

Effects of blue light on physiological and biochemical changes in germinating cabbage sprouts

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Sprouts as a natural food have been commonly consumed in different cultures, which are richer in health-promoting compounds than their mature counterparts. The purpose of this study was to investigate the effects of blue light on physiological and biochemical changes including sprout length, respiratory rate, free amino acids, ascorbic acid, glucosinolates, isothiocyanates and myrosinase activity in germinating cabbage sprouts. Furthermore, their correlation analysis was conducted to elucidate their changing patterns during germination and relationships with each other. The respiratory rate, free amino acids and ascorbic acid content were greatly enhanced by blue light compared to the darkness. Blue light could better retain glucosinolate content in cabbage sprouts than the darkness. More isothiocyanates formation was observed from cabbage sprouts under blue light, which was a result of the promoted myrosinase activity and higher glucosinolate content. These results indicate that blue light has selectively improved the nutritional compounds in germinated cabbage sprouts.

Key words: free amino acids, ascorbic acid, isothiocyanate, myrosinase

Introduction

Epidemiological studies have shown that dietary intake of *Brassica* vegetables, such as cabbage, broccoli, etc., can reduce the risks of chronic diseases such as atherosclerosis and cancer (Herr and Buchler 2010, Mandrich and Caputo 2020). These beneficial effects are attributed to glucosinolates, a group of thioglucosides that are compounds of an activated chemical defense found in *Brassica* vegetables (Wu et al. 2021). When plant tissues and cells are damaged, glucosinolates and myrosinase bond together to form hydrolyzed products like isothiocyanates (ITCs), nitriles, thiocyanates, epithionitriles and oxazolidines (Bones and Rossiter 2006, Tang et al. 2013). Isothiocyanates are reported to be one natural anticarcinogenic compound (Soundararajan and Kim 2018). Studies *in vitro* and *in vivo* demonstrated that ITCs had the ability to deactivate phase I enzymes and to activate phase II enzymes to inhibit carcinogenesis (Das et al. 2013).

Sprouts are believed to be a rich source of health-promoting compounds compared with their mature crops. Seed germination is a simple and effective way to improve nutritional compositions in plants, during which significant changes including interconversion and production of new compounds occurs (Oloyo 2004). Glucosinolate contents tend to decrease with time over the stages of germination (Pérez-Balibrea et al. 2011), the total glucosinolates concentration of young broccoli sprouts was 20-fold of mature broccoli tissues (Fahey et al. 1997). Previous study confirmed that germination led to significant changes in the content of glucoraphanin and its hydrolytic product, sulforaphane (Gu et al. 2012b). Germination also causes changes in other health-promoting compounds like ascorbic acid. Ascorbic acid content was greatly increased after germination (Martinez-Villaluenga et al. 2010), making sprouts a valuable source of vitamin C for human needs. Cabbage is one important *Brassica* vegetable, which is consumed worldwide and has been made into various kinds of food. Cabbage sprouts are recognised as wellness and health-promoting foods due to its high content of nutrients and bioactive compounds.

Lights can regulate plant growth and development, provide higher energy conversion efficiency, and also enhance the accumulation of bioactive compounds. It has been reported that broccoli sprouts grown in artificial light condition (16 h light/8 h dark photoperiod) had 33% higher content of total glucosinolates than those grown in the dark (Pérez-Balibrea et al. 2008). Blue light increased the height and weight of buckwheat sprouts compared to other LED lights (red and red + blue lights) (Lee et al. 2014). Yamada et al. (2003) found that the activity of myrosinase in radish hypocotyls was significantly enhanced within 10 min of blue light irradiation, and reached the highest at 30 min. Moreover, blue LED light resulted in the highest glucoraphanin content in sprouting Canola (Park et al. 2019). A short period of blue LED treatment before harvesting significantly increased the levels of β -carotene, total xanthophyll cycle pigments, glucoraphanin, aliphatic glucosinolates, essential micronutrients in broccoli microgreen bud tissue (Kopsell and Sams 2013). The blue light improved the expression of key enzyme-encoding genes in the

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aliphatic-GLs synthesis pathway, promoted the synthesis and accumulation of aliphatic-GLs, and improved the accumulation of secondary metabolites in broccoli sprouts (Xue et al. 2021). Similarly, our previous study found that blue light was superior to white, red, green, yellow and purple LED lights in improving the nutritional quality of broccoli sprouts (Zhuang et al. 2022). It can be seen that blue light has been proved to effectively regulate plant growth and development and promote the synthesis of secondary metabolites such as glucosinolates. However, to the most of our knowledge, the effects of blue light on glucosinolate content and isothiocyanates formation of germinating cabbage sprouts have not been investigated.

