Green and Red Light Reduces the Disease Severity by *Pseudomonas cichorii* JBC1 in Tomato Plants via Upregulation of Defense-Related Gene Expression

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Accepted for publication 7 October 2014.

ABSTRACT

Nagendran, R., and Lee, Y. H. 2015. Green and red light reduces the disease severity by *Pseudomonas cichorii* JBC1 in tomato plants via upregulation of defense-related gene expression. *Phytopathology* 105:412-418.

Light influences many physiological processes in most organisms. To investigate the influence of light on plant and pathogen interaction, we challenged tomato seedlings with *Pseudomonas cichorii* JBC1 by flood inoculation and incubated the seedlings under different light conditions. Tomato seedlings exposed to green or red light showed a significant reduction in disease incidence compared with those grown under white light or dark conditions. To understand the underlying mechanisms, we investigated the effects of each light wavelength on *P. cichorii* JBC1 and tomato plants. Treatment with various light wavelengths at

120 $\mu\text{mol m}^{-2}\text{s}^{-1}$ revealed no significant difference in growth, swarming motility, or biofilm formation of the pathogen. In addition, when we vacuum-infiltrated *P. cichorii* JBC1 into tomato plants, green and red light also suppressed disease incidence which indicated that the reduced disease severity was not from direct influence of light on the pathogen. Significant upregulation of the defense-related genes, phenylalanine ammonia-lyase (*PAL*) and pathogenesis-related protein 1a (*PR-1a*) was observed in *P. cichorii* JBC1-infected tomato seedlings grown under green or red light compared with seedlings grown under white light or dark conditions. The results of this study indicate that light conditions can influence plant defense mechanisms. In particular, green and red light increase the resistance of tomato plants to infection by *P. cichorii*.

Light is one of the major environmental signals that regulate the chemical and physiological processes in biological systems (30,44). For most bacteria, the light environment governs important lifestyle decisions including the choice between environmental and host-associated lifestyles (8). Recently, many reports have indicated that light influences both the defense response in the host and the virulence of the pathogens (11,31,38). Gomelsky and Hoff (8) reported that pathogenic bacteria use light to increase their virulence and assess their way to enter a new host. The authors further indicated that bacterial photoreceptors perceive different wavelengths of light from the environment and utilize it as a signal to transit between their environment and the pathogenic lifestyle. In addition, light regulates the surface attachment and biofilm formation of bacteria (31,32,35). For instance, the motility, attachment, and virulence in the hemibiotrophic plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 is influenced by light (37).

Light is also an indispensable factor for plant life, which not only regulates growth and developmental processes, but also has a discrete effect on disease development and defense mechanisms in plants (17). For instance, light amplified elicitor-induced phenylpropanoid phytoalexins are a precursor to biosynthesis in soybean cotyledons (10). In *Arabidopsis thaliana*, salicylic acid (SA) induced PR-1 accumulation and the development of hypersensitive response (HR) during an incompatible interaction were shown to be light- and phytochrome-dependent (4,7,48). Similarly, light of

a certain quantity induced a constitutive cell death phenotype in the *Arabidopsis* mutant *acd11* (2). In addition, plants acclimated to high light displayed increased resistance against *P. syringae* pv. *tomato* DC3000 both in tissues and in systemic leaves that were exposed directly to excess light (1,29). Islam et al. (14) reported that red light can induce systemic disease resistance against the root-knot nematode *Meloidogyne javanica* and *P. syringae* pv. *tomato* DC3000.

Red and blue light are crucial for photosynthetic metabolism while recent evidence signifies that green light also has a profound effect on plant processes (6,42). Green light sensors, phytochromes and cryptochromes, have been shown to adjust growth and development on par with red and blue light (6). Lin et al. (25) demonstrated that CRY1, a blue-UV-A photoreceptor, mediates responses in the green waveband. In addition, Gould et al. (9) reported that anthocyanins absorb more green light than red light and prevent photo degradation of light-labile plant defense secondary metabolites such as thiarubine A, which would otherwise degrade when exposed to visible light or UV radiation.

In view of the importance of light quality/quantity on the biological processes of bacteria and plants, understanding the influence of light on the interaction of pathogen and plant is essential for the design of new management strategies for plant diseases. However, the effects of light on bacterial pathogens and disease resistance still remain to be investigated. In this study, we report the role of various light wavelengths on disease development in tomato plants, and the lifestyle and virulence of its bacterial pathogen, *P. cichorii*. We demonstrated that light significantly alters the activation of defense-related genes. Furthermore, we suggest that green light is capable of up-regulating defense gene expression in tomato plants for the first time.

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*The e-Xtra logo stands for "electronic extra" and indicates that two supplementary figures are published online.

<http://dx.doi.org/10.1094/PHYTO-04-14-0108-R>
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MATERIALS AND METHODS

Bacterial strains. *P. cichorii* strain JBC1, which causes leaf spot on soybean, midrib rot on lettuce, and leaf spot and stem necrosis on tomato (46) was used in this experiment. The bacterial pathogen strains were picked from glycerol stock (-80°C) and inoculated in Luria-Bertani (LB) agar plates supplemented with vancomycin for *P. cichorii* JBC1 and cultured at 25°C as needed.

Design of light emitting diode panel. To investigate the effects of different light wavelengths on plant-microbe interactions, we designed and manufactured light emitting diode (LED) circuits (embedded in a plastic sheet of 8 cm diameter, 0.7 cm height) at ODTech Co. Ltd. (Jeonju, South Korea) as described by Yu and Lee (47). Each light source emitted photosynthetically active radiation, typically measured as photosynthetic photon flux density (PPFD), at a particular light wavelength such as red (wavelength range from 630 to 655 nm with a typical light emission at 645 nm), green (wavelength range from 497 to 544 nm with a typical light emission at 524 nm), blue (wavelength range from 448 to 475 nm with a typical light emission at 458 nm), and white (broad spectrum) light. The LED light sources were connected to a circuit box that allowed the control of light intensity for each wavelength. As each light source was 8 cm in diameter, it provided uniform light intensity from the center to the edges of a petri dish (9 cm diameter). The PPFD, which is quantified as $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ from each light source, was measured using a quantum sensor (LI-190 SB; Li-Cor, Lincoln, NE) as necessary.

