (DAS). However, there is still limited information about LED light effects on several important crops, especially on the germination and antioxidant content in the sprouts. The aim of this study was to investigate the combination of wavelengths with different light intensities from the LED lights on seed germination and accumulation of antioxidants in sprouts.

MATERIALS AND METHODS

1. Growth conditions and plant materials

The seeds of mungbean (SUT 4), rice (Khao Dawk Mali 105), sunflower (Aquara 6) and sesame (Ubon 6) were used as experimental plant materials. The experimental design was a completely randomized design (CRD) with three replications. The treatments comprised combinations of red (630 nm) and blue (470 nm) LED lights (7:3) at three light intensities (420, 200 and 145 μmol/m^2/s). The seeds germinated under dark condition were used as control. The temperature, relative humidity and duration of photoperiod were maintained at 25 °C, 70% RH and 16 h photoperiod, respectively. The germinated seeds were sprayed daily with distilled water.

Data were recorded for **α**–amylase activity, speed of germination, percentage of germination, fresh weight and total phenolic content.

1.1 Amylase activity was determined using the Kato-Noguchi and Macias method (Kato-Noguchi and Macías, 2005). One gram of germinated seeds was extracted in a mortar-pestle under liquid nitrogen add 1.5 ml extraction buffer. The mixture was centrifuged at 12,000 g for 30 min and then 0.5 ml A solution was added to the supernatant and incubated at 75 °C for 15 min. Subsequently, the B solution was added in the reaction mixture and incubated at 37 °C for 15 min 80 μl deionized water and 1.4 ml antrone was then added to the 20 μl reaction mixture, incubated at 90 °C for 17 min and measured with a spectrophotometer (Hitachi/UH–5300) at 625 nm. The standard curve was constructed from the D–glucose of 0.3125, 0.625, 1.25, 2.5, 5 and 10 μM. The solutions for the standard curve were prepared from a stock solution of 10 ml D–glucose mixed with 2 ml of distilled water. It was diluted to 0.3125, 0.625, 1.25, 2.5, 5 and 10 μM at 20 μl of solution by using 80 μl of distilled water with 1.4 ml of anthrone and boiled at 90 °C for 17 min, then left to cool at room temperature. The solutions were measured for **α**–amylase enzyme activity at 625 nm using spectrophotometer (Hitachi/UH–5300).

1.2 Speed of germination was recorded 2, 3, 4 and 5 days after seeding according to the following equation (1):

$$\text{Speed of germination} = n1/d1+n2/d2+n3/d3+\ldots \quad (1)$$

Where n = number of germinated seeds, d = number of days (Akbarian et al., 2016).

1.3 Percentage of germination and fresh weight were recorded at 5 days after seeding (Samuolienė et al., 2011).

1.4 Total phenolic content of sprouts was determined at 5 days after seeding. The total phenolic of the extracts were determined using the Folin and Ciocalteu reagent, following the method described by (Samuolienė et al., 2011). Calculation was done according to the following equation, Y = 0.0416x–0.0703. All determinations were carried out in triplicate.

2. Data analysis

Analysis of variance and significance of difference among means of amylase activity, germination speed, germination percentage and total phenolic content were tested by ANOVA and DMRT on mean values using SPSS v. 16 (Nie et al., 1970; Patel et al., 2017).

RESULTS

1. Amylase Activity

Studying the effect of light intensities showed significantly affected the activity of α–amylase enzyme in germinated rice and sunflower seeds. The highest enzyme activities of rice and sunflower were 0.580 and 0.149 µg/min, respectively at 145 µmol/m^2/s of light intensity. On the other hand, there were not affected activity of α–amylase enzyme in germinated sesame and mungbean seeds (Table 1).

2. Speed of seed germination, percentage of germination and fresh weight of sprouts

The germination speed of rice, sunflower and mungbean did not significantly differ among 4 light intensities and varied from 22.54–27.71 seeds/day in rice, 1.85–3.78 seeds/day in sunflower and 41.25–46.91 seeds/day in mungbean. The highest germination speed of sesame was recorded in dark condition (40.71 seeds/day) and at 200 µmol/m^2/s of light intensity (38.41 seeds/day), as shown in Table 1.