Therefore, the objective of this work was to investigate the effects of blue light on physiological and biochemical changes, including growth, respiratory rate, the contents of free amino acids, ascorbic acid, total glucosinolate and isothiocyanates, as well as the myrosinase activity and the correlation analysis between physiological and biochemical properties in germinating cabbage sprouts. This study might provide theoretical basis for the application of LED light in the industrialized production of cabbage sprouts.

Materials and methods

Materials

Cabbage seeds (cv. Xinxia) were purchased from Nanjing Jinshengda Seed Co., Ltd (Jiangsu, China). Sulforaphane standard, sinigrin standard and 1,2-benzenedithiol were obtained from Sigma-Aldrich (St Louis, MO, USA). Other chemicals and reagents to be used were of analytical grade and purchased from Shanghai Institute of Biochemistry (Shanghai, China).

Seed germination

Dry seeds were surface-sterilized for 15 min in 1.5% sodium hypochlorite and then rinsed with distilled water. After soaked in distilled water at 30 °C for 4 h, they were placed on a filter paper in Petri dishes (Ø 15 cm) filled with sterilized quartz sand and germinated at 30 °C under blue light for 1, 2, 3, 4 and 5 days. The plant was about 50 cm away from the light source and the light intensity was approximately $50 \mu\text{mol s}^{-1} \text{m}^{-2}$. Seeds germinated at 30 °C in the darkness were set as the control. At the end of germination, sprouts were carefully washed with distilled water, dried and flash-frozen in liquid nitrogen for further analyses.

Measurement of sprout length and dry weight

Sprout length was measured directly using a centimeter ruler. Twenty cabbage sprouts were taken as one sample.

Fresh cabbage sprouts with different growth days were dried to constant weight at 50 °C in drying oven and weighed. The results were expressed as mg/sprout.

Measurement of respiratory rate

One g of fresh cabbage sprouts was put into a sealed container at 28 °C for 1 h. An infra-red gas analyzer was used to determine its CO₂ concentration. Corresponding replicate sample was used for dry weight determination (50 °C, 3 h). The respiratory rate was expressed as $\mu\text{mol g}^{-1} \text{min}^{-1}$.

Free amino acids determination

The content of free amino acids was determined according to the method of Wang et al. (2011). Fresh cabbage sprouts (0.5 g) were ground with 5 ml 10% acetic acid, diluted to 25 ml and centrifuged for 15 min at 8000 g. 1 ml supernatant was mixed with 1 ml ammonium-free distilled water, 3 ml ninhydrin and 0.1 ml ascorbic acid, and then held 15 min in a boiling water bath. After cooling, the sample was diluted with 60% ethanol to 20 ml. With blank as the control, the absorbance at 570 nm was measured. The standard curve of amino acids was made with leucine, and the content of free amino acids in the sample was expressed by the content of amino nitrogen.

Where C is the content of amino hydrogen found on the standard curve, and V_T is the total volume of the sample, V_s is the sample volume taken during the determination, and M is the sample mass.

Ascorbic acid determination

Determination of ascorbic acid content was conducted as previously described (Volden et al. 2009). Briefly, 0.3 g of fresh cabbage sprouts was extracted with 5 ml of 1.0% (w/v) oxalic acid. After centrifuged at 10000 rpm for 10 min, the supernatant was filtered through a 0.45 μm membrane filter (Tianjin Navigator Lab Instrument Co., Ltd.) and then analyzed using a HPLC system (Agilent Technologies Co. Ltd., USA) equipped with a G1314B UV detector and a G1311A quat pump, using a reversed-phase C18 column (4.6 \times 250 mm, 5 μm , ZORBAX. Eclipse) at 254 nm. Ascorbic acid content was calculated by external standard curve and results are reported as mg kg⁻¹ FW.