Plant cultivation and bacterial inoculation assay. The seeds of a tomato plant (*Solanum lycopersicum* 'Seo Gwang') were surface-disinfected with 70% ethanol for 5 min followed by treatment with 20% commercial bleach, and then the seeds were rinsed with distilled water (DW) five times. The disinfected seeds were sown in Murashige and Skoog (MS) media fortified with 0.8% agar and the plates (16 seeds/plate) were incubated in a plant growth chamber (JS Research Inc., Gongju, Korea) for 4 to 5 days at 25°C with 16-h light and 8-h dark conditions. The MS agar plates containing tomato seedlings with 4 to 5 cm long hypocotyls and two to three leaflets were selected and used for the experiments.

To investigate the effects of light wavelength on the interaction between bacterial pathogen *P. cichorii* JBC1 and tomato plants, a seedling flood inoculation assay was carried out following Uppalapati et al. (43), with small modifications. *P. cichorii* JBC1 cultured in LB broth was adjusted to a concentration of $\text{OD}_{600} = 0.2$ (1×10^8 CFU/ml) in sterile DW supplemented with 0.025% (wt/vol) Silwet L-77. The bacterial suspension was flooded in MS agar plates such that the seedlings were submerged completely by the pathogen. The seedlings were incubated with slight mixing for 3 min and the bacterial suspensions were decanted from the plates. The lids of the MS agar plates were replaced with the base petri plates, and sealed firmly with parafilm. An LED circuit emitting a particular wavelength, red, green or white, was placed on the top of each plate, and adjusted to a particular distance (approximately 7 to 10 cm) such that it emitted light at an intensity of $120 \mu\text{mol m}^{-2}\text{s}^{-1}$ with a 16-h light/8-h dark photoperiod. The whole setup was covered by aluminum foil and transferred to a plant growth chamber maintained at 25°C . The infected seedlings were also placed in the dark.

After 4 days of incubation in different light conditions, the disease incidence in each plate was assessed by calculating the percentage of infected seedlings per plate and scored using a scale of 0 to 3, where $X_0 = 0$ to 25% infected seedlings (IS); $X_1 = 26$ to 50% IS; $X_2 = 51$ to 75% IS; and $X_3 = 76$ to 100% IS. Based on the scores of each set of seedlings, the amount of disease incidence of the seedlings under different light conditions was calculated using the following formula: $[\sum(\text{number of seedlings in each score} \times \text{numerical value of each score}) / 3 \times \text{total number of seedlings}] \times 100$. The experiment consisted of five replicates of 16 seeds each (~ 80 seedlings) and was repeated three times. In addition, bacterial

growth in tomato seedlings was determined by measuring the internal bacterial population. The leaves of infected tomato seedlings were collected, weighed, and surface sterilized with 15% H_2O_2 followed by rinsing with DW. The collected leaves were then homogenized in a pellet pestle with DW and the suspensions were serially diluted. A 100- μl aliquot of each diluted sample was spread on LB agar plates containing vancomycin, and the number of bacterial colonies in each plate was counted 24 h after incubation (HAI) at 25°C .

To assay the response of mature tomato plants to each light wavelength, we sprayed a cell suspension of *P. cichorii* JBC1 (1×10^8 CFU/ml) onto the leaves of 4-week-old tomato plants. Each light was illuminated 1 h prior to inoculation, and the inoculated plants were kept under different light wavelengths for 16 h per day (100 PPFD, 20 cm distance). Disease severity was recorded by adopting a scale from 0 to 5 (X_0 = no symptom; X_1 = up to 5% infected leaf area [ILA]; X_2 = 6 to 10% ILA; X_3 = 11 to 25% ILA; X_4 = 26 to 50% ILA; and X_5 = 51 to 100% ILA). The amount of disease incidence was calculated by using the following formula: $[\sum(\text{number of leaves } X_1 \times 1 + X_2 \times 3 + X_3 \times 5 + X_4 \times 7 + X_5 \times 9) / 5 \times \text{total number of leaves recorded}] \times 100$. Ten plants were tested for each treatment and symptoms were recorded from 50 leaves per treatment (five leaves/plant) 7 days after inoculation.

Effect of light wavelength on the growth of *P. cichorii* JBC1. The bacterial strain *P. cichorii* JBC1 was precultured in 10 ml of LB broth at 25°C and 180 rpm in darkness overnight, and 2 ml of the culture was inoculated in 20 ml of fresh LB broth (ca. 1×10^6 CFU/ml) in 100-ml conical flasks (Schott Duran, Germany). Each flask was laid on a light source and wrapped with aluminum foil to avoid the effect of ambient light. These flasks were incubated at 25°C and 180 rpm in a shaking incubator. White-, red-, and green-emitting diodes with an intensity of 120 and $240 \mu\text{mol m}^{-2}\text{s}^{-1}$ at a distance of 5 cm were used in the growth assay. The growth of each bacterial strain was spectrophotometrically monitored by measuring the optical density (OD) at 600 nm at 48 HAI. Control flasks were covered with aluminum foil without any light source. Based on the OD values, the growth of *P. cichorii* JBC1 at different wavelengths and intensities was evaluated. The light sources did not affect the temperature of the growth media significantly ($25.0 \pm 1^{\circ}\text{C}$). Each treatment consisted of three replicates and was repeated three times.

