

The Effect of Blue-light-emitting Diodes on Antioxidant Properties and Resistance to *Botrytis cinerea* in Tomato

Kangmin Kim^{1#}, Hee-Sun Kook^{1#}, Ye-Jin Jang¹, Wang-Hyu Lee², Seralathan Kamala-Kannan¹, Jong-Chan Chae^{1*} and Kui-Jae Lee^{1*}

¹Division of Biotechnology, Advanced Institute of Environment and Bioscience, Chonbuk National University, Iksan 570-752, Korea ²Department of Agricultural Biology, College of Agriculture and Life Sciences, Chonbuk National University, Jeonju 561-756, Korea #These authors contributed equally to this work

Abstract

In higher plants, blue-light is mainly perceived by cryptochromes and phototropins, which subsequently orchestrates phototropism, chloroplast relocation, stomatal opening, rapid inhibition of hypocotyl elongation and leaf expansion. Blue-light signaling is also known to mediate the plant responses to biotic stresses, but relevant mechanisms are largely unknown. Here, we demonstrated that blue LED (Light Emitting Diode)-driven inhibition of gray mold disease was highly correlated with the increases in cellular protectants like proline, antioxidants and ROS (Reactive Oxygen Species) scavenger activities. After twenty one days of exposure to various wavelengths of LED lights, blue-LED treated tomato displayed significant increases in proline accumulation in the leaves and stems, whereas red- and green-LED treated tomato exhibited the lower proline contents. Similarly, the blue-LED treatment increased the amount of polyphenolic compounds in tomatoes, compared to other wavelength of LED lights. The activities of various ROS (Reactive Oxygen Species) scavenging enzymes were also slightly increased under the blue-LED lighted conditions. Finally, blue-LED significantly suppressed symptom development of tomato infected by gray mold. Combined results suggest that blue LED light inhibits the development of gray mold disease, which can be mechanistically explained by the enhanced proline accumulation and antioxidative processes at least in partial.

Keywords: Tomato; LED (Light Emitting Diode); Antioxidative defense system; *Botrytis cinerea*, Blue light

Introduction

Tomato (Lycopersicon esculentum Mill.) is the one of major greenhouse vegetable crops throughout the world. In terms of nutritional value, tomato is an excellent source of vitamin A and C, carotenoids, a-tocopherol, as well as phenolic compounds as antioxidants [1]. However, the quantity or quality levels of such phytochemicals vary considerably depending on genotype, and/or environmental conditions [2]. In greenhouse environment, phytopathogenic attack leads to retarded growth, damages to cell viability and eventually reduction of plant productivity [3,4]. In particular, the gray mold is the most widespread fungal disease in plants and caused by Botrytis cinerea, a necrotrophic fungal pathogen attacking fruits, vegetables and flowers of horticultural crops [3]. This fungus has great adaptability under broad environmental conditions, and is well known to rapidly develop fungicide resistance lines [5]. In horticultural aspects, optimization of cultivation conditions, such as temperature, humidity and light regimes might mitigate disease via modulating metabolite levels, and/or cellular compositions.

Light critically regulates the plant growth by regulating the various morphological and physiological changes of grown plants [6,7]. Recently, light-emitting diode (LED) as artificial light source for plant growing in controlled-environment have a variety of advantages, such as small volume, durability, longevity and selectable narrow-waveband emissions [8]. A few studies using this technology have been carried out on the effect of the light spectral quality on the plant growth and morphogenesis, as well as physiological responses by photooxidative changes [7,9,10]. It has been reported that red light, mainly perceived by Phytochromes (Pyr), is important for shoot growth including stem elongation on strawberries [11]. Wang et al. [12] found that disease resistance to Sphaerotheca fuliginea in cucumber plants was induced by red light. On the other hand, blue lights are perceived by majorly two different receptor family, cryptochromes and phototropins. The activation of blue light signaling modulates the various physiological and developmental processes, such as phototropism, chloroplast relocation, stomatal opening, rapid inhibition of hypocotyl elongation and leaf expansion [13]. It is known that blue light also regulates the responses against biotic environmental stresses. *Arabidopsis CRY1* (*Cryptochrome1*) positively regulated resistance to *Pseudomonas syringae*, potentially *via* effector-triggered R protein-mediated local resistance [14]. In addition, stability of some R proteins is modulated by blue light receptors [15,16]. Nevertheless, the mechanistic basis underlying such blue light-driven protection is largely unknown.