Myrosinase activity determination

Myrosinase was extracted from cabbage sprouts according to the method by Kim et al. (2006) with some modifications. Cabbage sprouts (0.2 g) were homogenized with 3 ml of 0.1 mol l⁻¹ sodium-phosphate buffer (pH 6.5) in an ice bath. After centrifugation at 10000g for 15 min at 4 °C, the supernatants were used as the crude enzymes. The amounts of proteins in the crude enzymes were measured by the Bradford assay (Bradford 1976) using bovine serum albumin as a standard. The assays were conducted with 0.025 mmol l⁻¹ sinigrin and 200 μl of supernatants in a total volume of 100 μl . After incubation at 37 °C for 15 min, the reaction was stopped by incubating the samples in a boiling water bath for 5 min. The reaction mixture was diluted with distilled water, and the concentration of the remaining sinigrin was determined spectrophotometrically at 227 nm. One myrosinase unit corresponded to 1 nm sinigrin transformed per minute. The specific activity is expressed as units per milligram of protein.

Extraction and determination of glucosinolates

Glucosinolates assay was carried out according to the protocol of Guo et al. (2014) with minor modifications. Briefly, samples (500 mg) were extracted twice with 2 ml of 75% methanol at 80 °C for 15 min in a shaking heating bath. The supernatant was collected after centrifugation (5 min, 10000 g). 1 ml of the extract was applied to a 1 ml DEAE-Sephadex A-25 column and rinsed with 2 ml of 0.02 mol l⁻¹ sodium acetate. After addition of 200 μl of arylsulfatase solution and incubation for 16 h at 35 °C, the desulfoglucosinolates were eluted with 4 ml of Milli-Q water and filtered through a 0.45 μm membrane filter. Each sample (20 μl) was analyzed in an Agilent 1200 HPLC system, using an Eclipse XDB-C18 column (5 μm particle size, 4.6 \times 150 mm; Agilent Technologies Co. Ltd., USA) at 226 nm. Determination was conducted at a flow-rate of 1.0 ml min⁻¹ in a gradient starting with 0% acetonitrile for 1min, reaching 20% acetonitrile at 21 min, 0% acetonitrile at 26 min. Sinigrin (2-propenyl glucosinolate) (Sigma St. Louis, MO, USA) was used as an internal standard.

Extraction and determination of isothiocyanates formation

Isothiocyanates formation assay was performed as described previously (Jiao et al. 1998, Wang et al. 2015) isothiocyanate formation and myrosinase activity in cabbage sprouts with modifications. Briefly, 0.2 g of fresh cabbage seeds or sprouts were homogenized in 4 ml distilled water. The mixture was incubated at 37 °C for 4 h to completely release isothiocyanates from glucosinolates. After incubation, 3 ml of methylene dichloride was added and extracted for 30 min. The mixture was then centrifuged at 10000 rpm for 15 min and the organic phase containing isothiocyanates was carefully collected. The reaction solution consisted of 2 ml of methanol, 1.8 ml of 50 mmol l⁻¹ sodium borate buffer (pH 8.5), 0.2 ml of 7 mmol l⁻¹ 1, 2-benzenedithiol and 1 ml of the supernatant were incubated at 65 °C for 1 h. Then, they were cooled to room temperature and centrifuged at a low speed to sediment the insoluble materials. The 20 μl of supernatant was injected an Agilent 1200 HPLC system with an Eclipse XDB-C18 column (5 μm particle size, 4.6 \times 150 mm; Agilent Technologies Co. Ltd.). The isothiocyanate content was expressed as mg 100 g/FW of cabbage sprouts.

Statistical analysis

Experimental data were expressed as the mean \pm standard deviation (SD) with three replications (n = 3). SPSS 18.0 (SPSS Inc., Chicago, IL) was applied for the significant difference test.

Results

Effect of blue light on germination percentage, sprout length and dry weight

The seed germination was significantly stimulated under blue light. Blue light increased the germination percentage by 10.2% compared with the darkness (Fig. 1).