There were significant differences in the percentage of germination for rice and sunflower (Table 1) among light intensity treatments. The highest germination percentage for rice was found at the light intensity of 145 µmol/m^2/s (94%), but was not significantly different from light intensities of 200 and 400 µmol/m^2/s. While in sunflower, the light intensities of 145, 200 µmol/m^2/s and dark condition produced higher germination percentages than the light intensity of 400 µmol/m^2/s. However, there were not significant differences in the percentage of germination among light intensities in sesame and mungbean.

The highest fresh weight of rice sprout (4.14 g/plant) was found at the light intensity of 145 µmol/m^2/s but was not significantly different with light intensities of 200 and 400 µmol/m^2/s, while the lowest fresh weight (3.04 g/plant) was obtained from the dark condition. The maximum fresh weight of mungbean sprout was found in the dark condition (4.50 g/plant), while the fresh weight at light intensities of 200, 145 and 400 µmol/m^2/s were not significantly different (Table 1).

3. Total phenolic content

The highest total phenolic contents in rice and sunflower were found at the light intensity of 145 µmol/m^2/s but were not significant difference from light intensities of 200 and 400 µmol/m^2/s. While the dark condition produced the lowest total phenolic content in rice and sunflower (5.99 and 7.68 µg/mL, respectively). Similarly result was found in mungbean sprout i.e. the highest total phenolic content was obtained from a light intensity of 200 µmol/m^2/s (17.94 µg/mL) but were not significantly different from light intensities of 145 and 400 µmol/m^2/s (16.62 and 16.11 µg/mL, respectively). While the lowest total phenolic content was found under dark conditions with 6.47 µg/mL. The total phenolic content of sesame sprouts was not significantly different among light intensities and varied from 10.57–15.02 µg/mL.

DISCUSSION

Germination is the first step in the growth cycle of a plant, which determines the success of crop production. Various factors such as moisture, air, temperature and light can affect the germination of seeds. In terms of light, both light and dark conditions can affect the germination of seeds (Bewley et al., 2013). From this study, it indicated relationships between light intensities and germination rate of seeds, the speed of germination, amylase activity and total phenolic content. Previous studies suggested that there are differences in the responses of seed germination to light, some species require light for their seeds to germinate, whereas others do not (Lal and Sachan, 2017). Many studies have reported that the highest seed germination was observed with red light, while a low percentage of germination was observed with blue and yellow lights (Zhao et al., 2018). The results of this study confirmed these differences. While the germination percentage for rice and sunflower seeds can be induced with low RB light intensity through the induction of α–amylase activity, sesame and mungbean did not respond to light in terms of α–amylase activity and germination percentage. The result of the effect of red and blue light on the germination rate was still ambiguous. In most crop studies, germination rate is not associated with light/ dark conditions. In sesame, however, it appears that some levels of red and blue light intensity can cause a reduction of germination speed. These variations of germination, α–amylase activity and seed responses to light may be the result of varying seed reserve content for each species studied (Taiz and Zeiger, 2010). It may be interesting to further investigate the relationship between seed reserve content, germination and the effects of light on these crops.

Table 1. Effect of light intensities on amylase activity, percentage of germination, speed of germination, fresh weight and total phenolic content in rice, sunflower, sesame and mungbean sprouts.