Effect of light wavelength on swarming motility and biofilm formation. Swarming motility was assayed as described by Kraiselburd et al. (23) with minor modifications. Briefly, swarm agar plates were prepared with LB media fortified with 0.3% agar. The surfaces of the plates were dried for 30 min in a laminar flow hood before use. The *P. cichorii* JBC1 strain was grown overnight on LB broth in darkness at 25°C , and 2% of the overnight culture was transferred to fresh LB media and allowed to grow until the stationary phase and adjusted to $\text{OD}_{600} = 0.2$ (1×10^8 CFU/ml). A 3- μl volume of the suspension was added to the center of the swarming plates using a micropipette. The inoculated plates were incubated at 25°C under white, red, green, and blue light with an intensity of $120 \mu\text{mol m}^{-2}\text{s}^{-1}$, as well as in darkness. The swarming zone was observed and photographed 12 HAI, and the swarming motility was recorded by measuring colony diameter.

Static biofilm formation was evaluated following Merritt et al. (28) with small modifications. *P. cichorii* JBC1 was grown until the stationary phase and was diluted 100-fold with fresh LB medium supplemented with 1 mM MgSO_4 . A 100- μl aliquot of each diluted culture was transferred to individual wells of 96-well microtiter plates and incubated at 25°C for 48 h without shaking under the different light conditions, white, green, and red, with an intensity of $120 \mu\text{mol m}^{-2}\text{s}^{-1}$, as well as in darkness. After incubation, the bacterial cells were decanted and removed by washing with DW followed by staining the wells with 125 μl of 0.1% (wt/vol) crystal violet. After incubating for 10 min at room temperature, the wells were rinsed three times with DW and allowed to air-dry to remove excess water. The biofilm mass and the dye were solubilized by

adding 200 μ l of 30% acetic acid for 15 min at room temperature. A 125- μ l aliquot of each solubilized mixture was transferred to a fresh microtiter plate and the OD was measured at a wavelength of 595 nm. The experiment was performed in triplicate and repeated twice.

Vacuum infiltration assay. Four-week-old tomato plants were uniformly inoculated with *P. cichorii* JBC1 by vacuum infiltration following the protocol described by Katagiri et al. (20). Briefly, *P. cichorii* JBC1 cells from an overnight cultured LB plate were scraped off and washed twice with DW followed by resuspension in 10 mM MgCl₂. The concentration of the bacterial inoculum was adjusted to OD₆₀₀ = 0.002 (1×10^6 CFU/ml) using a spectrophotometer and 0.004% Silwet L-77 (40 μ l/liter) was added to the bacterial inoculums. The tomato plants were exposed to different light wavelengths 1 h prior to infection. The bacterial inocula were poured into a container followed by submerging the whole plant in the container except the pot. The vacuum pump was turned on after placing the inoculums with the plant inside the vacuum infiltration apparatus. After the vacuum pressure reached 20 inches of mercury, it was maintained for 1 min, at which point the vacuum pressure was released rapidly by releasing the valve stopcock. After infiltration, the leaves were allowed to air-dry for some time and kept inside the growth container under high humidity, and the LED panels were placed on top of the containers with the intensity of each light adjusted to 120 μ mol m⁻²s⁻¹. Disease symptoms were observed 7 days after incubation (DAI) at 25°C with a 16-h light/8-h dark photoperiod. Disease severity was evaluated by measuring the diseased area of the infected leaves with a 0 to 5 disease index scale: 0, no symptoms; 1, 0 to 20%; 2, 20 to 40%; 3, 40 to 60%; 4, 60 to 80%; and 5, over 80% of the diseased leaf area. On each leaf, the infection area was counted by the following formula: (the pixel of infected area/pixel of total leaf) $\times$ 100. The pixels of areas were measured using magnetic lasso tool

and histogram tool from Photoshop 7.0 (23). Average of disease index was calculated by using the following formula: Disease index = (Σ disease index number $\times$ number of leaves with similar disease index)/total leaves in the plant. We used three individual plants for each light treatment and conducted three independent experiments.

Quantitative polymerase chain reaction analysis for the expression of defense-related genes. The flood-inoculated tomato seedlings with *P. cichorii* JBC1 and noninoculated tomato seedlings were exposed to each light wavelength for 72 h, and leaves from five individual seedlings per plate were collected in liquid nitrogen. Total RNA was isolated using TRI reagent (Ambion, Life Technologies), and cDNA for each RNA sample was synthesized using a TOPscript cDNA Synthesis Kit (Enzynomics, Korea) according to the manufacturer's instruction. The expression of the *PAL* and *PR-1a* genes was assayed using a Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Science). The polymerase chain reaction (PCR) mixture included 10 μ l of SYBR Green Supermix, 1 μ l of diluted cDNA, and 0.1 μ M of each primer. The primers used for *PAL* gene expression were PAL-F 5'TGTCATCAACCTATCTCGTGGC3' and PAL-R 5'ATGAAGTTCGCCATTAGCACCC3. The primers used for *PR-1a* gene expression were PR-1a-F 5'CCGTGCCCAAGTCGGAGTCGGG3' and PR-1a-R 5'TGAAGTCACCACCACCCTTGGC3'. PCR cycling was performed as follows: preliminary denaturation at 95°C for 15 min and 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 15 s and elongation at 72°C for 20 s. The cycle threshold (CT) values were collected after the final melt. Melting curve analysis was performed to confirm the specificity of each primer for amplification at the end of each program. The Clathrin adaptor complex (*CAC*) gene of tomato was used as a control gene with unchanged expression upon infection. The primers used for *CAC* gene expression were CAC-F 5'CCTCCGTTGTGATGTAAC3' and CAC-R 5'ATTGGTGGAAAGTAACATCAT3'. The relative gene expression was calculated according to Livak and Schmittgen (26). The experiment was performed in triplicate and repeated three times.