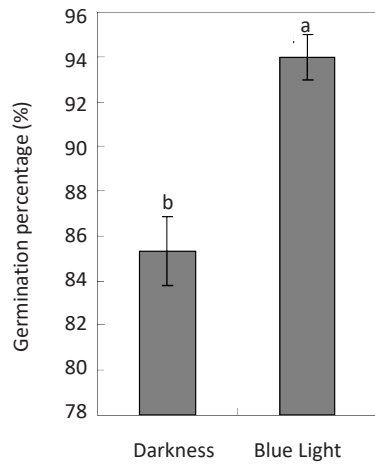


Fig. 1. Germination percentage of cabbage seeds (5-day) grown under blue light and the darkness. Data are the mean of 3 Petri dishes containing 80 seeds each. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Germination brought about a continuous rise in the sprout length, which were 5.62 and 9.68 cm at the end of 5-day germination of blue light and the darkness, respectively (Fig. 2). The sprout length of cabbage grew under blue light was less than that of the darkness and the disparity was enlarged with sprouts age. The highest disparity was 4.06 cm at 5-day old sprouts.

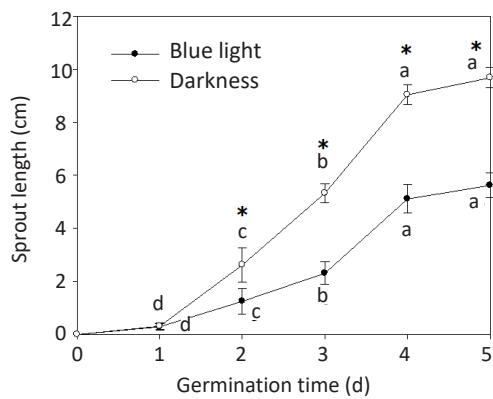


Fig. 2. The changing pattern of sprout length during (cm) germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$. * represents the data of blue light and darkness are significantly different at the same day of germination, the same as follows.

Dry weight of blue light-treated sprouts was heavier than that of control (Table 1). The dry weight varied from 1.87 to 3.64 mg/sprout during germination, reaching the highest value of 3.64 mg/sprout in 2-day old sprouts under blue light treatment, and enhanced by 33.33% compared to darkness.

Table 1. The changing pattern of dry weight (mg/sprout) during germination

| Germination time (d) | 1 | 2 | 3 | 4 | 5 |
|----------------------|-------------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|
| Blue light | 2.35 \pm 0.09 ^b | 3.64 \pm 0.07 ^a | 2.38 \pm 0.11 ^b | 2.20 \pm 0.15 ^b | 2.25 \pm 0.16 ^b |
| Darkness | 1.95 \pm 0.11 ^{bc} | 2.73 \pm 0.17 ^a | 1.87 \pm 0.09 ^c | 2.20 \pm 0.07 ^b | 2.00 \pm 0.21 ^{bc} |

Effect of blue light on respiratory rate

The respiratory rate of sprouts under blue light and the darkness both increased drastically by over 3 folds at the first 2 day of germination, and then gradually stabilized and remained at a high level (Fig. 3). However, the respiratory rate of sprouts grew under blue light was higher than the darkness during the whole germination period.

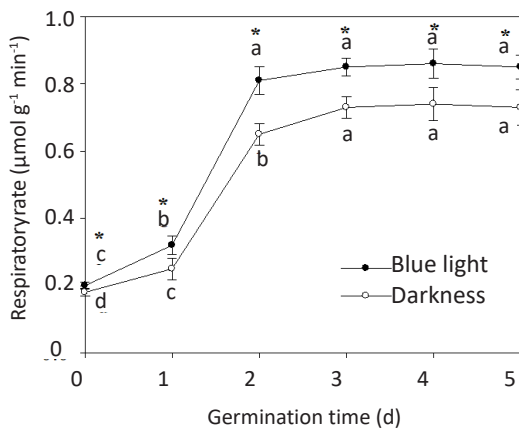


Fig. 3. The changing pattern of respiratory rate ($\mu\text{mol g}^{-1} \text{min}^{-1}$) during germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Effect of blue light on free amino acids content

The content of free amino acids in sprouts under blue light and the darkness both increased to the peak at 1-day of germination then decreased gradually before increasing again (Fig. 4). Blue light enhanced the content of free amino acids compared with the darkness.