Seed	Light intensity^1 (μmol/m^2/s)	α–amylase activity^2 (μg/min)	Germination (%)	Speed of germination (seeds/day)	Fresh weight (g/plant)	Total phenolic content (μg/mL)
Rice	Dark	0.105^b	82^b	27.71	3.04^b	5.99^b
	400	0.112^b	83^{ab}	23.08	3.64^{ab}	13.26^{ab}
	200	0.113^b	92^{ab}	23.41	3.77^{ab}	13.63^{ab}
	145	0.580^a	94^a	22.54	4.14^a	19.00^a
	F-test	*	*	ns	*	*
Sunflower	Dark	0.087^c	99^a	1.85	0.68	7.68^b
	400	0.117^b	95^b	2.76	0.45	15.61^a
	200	0.120^b	100^a	2.97	0.54	15.42^a
	145	0.149^a	100^a	3.78	0.47	15.97^a
	F-test	*	*	ns	ns	*
Sesame	Dark	0.113	100	40.71^a	0.59	10.57
	400	0.122	100	37.33^b	0.62	14.32
	200	0.138	100	38.41^a	0.56	15.02
	145	0.119	100	34.75^b	0.65	14.09
	F-test	ns	ns	*	ns	ns

Table 1. Effect of light intensities on amylase activity, percentage of germination, speed of germination, fresh weight and total phenolic content in rice, sunflower, sesame and mungbean sprouts. (continue)

Seed	Light intensity[1] (µmol/m²/s)	α–amylase activity[2] (µg/min)	Germination (%)	Speed of germination (seeds/day)	Fresh weight (g/plant)	Total phenolic content (µg/mL)
Mungbean	Dark	0.103	96	46.50	4.50^{a}	6.47^{b}
	400	0.130	99	41.25	2.09^{b}	16.11^{a}
	200	0.161	96	46.91	1.89^{b}	17.94^{a}
	145	0.130	99	46.50	2.02^{b}	16.62^{a}
	F-test	ns	ns	ns	*	*

[1] The combinations of LEDs were red and blue lights at a ratio of 7:3.

[2] The different letters within a column indicate significant differences at $p < 0.05$.

Light intensities also affect the growth and development of seedlings. In general, red and blue lights are the most important wavelengths of light for the physiological and morphological processes of the plant, including photosynthesis, stomatal regulation, germination, flowering, and biomass accumulation (Ohashi-Kaneko et al., 2006). Using supplemental light for seedlings could produce strong root growth and intense photosynthesis for vegetative growth (Bewley et al., 2013). The study of Ohashi-Kaneko et al. (2006) showed that red and blue light at 380 µmol/m²/s could induce the biomass of rice seedlings better than red light alone. This increment of biomass was due to an increasing leaf area as well as the roots of studied rice cultivars. Ryu et al. (2012) reported that *Taraxacum officinale* cv. had a higher number of leaves under LED light (blue and red, 6:4) than illuminated under fluorescent. Akbarian et al. (2016) reported that using blue and red LED light for ornamental plants could significantly increase root fresh weight compared to fluorescent light. The results of fresh weight found in this study seem to contradict the previous finding, except in rice seedlings, in which low red and blue light intensity at 145 µmol/m²/s can significantly induce fresh weight compared to dark conditions. On the other hand, the growth of mungbean seedlings was inhibited by light conditions, as seen from the reduced fresh weight.

Total phenolic compounds have been reported to increase under specific artificial wavelength conditions. Ajdanian et al. (2019) suggested that the combination of red and blue LED light can enhance not only vegetative growth of cress, but also the biochemical traits such as the amount of a, b and total chlorophyll, anthocyanin and phenolic contents. The study of Lobiuc et al. (2017) also showed an increment of phenolic compounds in basil microgreens. They also suggested that different cultivars responded differently to ratios of red and blue light. In this study, the total phenolic content of rice, sunflower and mungbean were significantly enhanced by a combination of red and blue light, regardless of intensity.

CONCLUSION

From this study, the maximum seed germination, α–amylase activity, seedling fresh weight, total phenolic content of rice was observed at a light intensity of 145 µmol/m²/s. For sunflower, the highest percentage of germination, fresh weight, and total phenolic content was found at light intensities of 145 and 200 µmol/m²/s. For mungbean and sesame seeds, the highest total phenolic content was found at a light intensity of 145 μmol/m^2/s, while other characteristics were not significantly different among light intensities.

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