RESULTS

Light wavelength influence on disease development by *P. cichorii* JBC1. To investigate the influence of light on the *P. cichorii* JBC1 and tomato interaction, the tomato seedlings were flood-inoculated with *P. cichorii* JBC1 and the symptom development in tomato seedlings under different light wavelengths was visually evaluated 4 DAI. The seedlings grown under white light exposure or in darkness showed severe necrotic lesions on the leaves and hypocotyls. However, the seedlings grown under red and green light showed only slight necrosis (Fig. 1). The amount of disease was significantly reduced when the seedlings were exposed to green light (26.5%) compared with the exposure of seedlings to red (49.5%) and white light (73.7%) or under darkness (95.5%). In addition, experiments with mature tomato plants also showed significant reduction in disease under red (8.5%) and green light (3.3%) compared with white light (24%) or darkness (85%) (Supplementary Fig. 1).

We further evaluated the in planta cell growth of *P. cichorii* JBC1 by quantifying internal populations in the tomato seedlings. Quantification results 4 DAI revealed that the bacterial growth in red and green light-treated tomato plants was significantly reduced to 8×10^8 and 1×10^6 CFU/g of leaf, respectively, compared with 3×10^9 and 2.8×10^{10} CFU/g of leaf in white light and in dark conditions, respectively.

Influence on the growth of *P. cichorii* JBC1 due to the different light conditions. To find out the reasons for the alteration of disease incidence due to different light treatments, we investigated the effect of various light wavelengths on the bacterial growth (Fig. 2). There was no significant change in the growth rate of *P. cichorii* JBC1 in various light conditions, namely red

Fig. 1. Effect of light wavelengths on *Pseudomonas cichorii* JBC1 disease severity in tomato seedlings. Four- to five-day-old tomato seedlings in MS agar plates (16 seedlings/plate) were flood-inoculated with *P. cichorii* JBC1 and incubated under red, green, and white light at an intensity of 120 μ mol m⁻²s⁻¹. **A**, Disease incidence in each plate was assessed 4 days after incubation using a score of 0 to 3 and calculated as described in the Materials and Methods. The in planta bacterial population under different light conditions was determined by homogenizing the infected leaves and plating on Luria-Bertani agar plates after serial dilution. The data obtained are the means $\pm$ SD from three independent experiments in five replicates. Means with the same letter are not significantly different according to Tukey's studentized range (honest significant difference) test ($P \leq 0.05$). **B**, The picture was taken after 3 days of bacterial and light treatment.

(8.5×10^8 CFU/ml), green (8.5×10^8 CFU/ml), and white (8.7×10^8 CFU/ml) at an intensity of $120 \mu\text{mol m}^{-2}\text{s}^{-1}$ or in darkness (8.7×10^8 CFU/ml). However, the bacterial growth was slightly inhibited when the intensity of each light wavelength was increased to $240 \mu\text{mol m}^{-2}\text{s}^{-1}$ (Fig. 2).

Influence on swarming motility and biofilm formation of *P. cichorii* JBC1 due to light wavelength. Light wavelength may have an influence on the dispersal and attachment of bacterial pathogens on the surface of tomato plants. Therefore, we investigated the effect of light wavelength at $120 \mu\text{mol m}^{-2}\text{s}^{-1}$ on the swarming motility and biofilm formation. The swarming motility on a 0.3% LB agar plate was slightly reduced, but it was insignificant when exposed to green light (7.5 cm) compared with dark (8.0 cm) and white light (8.0 cm) (Fig. 3).

To address the question of whether or not the light wavelength has an influence on the biofilm formation of *P. cichorii* JBC1, we incubated the *P. cichorii* JBC1 strain under red, green, and white light, as well as in darkness for 48 h. The biofilm formation in all of the treatments was similar: red (1.31 OD_{595 nm}), green (1.37 OD_{595 nm}), white (1.46 OD_{595 nm}), and dark (1.44 OD_{595 nm}) (Fig. 3).

The influence of the light wavelength on the entry of *P. cichorii* JBC1 to the tissues of tomato plants. Light conditions induce the opening/closing of the stomata in plants. To determine whether the changes in the disease development were influenced by the entry of *P. cichorii* JBC1 cells, we infiltrated bacterial cells into the leaves of tomato and cultivated the inoculated plants under various light wavelengths. Based on the average of infected area in each leaf, the disease symptoms and incidence in tomato plants grown under green light were significantly reduced (3.3%) compared with that of the plants grown under red light (18.5%) and white light (30.6%), or in darkness (67.4%) (Fig. 4). The alteration of disease incidence corresponded to the results from the flood inoculation assay.

Upregulation of the expression of defense-related genes under red and green light exposure. Following the results that light did not significantly influence the lifestyle of *P. cichorii* JBC1 or its entry into plant tissues, while the amount of disease by *P. cichorii* JBC1 in tomato plants was significantly altered by treatment with different light wavelengths, we sought to determine whether light could influence defense-related gene expression in tomato plants. To this end, we analyzed the expression of the *PR-1a* and *PAL* genes from *P. cichorii* JBC1 infected and uninfected tomato seedlings grown under different light conditions. Intriguingly, both *PAL* and *PR-1a* were upregulated in the *P. cichorii*

JBC1-infected tomato seedlings exposed to red and green light (Fig. 5). The *PAL* gene in infected seedlings was upregulated 7.1 ± 0.9 and 9.4 ± 0.9 fold higher compared with the uninfected seedlings when treated with red light and green light, respectively. In addition, the *PR-1a* gene in infected seedlings was also upregulated 3.9 ± 0.9 fold and 5.3 ± 0.9 fold higher than the uninfected seedlings under red and green light conditions, respectively. The amplification specificity was confirmed by melting curve analysis which showed a single peak (Supplementary Fig. 2).

DISCUSSION

The importance of light to the ecological and pathological processes of microbial pathogens and disease development in plants is becoming increasingly apparent (13,38). To understand the influence of light on plant and bacterial pathogen interaction, we inoculated 3- to 4-week-old tomato plants with bacterial pathogen *P. cichorii* JBC1, and cultivated under various light wavelengths. Interestingly, disease incidence was significantly reduced when the tomato plants were exposed to the green and red wavelengths. Following the observation, we assayed the disease incidence and in planta bacterial population using tomato seedlings challenged with *P. cichorii* JBC1 under different light wavelengths. Not surprisingly, the results (Fig. 1) showed significant reduction in disease incidence and internal bacterial growth in the tomato seedlings grown under green and red light.