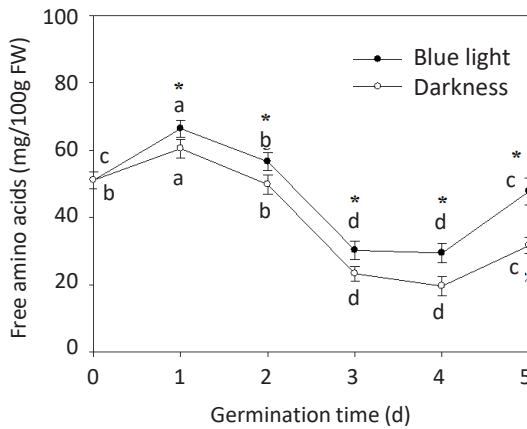


Fig. 4. The changing pattern of free amino acids ($\text{mg}/100 \text{g FW}$) during germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Effect of blue light on ascorbic acid content

The ascorbic acid content was very low ($5.4 \text{ mg kg}^{-1} \text{ FW}$) in ungerminated cabbage seeds (Fig. 5). Germination brought about a sharp rise in the ascorbic acid content of cabbage sprouts, reaching the highest value of 71.9 and $64.3 \text{ mg kg}^{-1} \text{ FW}$ under blue light and darkness, respectively. Hence, germination increased the ascorbic acid content by about 7 folds at the end of 5-day germination period. Blue light led to significantly more accumulation of ascorbic acid compared with the darkness. The ascorbic acid content in 1-day, 2-day and 3-day old sprouts were 90.5%, 103.6% and 74.5% higher than that of the darkness, respectively.

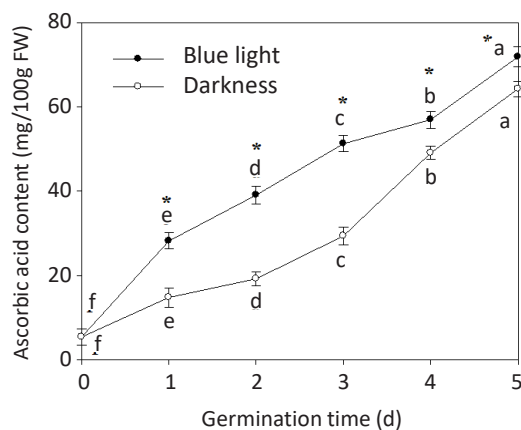


Fig. 5. The changing pattern of ascorbic acid ($\text{mg}/100 \text{g FW}$) during germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Effect of blue light on total glucosinolate content

Total glucosinolate content of cabbage sprouts under blue light and the darkness both decreased steadily with germination time although no significant decrease was observed from 2-day to 4-day of germination (Fig. 6). The content of total glucosinolates in cabbage sprouts decreased by about 60% at the end of the monitored germination period both under blue light and the darkness. However, blue light presented a positive effect on total glucosinolate content. Total glucosinolate content of 1-day cabbage sprouts were 70.6% higher than that of the darkness.

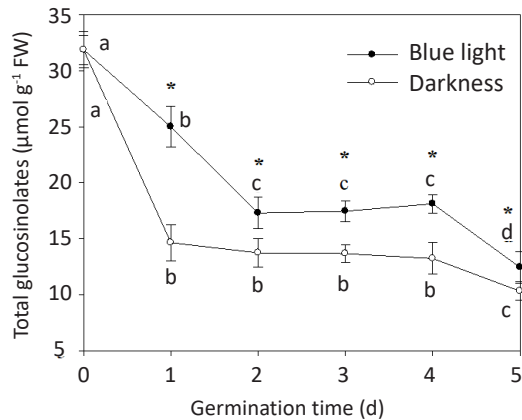


Fig. 6. The changing pattern of total glucosinolates (mmol/100 g FW) during germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Effect of blue light on isothiocyanates formation

Isothiocyanates formation of young cabbage sprouts under blue light and the darkness both increased with germination time during early germination period while decreased drastically afterwards (Fig. 7). However, there were still some differences between sprouts under blue light and the darkness. The highest isothiocyanates formation (14.44 $\mu\text{mol g}^{-1}$ FW) was obtained at 1-day old sprouts under blue light while that of the darkness was 11.21 $\mu\text{mol g}^{-1}$ FW at 2-day old sprouts. Sprouts grew under blue light had higher isothiocyanates formation as compared to the darkness.