In general, the oxidative burst involving generation of reactive oxygen species (ROS) is the earliest cellular responses, following recognition of a variety of bacterial and fungal pathogens [17]. The enhanced production of ROS is pre-requisite for hypersensitive response (HR) related to programmed cell death in systemic acquired resistance (SAR), but also damage to the major cellular components, such as DNA, lipids and proteins [18]. In a systemic tissues, in order to minimize oxidative stress by excess ROS, plants have developed detoxifying mechanisms consisting of antioxidants and some ROS scavenging enzymes, such as peroxidase, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) [18]. Besides acting as an osmoprotectant, proline

*Corresponding authors: Kui-Jae Lee, Division of Biotechnology, Advanced Institute of Environment and Bioscience, Chonbuk National University, Iksan 570-752, Republic of Korea, Korea, Tel: +82-63-850-0836; Fax: +82-63-850-0834; E-mail: leekj@jbnu.ac.kr

Jong-Chan Chae, Division of Biotechnology, Advanced Institute of Environment and Bioscience, Chonbuk National University, Iksan 570-752, Korea, E-mail: chae@jbnu.ac.kr

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also plays antioxidative roles by bringing concentrations of ROS within compatible ranges under the stressed conditions [19]. On the other hand, certain wavelengths of lights have been reported to increase antioxidative actions against abiotic challenges. In broad bean leaves, for instance, significantly increase of catalase activity under red light contributed to scavenging of hydrogen peroxide generated by *Botrytis cinerea* infection [20]. In addition, enhanced activities of CAT, as well as increased contents of total polyphenol and proline were observed in flax resistant to powdery mildew [21]. Regardless of these reports, the effects of light quality on the antioxidative status of plants are still largely open questions.

Here, we examined the roles of white-, blue-, red- and green-LED into the growth of tomato and analyzed the contents of proline, total phenol and the activities of antioxidant enzymes. Moreover, to shed light on the correlation between increased antioxdative capacity driven by LED lights and resistance to pathogenic attacks, we monitored the disease development of tomato under blue-LED lights.

Materials and Methods

Plant growth conditions

Tomato (cv. Toy-mini tomato) seeds were surface-sterilized in 70% ethanol for 5 min and in 2% sodium hypochlorite for 20 min, followed by several rounds of washing with sterile water. Seeds were transferred to two sheets of sterile filter paper moistened with deionized water, and then germinated at 25°C under dark condition for three days. The uniformly germinated seedlings were transplanted into plastic culture tray (25×50×5 cm, the outer size of tray) containing peat-vermiculite media (Uddeumi, Sunghwa Co., Korea), and were grown for two weeks. For nutrition supply, the half-strength Hoagland solution [22] was irrigated. Growth chamber was set as the photoperiod of 18 hr and 22 \pm 1°C under a relative humidity 60-65% with a photon flux density of 150 µmol/m²s. The pH of nutrient solution was maintained at 5.8. At two weeks after transplanting, the tomato seedlings were planted into plastic pots (10×9 cm²), at a density of one plant per pot and grown in phytotron chamber (130×60×180 cm, Woniltech, Ltd., Korea), for twenty one days under different light-emitting diode (LED) conditions. After morphological measurements, the leaves and stems tissues of tomato were ground to fine powder in liquid nitrogen for biochemical analyses.