Various cellular responses of microorganisms are light-dependent (30,31). Prokaryotes detect the visible light environment using photosensory receptors and regulate physiological processes for adaptation to the environment (34). The growth of *P. cichorii* JBC1 under green, red, and white light at 120 PPFD was similar to the growth of *P. cichorii* JBC1 incubated in darkness (Fig. 2), which indicates that different light wavelengths at 120 PPFD did not

Fig. 2. Effect of light wavelength on the growth of *Pseudomonas cichorii* JBC1. Bacterial cells (1×10^6 CFU/ml) in 20 ml of Luria-Bertani were cultured under exposure to red, green, and white light at 120 and $240 \mu\text{mol m}^{-2}\text{s}^{-1}$ or in darkness for 48 h. The bacterial growth under each wavelength was determined by measuring the optical density at 600 nm (OD_{600nm}). Results are means $\pm$ SD from three independent experiments in triplicate. Means with the same letter are not significantly different according to Tukey's studentized range (honest significant difference) test ($P \leq 0.05$).

Fig. 3. Effect of light on the swarming motility and biofilm formation of *Pseudomonas cichorii* JBC1. For the swarming motility assay, bacterial cells were inoculated on 0.3% agar Luria-Bertani medium and incubated at 25°C under red, green, and white light conditions at $120 \mu\text{mol m}^{-2}\text{s}^{-1}$ or in darkness for 12 h. **A**, After incubation, the swarming diameter under each light was evaluated. Biofilms formed in 96 well microtiter plates under different light conditions were stained with crystal violet and quantified using a microtiter plate reader at 595 nm , 48 h after incubation (HAI) at 25°C . Each bar represents means $\pm$ SD from three independent experiments in triplicate. Means with the same letter are not significantly different at $P \leq 0.05$ according to Tukey's studentized range (honest significant difference) test. **B**, Swarming motility of *P. cichorii* JBC1 under different light conditions. The pictures were taken 12 HAI.

significantly influence the growth of *P. cichorii* JBC1. In previous reports, the growth of *P. syringae* pv. *tomato* DC3000 was not affected by different light conditions (36), which is very similar to the results of the present study. Though the growth of *P. cichorii* JBC1 at 240 PPFD was slightly inhibited, we believe that the change in disease severity at 120 PPFD was not due to the negative effect of light on bacterial growth.

The motility of bacteria plays an important role in plant-bacterium interactions by allowing efficient dispersion and infectivity. For instance, motility increased the infectivity of

P. syringae pv. *phaseolicola* (33), *P. syringae* pv. *glycinea* (12), and *Ralstonia solanaceae* (41). Our results indicate that the swarming distance of *P. cichorii* JBC1 was similar for all wavelengths of light at 120 PPFD (Fig. 3). Though the motility was reduced by 240 PPFD in our preliminary studies, the results indicate that different light wavelengths at 120 PPFD did not significantly influence motility. Prior to host infection, bacteria physically interact with the plant by adhering to and colonizing the epiphytic surface of the plant, forming biofilms (36). The results of the present study indicated that different wavelengths of light at 120 PPFD did not significantly affect the biofilm formation of *P. cichorii* JBC1 (Fig. 3). Taken together, the growth, swarming motility, and biofilm formation of *P. cichorii* JBC1 was not significantly altered by light wavelengths at 120 PPFD. Therefore, the decrease in the disease severity in tomato seedlings cultivated under green and red light may not be from direct negative effects on *P. cichorii* JBC1.

Stomata opening and closure plays a major role in the entry of a pathogen into a host plant. In order to investigate whether disease reduction under green light was due to differences in the entry of *P. cichorii* JBC1 into hosts, we infiltrated tomato plants with *P. cichorii* JBC1 cells to ensure entry regardless of stomatal opening. Intriguingly, the disease development on leaves of tomato plants grown under green light remained significantly reduced (Fig. 4) compared with those under red and white light or in darkness, which corresponds to the results observed from the flood inoculation assay (Fig. 1) and 4-week-old plants. Río-Álvarez et al. (37) reported that white and blue light inhibited the motility of *P. syringae* pv. *tomato* DC3000 while promoting its attachment to plant leaves. In addition, they indicated that light-controlled changes during the epiphytic stage cause a reduction in virulence, highlighting the relevance of motility during the entry process into the plant. However, the results of this study suggested that light regulation of stomatal opening/closing may not be the cause of disease suppression in tomato and *P. cichorii* interaction under green light.

To battle against pathogens upon infection, plants induce defense responses which include activation of the SA- and jasmonic acid-mediated defense pathways, which in turn increase reactive oxygen species (ROS) production, phytoalexin accumulation, HR, and/or upregulation of PR protein expression. Light plays an important role in plant defense against pathogens and is required for the activation of several defense genes and the regulation of cell death response (4,19,38). Light signals are perceived and transduced into other downstream cellular responses by a family of photoreceptors that

Fig. 4. Effect of light on disease development by *Pseudomonas cichorii* JBC1. A cell suspension of *P. cichorii* JBC1 (1×10^6 CFU/ml) was vacuum infiltrated into the leaves of 4-week-old tomato plants. Each light was illuminated 1 h prior to inoculation, and the inoculated plants were kept under different light wavelengths for 16 h a day (120 photosynthetic photon flux density [PPFD], 20 cm distance). **A**, The disease severity in each sample was measured by a direct estimation of percent disease per leaf after incubation. The data obtained are the means $\pm$ SD from three independent experiments in three replicates. Means with the same letter are not significantly different according to Tukey's studentized range (honest significant difference) test ($P \leq 0.05$). **B**, Disease symptoms were observed and photographed 7 days after incubation.