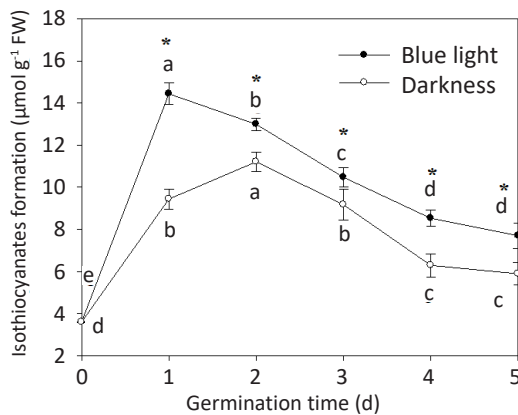


Fig. 7. The changing pattern of isothiocyanates formation ($\mu\text{mol g}^{-1}$ FW) during germination. Each point was expressed as mean \pm SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Effect of blue light on myrosinase activity

Myrosinase activity of cabbage sprouts under blue light and the darkness both increased to the highest at 1-day of germination and decreased afterwards till kept stable (Fig. 8). Blue light significantly promoted the activity of myrosinase compared with the darkness. The highest myrosinase activity (1-day old sprouts) of sprouts under blue light was 27.9% higher than that of the darkness.

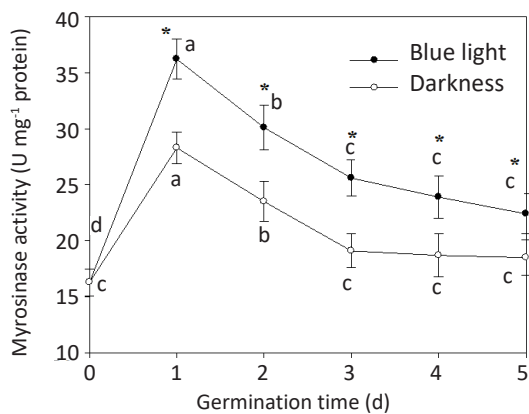


Fig. 8. The changing pattern of myrosinase activity (U mg⁻¹ protein) during germination. Each point was expressed as mean ± SD. Values not sharing a common letter are significantly different at $p < 0.05$.

Correlation analysis between physiological and biochemical properties under blue light

Table 2 presents the correlation analysis between physiological and biochemical properties of cabbage sprouts under blue light. It was found that glucosinolate content significantly correlated negatively with sprout length, respiratory rate and ascorbic acid. Respiratory rate and sprout length were significantly correlated with each other and the correlation coefficient was 0.759. Ascorbic acid content significantly correlated positively with sprout length and respiratory rate. Isothiocyanates formation significantly correlated positively with myrosinase activity and correlated negatively with glucosinolate content.

Table 2. Correlation analysis between physiological and biochemical properties of cabbage sprouts under blue light

| | SL | RR | FAA | GLs | AA | MA |
|------|---------|---------|--------|---------|-------|--------|
| RR | 0.759* | | | | | |
| FAA | -0.594 | -0.611 | | | | |
| GLs | -0.779* | -0.936* | 0.373 | | | |
| AA | 0.905* | 0.887* | -0.490 | -0.954* | | |
| MA | -0.245 | 0.040 | 0.477 | -0.181 | 0.075 | |
| ITCs | -0.186 | 0.205 | 0.365 | -0.311 | 0.171 | 0.978* |

SL = Sprout length; RR = Respiratory rate; FAA = Free amino acids; GLs = Glucosinolates; ITCs = isothiocyanates; AA = Ascorbic acid; MA = Myrosinase activity; *significant at $p < 0.05$