Light treatment conditions

Each lighting treatment was conducted in separately controlled chambers (ODTech, Ltd., Korea), to be free from spectral interference among treatments. The LED array chambers were programmed to provide an 18 h light/6 h dark photoperiod at photosynthetic photon flux (PPF) maintained of approximately 150 μ mol/m²s. All tomato plants were grown under four different light sources with broad-spectrum-white LED (BSWL, 420-680 nm) as a control, blue LED (460 nm), red LED (635 nm) and green LED (520 nm). Light quality and quantity were estimated using a Testo545 light meter (Testo, Germany).

Determination of proline content

Determination of free proline content was performed as previously described [23]. Tomato leaf and stem samples (0.5 g) were homogenized in 3% (w/v) sulfosalicylic acid and filtered through filer paper. Filtrate (2 mL) was reacted with acid ninhydrin (2 mL) and 30% glacial acetic acid (2 mL), and then heated at 100°C for 1 h. The reaction was extracted with 4 mL toluene for 30 min at room temperature; and the absorbance of the toluene fraction aspired from the liquid phase was measured at

520 nm. The proline concentration was determined based on a standard curve drawn with pure proline and expressed as μ mol proline g^{-1} FW.

Determination of total phenolic compounds

The amount of total phenolics was determined using the Folin-Ciocalteu method [24]. Tomato leaf and stem samples (0.5 g) from each LED treatment were stirred slightly in 10 mL of 80% aqueous methanol. The suspension was sonicated for 5 min and collected by centrifugation. Samples (500 μ L) were reacted with Folin-Ciocalteu's reagent (2.5 mL) and 7.5% sodium carbonate (2 mL) at room temperature for 30 min. The absorbance of the reaction product was measured at 765 nm. The total phenolic concentration was determined using gallic acid as a standard, and expressed as gallic acid equivalents in milligrams per gram of dry matter.

Antioxidant enzyme analysis

The activities of SOD, CAT, APX and GR were determined spectrophotometrically. SOD activity was assayed at 560 nm by determining the inhibition rate of nitroblue tetrazolium reduction, with xanthine oxidase as a hydrogen peroxide generating agent [25,26]. CAT activity was assayed at 240 nm by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules [27]. APX activity was determined at 290 nm following the oxidation of ascorbate to dehydroascorbate, as described by Nakano and Asada [28]. GR activity was determined at 340 nm by measuring the reduction kinetics of oxidized glutathione [29].

Detached leaf assay

Botrytis cinerea (No. 40574) was obtained from the Korean Agricultural Culture Collection (KACC; Suwon, Korea). B. cinerea were incubated on potato dextrose agar (PDA) medium (MB Cell, Los Angeles, USA) containing 4% potato starch, 20% dextrose and 15% agar at 24°C in the dark. After two weeks, spores on the medium were suspended with sterilized distilled water (DW). Spore concentrations were adjusted to the approximately 5.7×10⁵ mL⁻¹ using a hemocytometer. If not otherwise stated, the spore suspension was used at the same concentration throughout the experiments. Detached leaves from 4-week-old tomato plants were washed with DW and placed on wet filter paper in Petri dishes. The leaves were inoculated with 10 µL drops of the *B. cinerea* spore suspension $(5.7 \times 10^5 \text{ mL}^{-1})$, and then kept under blue or broad-spectrum-white LED light conditions for 10 days. Disease severity on leaves infected with B. cinerea was visibly assessed on a scale of 0 ('no symptoms') to 4 ('51 to 100% symptoms'), according to the method of Rajkumar et al. [30].

Statistical analysis

Data were analyzed by a general linear model and multiple comparisons among the treatments were conducted by Tukey's honestly significant difference (HSD), using the statistical analysis program, Statistix (Statistix 9 Analytical Software, USA). The significance of differences among samples was determined at 95% confidence level.