Fig. 5. Expression of defense-related genes in tomato plants by quantitative polymerase chain reaction (qPCR) analysis. The leaves of tomato seedlings flood-inoculated with *Pseudomonas cichorii* JBC1 were harvested 72 h after incubation under different light conditions at $120 \mu\text{mol m}^{-2}\text{s}^{-1}$ or in darkness. The total RNA was extracted, reverse transcribed, and qPCR analysis was performed with primers for *PAL* and *PR-1a* while *CAC* was used as a housekeeping gene for internal control. Using the cycle threshold (CT) values obtained from qPCR, fold differences were normalized to the expression of defense-related genes under white light and calculated using the $\Delta\Delta\text{CT}$ method. The difference of **A**, *PAL* and **B**, *PR-1a* gene expression was compared with uninfected control. The graph presents the means $\pm$ SD from three independent experiments in triplicate. Means with the same letter are not significantly different according to Tukey's studentized range (honest significant difference) test ($P \leq 0.05$).

includes phytochrome, cryptochrome, phototropin, and several others (21,45). Among them, phytochromes, which absorb red/far-red light, influenced defense responses such as hypersensitive cell death, SA-induced PR-1 accumulation, and systemic acquired resistance in *Arabidopsis* (7,11,18). Mutations in the photoreceptors phytochrome A (PhyA) or B (PhyB) not only compromised the HR but also repressed the expression of *PR-1* gene, which increased susceptibility to the avirulent strain *P. syringae* (7). Furthermore, the constitutive shade-avoidance 1 mutant (*csa1*), which showed a dominant negative effect on phytochrome signaling also suppressed resistance against pathogenic *P. syringae* strain, which indicates phytochrome-mediated defense response (5). However, HR-associated programmed cell death triggered by *Turnip crinkle virus* was dependent on light, but independent of the PhyA and PhyB (4).

In addition, chloroplasts, which take part in ROS production in plant cell, were capable of inducing defense responses by triggering and/or mediating ROS signal during interaction with pathogens (15,16,29). For instance, functional chloroplasts were required for the HR, which indicating participation of redox status of the chloroplast for light-dependent plant immunity (7). Furthermore, in *Arabidopsis*, the *lesion simulating disease 1* (*lsd1*) mutant fails to limit the spread of HR under long (>16 h) photoperiods or after infection with an avirulent pathogen. This result suggests that *lsd1* fails to upregulate genes encoding Cu/Zn superoxide, which act as antioxidant enzymes in chloroplasts (27). Further analysis using *Arabidopsis* mutants indicated that HR, light acclimation, and light-dependent defense responses are initiated, at least in part, by redox changes of the plastoquinone pool (18,29).

Pathogen-induced activation of phenylpropanoid biosynthesis is another major defense pathway controlled by light. *Arabidopsis* plants inoculated in darkness with an avirulent strain of *P. syringae* were not able to substantially accumulate the phenolic metabolite SA and failed to induce expression of the key phenylpropanoid pathway enzyme PAL (48). However, our understanding of the qualitative and quantitative effects of light on disease resistance of plants to bacterial pathogens is still limited.

The lack of a significant influence of light on the growth of *P. cichorii* JBC1 and entry to the plant apoplasts led us to further investigate the effects of light wavelength on defense-related gene expression in tomato plants infected with *P. cichorii* JBC1 and uninfected control. The expression of the *PAL* and *PR-1a* genes was significantly upregulated in infected tomato seedlings grown under green and red light compared with those under white light or in darkness (Fig. 5). However, no significant elevation of *PAL* and *PR-1a* genes was observed in uninfected tomato seedlings. In addition, when we vacuum-infiltrated tomato plants with *P. cichorii* JBC1, the disease incidence was also reduced under green and red light (Fig. 4). Taken together, the results suggested that red and green light initially trigger the priming of *PAL* activity and *PR-1a* synthesis for resistance following *P. cichorii* infection. Genoud et al. (7) previously reported the strong reduction of SA-induced PR gene expression in *Arabidopsis* by *P. syringae* pv. *tomato* DC3000 under dark conditions, which is similar to our results.

Many previous studies indicated that red light induces resistance in broad bean against *Botrytis cinerea* (22), in *Arabidopsis* against *P. syringae* pv. *tomato* DC3000 (14), and in rice against *Magnaporthe grisea* (39). Moreover, green light, which is perceived by phytochromes and cryptochromes, has been reported to play an important role in plant growth and development (6,45). Green light also reversed blue light stimulated stomatal opening in a diverse range of plant species (40). Although more investigation is necessary to understand the molecular processes involved in green light perception and signaling for disease resistance, the results of this study indicate that green light significantly reduces disease severity in tomato plants by modulating defense-related gene expression, which is the first report on this phenomenon to our knowledge.

Considering the results of previous literature, an important regulatory function of light plays pivotal roles in different disease

resistance responses. Especially the interaction between green light and plant responses underpinning defense signaling mechanisms promises to be an attractive research area. Moreover, the results of this study suggested that light quality and quantity need to be carefully controlled when we culture microorganisms or assay disease resistance of crops in routine experiments. Moreover, light influenced on growth of human pathogens and senescence of fresh vegetables during postharvest storage, which indicated the possibility of specific light wavelengths to control postharvest spoilage (3,24). We strongly believe that plant responses including disease resistance can be modulated by regulating light quality and quantity. However, we need more understanding on influence of each light and coactions of the lights.

In summary, the results of the present study demonstrated that tomato plants grown under green and red light suppress disease development by regulating defense-related gene expression. However, we strongly believe that the influence of light on the pathogenesis of microorganisms is quite variable. In addition, the influence of light on defense signaling networks still remains to be answered. To answer these questions, we would like to attempt to identify the green light responsive genes, receptors, and transcription factors which mediate active resistance against *P. cichorii* JBC1 infection using high throughput transcriptomic approaches. Furthermore, we will characterize the identified genes using gain and loss of function approaches to acquire an in-depth understanding of how green light can suppress disease severity.