Discussion

The germination of cabbage seeds was accelerated under blue light (Fig. 1), and blue light significantly promoted dry matter accumulation (Table 1). However, the sprout length was less than that of the darkness (Fig. 2). Despite of this fact, it was noticed that the sprouts under blue light had more strong stems and big, thick leaves than sprouts under the darkness (Carvalho et al. 2014, Lee et al. 2014). This kind of inhibition by blue light was also found in the seedling growth of *Arabidopsis* (Parks et al. 1998) and the inhibition phase is believed to begin with an anion-channel-mediated depolarization of the plasma membrane (Parks et al. 2001). The application of blue light encouraged the synthesis of chlorophyll and chloroplast movement (Poudel et al. 2007), which might promote the accumulation of dry matters at the sacrifice of sprout elongation. Another possibility was the physiological metabolism of sprouts under blue light was more vigorous than the darkness since their respiratory rate was higher (Fig. 3). Therefore, more nutrients and bioenergy were consumed, decreasing biomass accumulation (Samuoliene et al. 2020), and as a consequence, there was less to be supplied for sprout elongation.

Water absorption was the predominant activity during the early period of germination, when the enzymes in seeds were activated and so the respiratory rate increased as a starting symbol of seed germination (Chugh and Sawhney 1996). A similar changing pattern was also observed by Gu et al. (2012a). In the present study, the respiratory rate of sprouts increased significantly by over 3 folds during the first 2 days of germination and kept

stable afterwards (Fig. 3). In addition, sprout length increased with the increment in respiratory rate and they were significantly correlated (Table 2). Blue light enhanced the respiratory rate of sprouts which might be a result of photosynthesis. During photosynthesis process, more complicated metabolism of inner substances occurred including degradation of stored compounds and biosynthesis of new ones. The exchange rate between oxygen and carbon dioxide was higher. Zeiger et al. (2010) reported an increasing stomatal response was observed after the intact leaves of *Commelina communis* were irradiated with blue light. The above two suggestions might explain the higher respiratory rate of sprouts under blue light in the present study.

Glucosinolates biosynthesis consists of three stages including the elongation of side chain amino acids, the addition of glucone moiety and further modifications of secondary side-chain (Grubb and Abel 2006). In *Brassica* plants, sulfur application could increase aliphatic and indole glucosinolate contents, which was highly supposed to be the fact that aliphatic glucosinolates are derived from the sulfur-containing amino acid methionine, while the indole ones are biosynthesized from tryptophan (Gu et al. 2012b). In this study, seeds initiated their germination at the first day, during which substances (crude protein, crude fat, starch etc.) stored in the seeds were consumed to supply sprout growth. Hence, being the hydrolyzed products of protein, free amino acids accumulated. However, after germinating for 1 day, a decrease in the content of free amino acids was observed. This could be due to the totally utilization of crude protein by sprouts growth or selectively biosynthesis of glucosinolates using amino acids inside the sprouts. The content of free amino acids in sprouts under blue light was higher than that of the darkness, which might also be the result of photosynthesis induced by blue light.

No or extremely low ascorbic acid content was detected in ingeminated seeds such as broccoli (Pérez-Balibrea et al. 2011) and soybean (Xu et al. 2005). Germination brought about a sharp and constant rise in the ascorbic acid content of cabbage sprouts in this study (Fig. 5), during which the biosynthesis of ascorbic acid was reactivated (Xu et al. 2005). In contrast, some other studies found that the ascorbic acid content increased to a peak value at a certain germination time, after which it would decrease to the original value (Pérez-Balibrea et al. 2008, Xu et al. 2005). Therefore, a suitable germination time could exhibit the potential of accumulating ascorbic acid in cabbage sprouts. Blue light greatly accelerated the ascorbic acid content in cabbage sprouts than the darkness. The possible explanation of this phenomenon was the biosynthesis of secondary metabolites and photoprotection induced by blue light (Pérez-Balibrea et al. 2008).