Results and Discussion

In many plant species, proline is a major organic osmolyte that maintains osmotic balances, induces expression of stress responsive genes and functions to stabilize sub-cellular structures, scavenges free radicals and buffers cellular redox potential under stress conditions [31]. To explore the effect of different wavelength of light on the accumulation of proline, we quantitated the amount of proline in leaves and stem of tomato grown under LED light having different wavelengths. There was



Figure 1: The content of proline in the leaves and stems of tomato grown for 21 days under light-emitting diode (LED) lights. Control represents broadspectrum-white LED. Error bars represents the standard deviation (n=3). Bars with the same low case letter are not significantly different (p>0.05), as assessed by Tukey's honestly significant difference.

a considerable difference in the content of proline of tomato seedlings lightened with different LED sources. Compared to broad-spectrum-white LED (BSWL), when tomato seedlings were treated with blue-LED, proline contents were increased in leaves and stems by about 296% and 127%, respectively. Each differences were statistically significant based on Tukey's HSD test with α =0.05 (p<0.05). In contrast, red and green LED lights significantly decreased the amount of accumulated proline (p<0.05, Figure 1), compared to BSWL emitted conditions.

We also investigated the antioxidant capacity of tomato leaves and stems cultivated under different LEDs. In plants, antioxidant defense systems include various antioxidants, such as carotenoids, tocopherol, flavonoids, ascorbate and phenolic compounds, which play important roles in protection from photooxidative damage [11,21]. To learn how different wavelengths of light modulate antioxidation capacity in partial, we measured the contents of phenolic compounds from tomato grown under different colored LED lights. When blue LED was engaged, the content of total phenolic compounds both in leaves (1.3 fold) and stems (1.2 fold) was significantly increased (Tukey's test with α =0.05, p<0.05), compared to BSWL conditions (Figure 2). On the other hand, under red and green light conditions, the content of total phenolic compounds of leaves showed a similar levels (Tukey's test with α =0.05) to BSWL conditions. However, in stems, red and green LED significantly decreased the contents of phenolic compounds by 49% and 37%, respectively, compared to BSWL LED. Luthria et al. [32] reported that the quantity and composition of phenolic compounds in plants bearing edible fruits is significantly influenced by the quality of light. Furthermore, Johkan et al. [33] reported that the content of phenolic compounds was increased in red leaf lettuce, as a result of supplemental blue light radiation [33]. Our results are in agreement with data published by Johkan et al. [33], in which the content of polyphenols and antioxidant activity were shown to be greatly increased in lettuce seedlings treated with blue LED light. Taken together, our results clearly indicated that the contents of proline and total phenolic compounds in leaves and stems of tomato plants was considerably influenced by the spectral quality of LEDs. Especially, blue-LED dramatically increased the contents of proline and phenolic compounds in vegetative tissues in plants.

Page 3 of 5

We also assessed the activity of antioxidant enzymes (e.g. SOD, CAT, APX, and GR) of tomato grown under each LED lights (Figure 3). In our study, no significant differences (Tukey's HSD test with α =0.05) between the BSWL, blue, red and green light treatments were found for SOD activity in tomato leaves. However, in the case of stem, SOD activity was significantly increased by 29% under blue-LED treatment, while SOD activities under the red- and green-LED treated stems were decreased by 16% and 35%, respectively, compared to BSWL LED treatment as a control (Tukey's HSD test with α =0.05, Figure 3A and 3B). Under the red- and green-LED treatment, however, CAT activity in leaves and stems were noticeably decreased by about 28% to 18% and 63% to 39%, respectively, compared to BSWL LED light (Tukey's HSD test with α =0.05, Figure 3C and 3D). In contrast, CAT activity in blue LED-treated tomato was increased about 15% in leaves compared to that in BSWL LED light treatment (Tukey's HSD test with α =0.05). Being similar to our results, Schmidt et al. [34] reported that the activation of catalase enzyme in winter rye leaves was more enhances by blue light



Figure 2: The content of total polyphenolic compounds in the leaves and stems of tomato grown for 21 days under light-emitting diode (LED) lights. Control represents broad-spectrum-white LED. Error bars represents the standard deviation (n=3). Bars with the same low case letter are not significantly different (p>0.05), as assessed by Tukey's honestly significant difference.