ACKNOWLEDGMENTS

We gratefully acknowledge a grant from the Basic Science Research program (Project No. 2014R1A1A4A01003957) and Basic Research Laboratory Program (Project No. 2011-0020202) through the National Research Foundation of Korea (NRF) funded by the Ministry of Education and Science, ICT and Future Planning.

LITERATURE CITED

1. Bechtold, U., Karpinski, S., and Mullineaux, P. M. 2005. The influence of the light environment and photosynthesis on oxidative signaling responses in plant-biotrophic pathogen interactions. *Plant Cell Environ.* 28: 1046-1055.
2. Brodersen, P., Petersen, M., Pike, H. M., Olszak, B., Skov, S., Odum, N., Jorgensen, L. B., Brown, R. E., and Mundy, J. 2002. Knockout of *Arabidopsis* accelerated-cell-death11 encoding a sphingosine transfer protein causes activation of programmed cell death and defense. *Genes Dev.* 16: 490-502.
3. Büchert, A. M., Lobato, M. E. G., Villarreal, N. M., Civello, P. M., and Martinez, G. A. 2011. Effect of visible light treatments on postharvest senescence of broccoli (*Brassica oleracea* L.). *J. Sci. Food Agric.* 91: 355-361.
4. Chandra-Shekhara, A. C., Gupte, M., Navarre, D., Raina, S., Raina, R., Klessig, D., and Kachroo, P. 2006. Light-dependent hypersensitive response and resistance signaling against Turnip Crinkle Virus in *Arabidopsis*. *Plant J.* 45:320-334.
5. Faigon-Soverna, A., Harmon, F. G., Storani, L., Karayekov, E., Staneloni, R. J., Gassmann, W., Mas, P., Casal, J. J., Kay, S. A., and Yaovsky, M. J. 2006. A constitutive shade-avoidance mutant implicates TIR-NBS-LRR proteins in *Arabidopsis* photomorphogenic development. *Plant Cell* 18: 2919-2928.
6. Folta, K. M., and Maruhnich, S. A. 2007. Green light: A signal to slow down or stop. *J. Exp. Bot.* 58:3099-3111.
7. Genoud, T., Buchala, A. J., Chua, N. H., and Metraux, J. P. 2002. Phytochrome signalling modulates the SA-perceptive pathway in *Arabidopsis*. *Plant J.* 31:87-95.
8. Gomelsky, M., and Hoff, W. D. 2011. Light helps bacteria make important lifestyle decisions. *Trends Microbiol.* 19:441-448.
9. Gould, K. S., Dudle, D. A., and Neufeld, H. S. 2010. Why some stems are red: Causal anthocyanins shield photosystem II against high light stress. *J. Exp. Bot.* 61:2707-2717.
10. Graham, T. L., and Graham, M. Y. 1996. Signaling in soybean phenylpropanoid responses (dissection of primary, secondary, and conditioning effects of light, wounding, and elicitor treatments). *Plant Physiol.* 110: 1123-1133.