Glucosinolates in *Brassica* plants were reported to come from two different processes including glucosinolates biosynthesis induced by outside inducers and hydrolysis by inner enzyme myrosinase (Mithen et al. 2000). Total glucosinolate content in *Brassica* plants usually tends to decrease with plant growth (Gu et al. 2012b), which was also found in our study. Glucosinolate content significantly correlated negatively with sprout length, respiratory rate and ascorbic acid (Table 2). This result strongly suggested that glucosinolate content decreased with sprout growth, during which sprout length, respiratory rate and ascorbic acid content increased (Fig. 2, 3 and 5). Many studies have been conducted to enhance and retain glucosinolates. Broccoli sprouts grew in artificial light condition (16 h light/8 h dark photoperiod) had 33% higher content of total glucosinolates than those in the dark (Pérez-Balibrea et al. 2008). Salt stress (100 mM of NaCl treatment) significantly increased total glucosinolate content of 5- and 7-day-old radish sprouts (Yuan et al. 2010). Sucrose and mannitol could also accumulate total glucosinolates in broccoli sprouts (Guo et al. 2011). Glucosinolates may act as a response to external factors of environment such as light and temperature (Del Carmen Martinez-Ballesta et al. 2013). The present study showed that blue light leads to better retention of total glucosinolate content in cabbage sprouts, in comparison with the darkness during germination (Fig. 6). Similarly, the results of Chen et al. (2020) showed that that the blue light application had a significant positive effect on GS accumulation in Chinese kale. It is possible that blue light favored the biosynthesis of glucosinolates or inhibited some degradative pathways, which needs further investigation.

Isothiocyanates are hydrolyzed from glucosinolates by the enzyme myrosinase when cabbage sprouts are crushed. Hence, its formation content should be directly associated with the content of glucosinolates in sprouts as well as the activity of myrosinase. Isothiocyanates formation in cabbage sprouts increased drastically to the highest at 1-day of germination while decreased afterwards (Fig. 7). This could be attributed to the fact that myrosinase activity was the highest at 1-day of germination (Fig. 8) although the substrate glucosinolate content reduced. After germinating for 1 day, isothiocyanates formation decreased constantly, which was a consequence of the decrease in myrosinase activity and glucosinolate content. It was noticed that isothiocyanates formation significantly correlated positively with myrosinase activity and correlated negatively with glucosinolate content (Table 2). Previous studies demonstrated that high pressure (Van Eylen et al. 2009) and sucrose and mannitol (Guo et al. 2011) could increase isothiocyanates formation. The present results showed that blue light enhanced isothiocyanates formation compared with the darkness (Fig. 7). One possibility could be higher glucosinolate content of sprouts

under blue light (Fig. 6) results in higher isothiocyanates formation, another might be the stimulation of myrosinase activity by blue light (Yamada et al. 2003).

Myrosinase activity of cabbage sprouts increased to day 1 of germination while decreased subsequently till seeds level (Fig. 8). This kind of variation was also found in young broccoli seedlings (Williams et al. 2008). Myrosinase activity varies with plant species, organ and development stage (Bhat and Vyas 2019), where the activity increases during the first 2 days of germination and then decreases with growth was commonly observed (Phelan et al. 1984, Bones 1990). Glucose treatment inhibited the activity of myrosinase in Chinese kale and pak choi sprouts while highly accelerated that in radish sprouts (Wei et al. 2011). The myrosinase activity of cabbage sprouts grown under 176 mM sucrose was significantly inhibited and it was assumed that sucrose may regulate myrosinase activity in cabbage sprouts as an elicitor signal (Guo et al. 2011). Our study found that blue light greatly promote myrosinase activity in cabbage sprouts in comparison with the darkness, which might attribute to the promotion in gene expression of myrosinase induced by phototropic stimulation under blue light (Yamada et al. 2003). However, since the study of Yamada et al. (2003) was carried out on radish hypocotyls and our study firstly reported that blue light accelerated myrosinase activity in cabbage sprouts, more investigation on the molecular mechanisms involved are warranted to be carried out.

Conclusion

Cabbage sprouts as a natural food and valuable dietary supplement are rich in phytochemicals, containing an especially high level of ascorbic acid and glucosinolates. In addition, it exhibits the potential of rich isothiocyanates formation. The content of free amino acids, glucosinolates, isothiocyanates formation and ascorbic acid in cabbage sprouts were enhanced differently under blue light. The related mechanism will be investigated in further study. These results suggested that blue light treated with cabbage sprouts could be exploited as a functional food for fresh consumption or as a source of bioactive phytochemicals with potential industrial applications.

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