Page 4 of 5



tomato grown for 21 days under light-emitting diode (LED) lights. (A,B) superoxide dismutase (SOD); (C,D) catalase (CAT); (E,F) ascorbate peroxidase (APX); and (G,H) glutathione reductase (GR). Control represents broad-spectrum-white LED. Error bars represents the standard deviation (n=3). Bars with the same low case letter are not significantly different (p>0.05), as assessed by Tukey's honestly significant difference.

treatment than that in red or far-red light treatment. APX activity in blue-LED light treatment was also increased in leaves and stems by 7% and 13%, respectively, compared to that in BSWL LED light treatment (Tukey's HSD test with α =0.05, Figures 3E and 3F). In addition, in the case of GR activity of leaves and stems, the blue-LED treatment increased 1.4-fold and 2.1-folds (in leaves), 2.2-fold and 4.2-fold (in stems), compared to BSWL LED control (Tukey's HSD test with α =0.05, Figure 3G and 3H). In the leaves of tall fescue, Xu et al. [35] found that the activities of catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are increased by light treatment. Combined together, results of the present study indicated that blue-containing LED radiation had positive effects on the action of antioxidant defense mechanisms in tomato seedlings.

Inferred from the outstanding effects of blue-LED on proline contents and antioxidation capacities, we examined whether blue-LED light treatment increased defense ability of tomato to gray mold disease caused by *B. cinerea.* Under the blue-LED light- and BSWL-treated tomato leaves, disease incidence of gray mold was 0.67 and

3.33, respectively. Such differences are statistically significant based on (Tukey's HSD test with α =0.05, Figures 4A and 4B). In tomato leaves, Kuzniak and Sklodowska [36] found that activity increases of peroxisomal antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) can contribute to the inhibition of pathogen-induced leaf senescence by Botrytis cinerea infection. Khanam et al. [37] reported that enhanced catalase activity under red light treatment contributes to the inhibition of lesion formation and fungal development on broad bean leaves infected with Botrytis cinerea. In addition, Grote and Claussen [38] reported that the proline content in tomato leaves is increased by pathogen attack such as phytophthora nicotianae, as well as light intensity. Thus, numerous studies have suggested that physiological resistance of plants to environmental stresses, including pathogen attack, is closely connected with specific light treatments, as well as effective antioxidative mechanisms. Similarly, our results suggest that blue-LED light suppress the development of gray mold, and/or the propagation of B. cinerea in tomato potentially via enhanced accumulation of proline and antioxidative responses.

In conclusion, current study suggests that blue-LED is highly efficient to protect crop plants from pathogenic attacks, at least where artificial lights are applied as main light sources. In mechanistic level, such advantages of blue-LED are ascribed into the increased production



Figure 4: The effect of blue LED light on the resistance of tomato to B. *cinerea*. (A) Development of brown lesion in 4-week-old tomato seedlings infected by B. cinerea. Tomato leaves were inoculated with B. *cinerea* spores and then kept under broad-spectrum-white LED (control) and blue LED lights for 10 days. (B) Incidence of disease (mean \pm SD) was quantitatively assessed by the following indices: 0: no symptoms, 1: 1-12% lesion, 2: 13-25%, 3: 26-50%, and 4: 51-100%. Error bars represents the standard deviation (n=3). Bars with the same low case letter are not significantly different (*p*>0.05), as assessed by Tukey's honestly significant difference.

of osmoprotectants and antioxidants, including ROS scavenging enzymes. Nevertheless, it must be prompted which signaling pathway are activated by blue light to gain more insight into the light wavelengthdependent developmental modifications in plants.

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Page 5 of 5

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