11. Griebel, T., and Zeier, J. 2008. Light regulation and daytime dependency of inducible plant defenses in *Arabidopsis*: Phytochrome signaling controls systemic acquired resistance rather than local defense. *Plant Physiol.* 147:790-801.
12. Hattermann, D. R., and Ries, S. M. 1989. Motility of *Pseudomonas syringae* pv. *glycinea* and its role in infection. *Phytopathology* 79:284-289.
13. Idnurm, A., and Crosson, S. 2009. The photobiology of microbial pathogenesis. *PLoS Pathog.* 5:e1000470.
14. Islam, S. Z., Babadoost, M., Bekal, S., and Lambert, K. 2008. Red light-induced systemic disease resistance against root-knot nematode *Meloidogyne javanica* and *Pseudomonas syringae* pv. *tomato* DC 3000. *J. Phytopathol.* 156:708-714.
15. Joo, J. H., Wang, S., Chen, J. G., Jones, A. M., and Fedoroff, N. V. 2005. Different signaling and cell death roles of heterotrimeric G protein alpha and beta subunits in the *Arabidopsis* oxidative stress response to ozone. *Plant Cell* 17:957-970.
16. Kachroo, P., Lapchyk, L., Fukushige, H., Hildebrand, D., Klessig, D., and Kachroo, P. 2003. Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the *Arabidopsis* *ssi2* mutant. *Plant Cell* 15:2952-2965.
17. Kangasjärvi, S., Neukermans, J., Li, S., Aro, E. M., and Noctor, G. 2012. Photosynthesis, photorespiration, and light signaling in defense responses. *J. Exp. Bot.* 63:1619-1636.
18. Karpinski, S., Gabrys, H., Mateo, A., Karpinska, B., and Mullineaux, P. M. 2003. Light perception in plant disease defence signaling. *Curr. Opin. Plant Biol.* 6:390-396.
19. Karpinski, S., Reynolds, H., Karpinska, B., Wingsle, G., Creissen, G., and Mullineaux, P. M. 1999. Systemic signaling and acclimation in response to excess excitation energy in *Arabidopsis*. *Science* 284:654-657.
20. Katagiri, F., Thilmony, R., and He, S. Y. 2002. The *Arabidopsis thaliana*-*Pseudomonas syringae* Interaction. *Arabidopsis Book* 1:29-35.
21. Kazan, K., and Manners, J. M. 2011. The interplay between light and jasmonate signaling during defense and development. *J. Exp. Bot.* 62:4087-4100.
22. Khanam, N. N., Ueno, M., Kihara, J., Honda, Y., and Arase, S. 2005. Suppression of red light-induced resistance in broad beans to *Botrytis cinerea* by salicylic acid. *Physiol. Mol. Plant Pathol.* 65:20-29.
23. Kraiselburd, I., Alet, A. I., Tondo, M. L., Petrocelli, S., Daurelio, L. D., Monzon, J. 2012. A LOV protein modulates the physiological attributes of *Xanthomonas axonopodis* pv. *citri* relevant for host plant colonization. *PLoS ONE* 7:e38226.
24. Kroupitski, Y., Pinto, R., Brandl, M. T., Belausov, E., and Sela, S. 2009. Interactions of *Salmonella enterica* with lettuce leaves. *J. Appl. Microbiol.* 106:1876-1885.
25. Lin, C., Ahmad, M., Gordon, D., and Cashmore, A. R. 1995. Expression of an *Arabidopsis* cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. *Proc. Natl. Acad. Sci. USA* 92:8423-8427.
26. Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402-408.
27. Mateo, A., Mühlenbock, P., Rustèrucci, C., Chang, C. C.-C., Misalski, Z., Karpinska, B., Parker, Millineaux, P. M., and Karpinska, S. 2004. LESION SIMULATING DISEASE 1 is required for acclimation to conditions that promote excess excitation energy. *Plant Physiol.* 136:2818-2830.
28. Merritt, J. H., Kadouri, D. E., and O'Toole, G. A. 2005. Growing and analyzing static biofilms. Pages 1B.1.1-1B.1.17 in: *Current Protocols in Microbiology*. R. Coico, T. Kowalik, J. Quarles, B. Stevenson, and R. Taylor, eds. John Wiley & Sons, Hoboken, NJ.
29. Muhlenbock, P., Szechyńska-Hebda, M., Płaszczyca, M., Baudo, M., Mateo, A., Mullineaux, P. M., Parker, J. E., Karpinska, B., and Karpinski, S. 2008. Chloroplast signaling and LESION SIMULATING DISEASE1 regulate crosstalk between light acclimation and immunity in *Arabidopsis*. *Plant Cell* 20:2339-2356.
30. Mussi, M. A., Gaddy, J. A., Cabruja, M., Arivett, B. A., Viale, A. M., Rasia, R., and Actis, L. A. 2010. The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. *J. Bacteriol.* 192:6336-6345.
31. Oberpichler, I., Rosen, R., Rasouly, A., Vugman, M., Ron, E. Z., and Lamparter, T. 2008. Light affects motility and infectivity of *Agrobacterium tumefaciens*. *Environ. Microbiol.* 10:2020-2029.
32. Ondrusch, N., and Kreft, J. 2011. Blue and red light modulates SigB-dependent gene transcription, swimming motility and invasiveness in *Listeria monocytogenes*. *PLoS ONE* 6:e16151.
33. Panopoulos, N., and Schroth, M. N. 1974. Role of flagellar motility in the invasion of bean leaves by *Pseudomonas phaseolicola*. *Phytopathology* 64:1389-1397.
34. Purcell, E. B., and Crosson, S. 2008. Photoregulation in prokaryotes. *Curr. Opin. Microbiol.* 11:168-178.
35. Purcell, E. B., Siegal-Gaskins, D., Rawling, D. C., Fiebig, A., and Crosson, S. 2007. A photosensory two-component system regulates bacterial cell attachment. *Proc. Natl. Acad. Sci. USA* 104:18241-18246.
36. Ramey, B. E., Koutsoudis, M., von Bodman, S. B., and Fuqua, C. 2004. Biofilm formation in plant-microbe associations. *Curr. Opin. Microbiol.* 7:602-609.
37. Río-Álvarez, I., Rodríguez-Herva, J. J., Martínez, P. M., González-Melendi, P., García-Casado, G., Rodríguez-Palenzuela, P., and López-Solanilla, E. 2014. Light regulates motility, attachment and virulence in the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Environ. Microbiol.* 16:2072-2085.
38. Roden, L. C., and Ingle, R. A. 2009. Lights, rhythms, infection: The role of light and the circadian clock in determining the outcome of plant-pathogen interactions. *Plant Cell* 21:2546-2552.
39. Shirasawa, H., Ueno, M., Kihara, J., and Arase, S. 2012. Protective effect of red light against blast disease caused by *Magnaporthe oryzae* in rice. *Crop Prot.* 39:41-44.
40. Talbott, L. D., Nikolova, G., Ortiz, A., Shmayevich, I., and Zeiger, E. 2002. Green light reversal of blue-light-stimulated stomatal opening is found in a diversity of plant species. *Am. J. Bot.* 89:366-368.
41. Tans-Kersten, J., Huang, H., and Allen, C. 2001. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.* 183:3597-3605.
42. Terashima, I., Fujita, T., Inoue, T., Chow, W. S., and Oguchi, R. 2009. Green light drives leaf photosynthesis more efficiently than red light in strong white light: Revisiting the enigmatic question of why leaves are green. *Plant Cell Physiol.* 50:684-697.
43. Uppalapati, S. R., Ishiga, Y., Wangdi, T., Urbanczyk-Wochniak, E., Ishiga, T., Mysore, K. S., and Bender, C. L. 2008. Pathogenicity of *Pseudomonas syringae* pv. *tomato* on tomato seedlings: Phenotypic and gene expression analyses of the virulence function of coronatine. *Mol. Plant-Microbe Interact.* 21:383-395.
44. van der Horst, M. A., Key, J., and Hellingwerf, K. J. 2007. Photosensing in chemotrophic, non-phototrophic bacteria: Let there be light sensing too. *Trends Microbiol.* 15:554-562.
45. Wang, Y., and Folta, K. M. 2013. Contributions of green light to plant growth and development. *Am. J. Bot.* 100:70-78.
46. Yu, S. M., and Lee, Y. H. 2012. First report of *Pseudomonas cichorii* associated with leaf spot on soybean in South Korea. *Plant Dis.* 96:142.
47. Yu, S. M., and Lee, Y. H. 2013. Effect of light quality on *Bacillus amyloliquefaciens* JBC36 and its biocontrol efficacy. *Biol. Control* 64:203-210.
48. Zeier, J., Pink, B., Mueller, M. J., and Berger, S. 2004. Light conditions influence specific defense responses in incompatible plant-pathogen interactions: Uncoupling systemic resistance from salicylic acid and PR-1 accumulation. *Planta* 219:673-683.